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Topical Microbicides in HIV Prevention: State of the Promise

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Abstract

HIV topical microbicides are products with anti-HIV activity, generally incorporating a direct-acting antiretroviral agent, that when applied to the vagina or rectum have the potential to prevent the sexual acquisition of HIV in women and men. Topical microbicides may meet the prevention needs of individuals and groups for whom oral daily forms of pre-exposure prophylaxis (PrEP) have not been acceptable. Microbicides can provide personal control over HIV prevention and offer the possibility of discreet use, qualities that may be particularly important for receptive partners in sexual relationships such as women and transgender women and men, who together account for the clear majority of new HIV infections worldwide. Although the promise of such a product emerged nearly three decades ago, proof of concept has been demonstrated only within the last decade. A robust pipeline of microbicidal gels, films, inserts, and rings has been evaluated in multiple studies among at-risk women and men, and refinement of products for ease of use, reversibility, and high safety is the priority for the field.

CONTEXT

The Joint United Nations Program on HIV/AIDS (UNAIDS) estimates that 38 million persons are currently living with HIV and 1.7 million are newly infected annually (1). The development and wide-scale roll-out of effective antiretroviral medications for persons living with HIV have revolutionized treatment worldwide, but only 23 million of the 38 million persons living currently with HIV are accessing effective treatment and new infections each year continue to outpace the number of infected persons initiating antiretroviral therapy. Over half of those newly infected with HIV each year are women, and adolescent girls and young women aged 15–24 account for 6,200 new infections each week globally, most in sub-Saharan Africa. Indeed, women in some African settings have among the highest rates of HIV infection in any population worldwide, and recent evidence has demonstrated that HIV incidence in young African women in some settings has seen no substantive decline over the past decade (2). Transgender women and men across settings have greater prevalence and incidence of HIV and often lesser access to prevention and treatment services (3). Worldwide, men who have sex with men (MSM) have >20-fold higher risk of HIV acquisition than men from the general population (4, 5).

The past decade has seen the development and scale-up of highly effective and safe prevention tools, most notably using antiretroviral medications as both pre-exposure prophylaxis (PrEP) by uninfected persons to prevent HIV acquisition and as treatment for persons living with HIV to eliminate infectiousness. The first PrEP approach to demonstrate HIV prevention efficacy and gain regulatory approval and normative guidance recommendations used oral tablets containing the antiretroviral tenofovir disoproxil fumarate (TDF), alone or in combination with emtricitabine (FTC) (6–8). Global implementation of TDF/FTC PrEP currently extends to >300,000 persons (**https://www.prepwatch.org/resource/global-prep-tracker/**), a substantial expansion in less than a decade since definitive evidence of efficacy, but still far short of the UNAIDS 2020 target of three million persons. Antiretroviral PrEP and treatment add to longer-standing HIV prevention strategies, including testing (and testing of partners), condom use and behavioral risk reduction, voluntary medical male circumcision, harm reduction interventions for persons who inject drugs, and treatment and prevention of other sexually transmitted infections. In settings where the full complement of prevention strategies has been implemented at the population level, marked reductions in HIV incidence have been observed, even with less than perfect uptake of the prevention components at the individual level, demonstrating that prevention impact at scale is possible (9). However, marginalized groups bear a disproportionate burden of new infections, and prevention tools are not provided to an adequate degree to such groups in most settings, with stigma and discrimination impeding prevention access. Thus, new prevention options, particularly ones that can be delivered at scale and to populations not currently benefiting from prevention access, are needed.

TOPICAL MICROBICIDES FOR HIV PREVENTION: THE PROMISE

Topical microbicides can be applied to the vagina or anus and rectum to prevent the sexual acquisition of HIV. The concept was first proposed nearly three decades ago (10), and the promise of a topical microbicide for HIV protection was driven by the desire for a prevention tool that would be under the control of women. At that time, as now, most prevention tools that engage women are under the control of male partners—for example, condoms provide substantial HIV protection but are inadequate prevention for many women who are unable to negotiate use with their partners. Since the first proposal of the idea of a topical microbicide, the concept has been extended from cisgender women to MSM and to transgender women and men, as all receptive partners

in sexual relationships share a common challenge of achieving HIV protection when prevention tools are under the control of partners.

First-generation microbicides were gels containing detergents, acidifiers, or large molecules without direct antiviral activity against HIV, and none of these products proved effective in preventing HIV transmission. The topical microbicide field has subsequently shifted to products containing direct-acting antiretroviral agents, and the first antiretroviral evaluated was a gel product containing 1% tenofovir. The first suggestion that a topical microbicide could provide protection against HIV occurred in 2010 when a phase II evaluation of a vaginal tenofovir gel demonstrated evidence of HIV protection (11); however, this result was not confirmed in subsequent studies of this product, in large part due to low adherence. The gel was prescribed to be applied either daily or both before and after sex or daily, and it was not advanced to licensure and scale-up (12, 13).

DAPIVIRINE VAGINAL RING FOR HIV PREVENTION IN WOMEN

In 2016, a flexible silicone matrix vaginal ring containing the nonnucleoside HIV reverse transcriptase inhibitor dapivirine, worn for a month at a time, was demonstrated in two phase III placebo-controlled trials to reduce HIV infection in women by ~30% compared to placebo (14, 15), with evidence of greater protection among subgroups with objective evidence of greater adherence to the product (14). Specifically, HIV protection exceeded 50% in women older than 21 years, who had evidence overall of better adherence than women aged 18–21, and more detailed analyses using residual levels in rings that had been distributed and returned each month associated the highest levels of ring use with HIV protection, on the order of 75–92% (16). Among those acquiring HIV, there was no evidence to suggest selection of antiretroviral resistance by dapivirine exposure (14, 17). These findings—specifically that HIV protection occurred when the product was used and that antiretroviral resistance was uncommon—were consistent with those from the licensure trials of oral FTC/TDF PrEP. Although FTC/TDF PrEP has a strong safety profile, its use is associated with mild gastrointestinal symptoms and reversible laboratory abnormalities in renal function in a subset of subjects (18); the dapivirine vaginal ring, in contrast, had no side effects or laboratory anomalies consistently associated with use.

Vaginal rings have been used to provide sustained and controlled release of various medications including contraception, and thus the extension to a sustained-release antiretroviral-containing microbicide for HIV prevention built upon an already-tested technology. Similarly, the concept of chemoprophylaxis is one that has analogies in infectious disease prevention, for example against malaria for travelers, so the application of oral antiretroviral PrEP against HIV, proven for systemically acting antiretroviral pills, could be extended easily to microbicides. That said, for prevention of a systemic infectious disease like HIV, there had been no prior example of the ability of topically applied prophylaxis to be preventative. Thus, the confirmation, in two large studies, that topical delivery of a PrEP agent could protect against HIV was a critical proof of concept for microbicides.

Importantly, the dapivirine vaginal ring has been tested across a series of trials that encompass a woman's life cycle (**Table 1**). Placebo-controlled trials demonstrated HIV protection, safety, and tolerability. Recently completed open-label extension studies (19, 20), which provided the dapivirine vaginal ring to individuals who had participated in the placebo-controlled trials, found objective evidence of greater adherence to and persistence of use of the ring—than in the prior placebo-controlled trials, a finding that had also been seen in open-label extension studies of FTC/TDF PrEP (21). Completed and planned studies evaluate the dapivirine vaginal ring in adolescent girls and young women, postmenopausal women, women who become pregnant while using the ring, women who start using the ring while pregnant, women who choose to use contraception, and women who are breastfeeding a child. The planned MTN-042 trial

Table 1 The dapivirine vaginal ring: evaluation across the life cycle

Population	Trial design	Trial name and size	Location	Status/findings	References
Women aged 18–45 years, using effective contraception	Randomized, double-blind, placebo-controlled trial	MTN-020/ ASPIRE ($n = 2,629$) IPM 027/The Ring Study ($n = 1,959$)	Malawi, South Africa, Uganda, Zimbabwe	Completed. Demonstration of efficacy and high safety profile = proof of concept for topical microbicides through two confirmatory trials. HIV incidence compared to placebo was 27% and 31% in the two trials, respectively, with >50% HIV protection in analyses among subgroups with objective evidence of greater adherence	14, 15
Women aged 18–45 years, using effective contraception	Open-label extension trial (limited to participants from the placebo-controlled trials)	MTN-025/ HOPE ($n = 1,456$) IPM 032/ DREAM ($n = 941$)	Malawi, South Africa, Uganda, Zimbabwe	Completed. Higher adherence, by objective measures, than in the placebo-controlled trials. High persistence of use over 12 months. HIV reduction estimated at 39% and 63% in the two trials, respectively, compared to a counterfactual estimate. Similar safety profile to the placebo-controlled trials	19, 20
Adolescent girls and young women aged 15–17 years	Randomized, double-blind, placebo-controlled trial	MTN-023/ IPM 030 ($n = 96$)	United States	Completed. Safe, well tolerated, and acceptable in adolescents	85
Post-menopausal women aged 45–65 years	Randomized, double-blind, placebo-controlled trial	MTN-024/ IPM 031 ($n = 96$)	United States	Safe, well tolerated, and acceptable in postmenopausal women. Plasma concentrations were comparable to reproductive-age women	86
Women who became pregnant while using the dapivirine vaginal ring	Randomized, double-blind, placebo-controlled trial	Subset from MTN-020/ ASPIRE ($n = 169$)	Malawi, South Africa, Uganda, Zimbabwe	Completed. Similar distribution of pregnancy outcomes and no difference in the frequency or pattern of congenital anomalies or infant growth for those assigned dapivirine vaginal ring or placebo. No interference with contraception	87, 88
Women initiating the dapivirine vaginal ring during pregnancy	Randomized, open-label trial (compared to FTC/TDF PrEP)	MTN-042 ($n = 750$)	Malawi, South Africa, Uganda, Zimbabwe	Pending. Anticipated initiation Q4 2019. **https://mtnstopshiv.org/research/studies/mtn-042**	None
Women breastfeeding a child	Open-label trials	MTN-029/ IPM 039 ($n = 16$) MTN-043 ($n = 200$)	Malawi, South Africa, Uganda, United States, Zimbabwe	MTN-029/IPM 039 completed. Low concentrations of dapivirine in breastmilk with low estimated daily levels of infant dapivirine exposure. Anticipated start of MTN-043 Q4 2019/Q1 2020. **https://mtnstopshiv.org/research/studies/mtn-043**	89

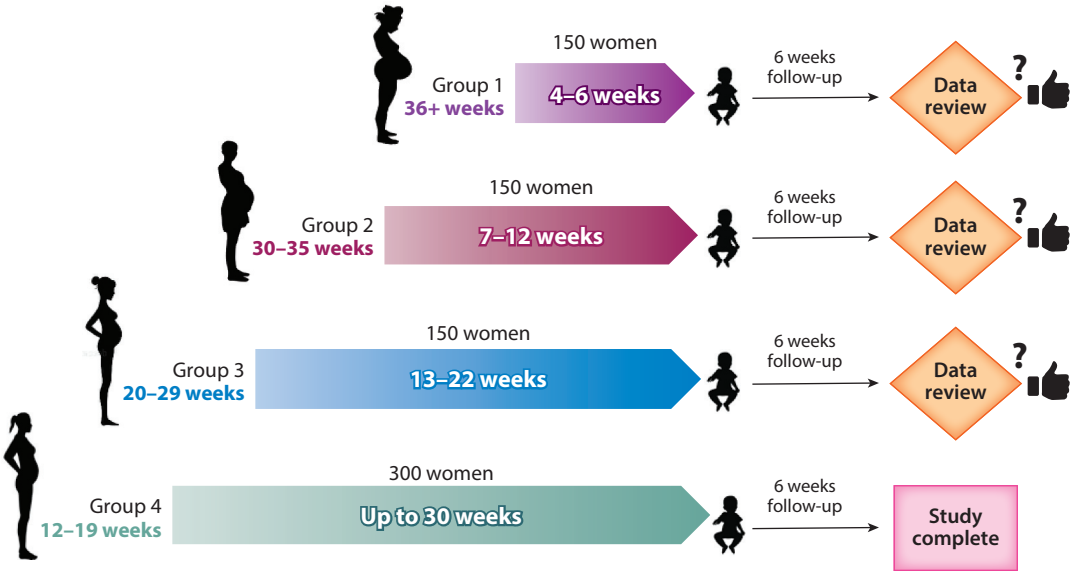

Figure 1

MTN-042 (**https://mtnstopshiv.org/research/studies/mtn-042**) is a planned open-label, multisite, randomized trial of safety and pharmacokinetics of the dapivirine vaginal ring versus FTC/TDF oral PrEP (2:1 assignment) when used during pregnancy. Approximately 750 women aged 18–45 years who have an uncomplicated singleton pregnancy will be enrolled in sequential cohorts, starting in later pregnancy and, if safety is demonstrated, moving earlier. Abbreviations: FTC/TDF, tenofovir disoproxil fumarate in combination with emtricitabine; PrEP, pre-exposure prophylaxis.

(**https://mtnstopshiv.org/research/studies/mtn-042**), which will directly compare the safety and pharmacokinetics of the dapivirine vaginal ring versus oral FTC/TDF PrEP when used during pregnancy, offers a novel design for evaluation of medications in pregnant women (**Figure 1**), with sequential evaluation beginning in women in later pregnancy and moving into the early second trimester. The dearth of rigorous research of new medications in pregnant women is a perennial challenge for drug development but is of particular concern for HIV prevention, given the large number of HIV infections that occur in women of childbearing age globally and the repeatedly documented observation that women have greater susceptibility to HIV infection during pregnancy and the postpartum period (22). Of note, the MTN-042 trial will also provide some of the most rigorous assessment to date of FTC/TDF PrEP pill use in pregnant women.

An array of new microbicide products has been evaluated in multiple studies among at-risk women and men, and microbicide products form a substantial fraction of novel PrEP agents in the pipeline for new product development (**https://www.avac.org/infographic/future-arv-based-prevention**). For vaginal products, substantial activity is being devoted to ring delivery, given the example of successful and safe HIV prevention with dapivirine; however, other formulations, including vaginal films and tablets, are in later-phase preclinical development. Notably, rectal microbicide products have benefited from substantial innovation in both preclinical science and advancement to early-phase clinical testing in the past few years.

RECTAL MICROBICIDES: PRODUCT INNOVATION

The development of rectal microbicide products lagged vaginal product development by a decade. As with vaginal products, the focus has been on directly acting antiretroviral active pharmaceutical

Table 2 Recently completed and actively enrolling phase I clinical studies of rectal microbicides

Study[a]	Active ingredient	Formulation	Status
MTN-026[b]	dapivirine	gel (HEC)	completed, in data analysis
MTN-033[b]	dapivirine	gel applicator and gel as lubricant	completed, in data analysis
MTN-035[d]	placebo	insert, suppository douche	enrolling
MTN-037[c]	MIV-150, Zn^{++}, carrageenan	gel applicator	completed, in data analysis
MTN-039[d]	elvitegravir, tenofovir alafenamide	Fast-dissolving insert	enrolling
DREAM-01 U19[e]	tenofovir (single dose escalation)	douche	completed, in data analysis
DREAM-02 U19[e]	tenofovir (sex effects)	douche	enrolling
DREAM-03 U19[e]	tenofovir (multiple dose)	douche	enrolling
ImQuest U19[e]	IQP 0528	gel applicator	completed, in data analysis
PREVENT U19[e]	Griffithsin	douche	enrolling
Orion Biotechnology	OB-002H (CCR5 antagonist)	gel applicator	enrolling

[a]All studies include assessments of toxicity, pharmacokinetics, pharmacodynamics, and acceptability, except MTN-035 (placebo products only).
[b]Microbicide Trials Network (MTN) and International Partnership for Microbicides partnership.
[c]Microbicide Trials Network (MTN) and Population Council partnership.
[d]Microbicide Trials Network (MTN) and Contraception Research and Development (CONRAD) partnership.
[e]U19 funded by Integrated Pre-Clinical/Clinical HIV Topical Microbicides Program of NIH.
Abbreviation: HEC, hydroxyethyl cellulose.

ingredients. Only one rectal microbicide (tenofovir, formulated as gel) has been advanced to a phase II trial (MTN-017) (23–25), and no rectal microbicide has been tested for protective efficacy. At least eight products have completed phase I testing—most in the past 12 months—in development programs benefiting from earlier vaginal microbicide development and more recent scientific methodologic advancements (**Table 2**). The rectal microbicide field has thus advanced tremendously in recent years, and new product development is proceeding quickly.

Novel Methodologies for Rectal Microbicide Advancement

New methods that have been applied to rectal microbicide development have included not only laboratory work focused on novel pharmacokinetic and pharmacodynamic studies but also behavioral and social science focused on acceptability across populations.

Small intensive pharmacokinetic studies established concentration–response relationships in numerous oral tenofovir PrEP trials. Using sparse biopsy sampling methods, in which each individual contributed biopsies at only some sample times (minimizing subject burden), a composite with rich tissue sampling was provided alongside traditional blood sampling. Subsequently, methods directly assessed tissue $CD4^+$ cell subsets to describe active intracellular analytes of TDF and FTC along with deoxynucleotide triphosphates with which they compete for HIV reverse transcriptase binding (26–28). This enabled multicompartment models that, combined with viral dynamic models, enabled clinical trial simulation to define optimized dosing and formulation for rectal microbicides (27, 29, 30). To assure microbicide distribution within the colorectal lumen that matched or exceeded the area of HIV exposure, the colorectal distribution of radiolabeled "HIV surrogates" was visualized using single photon emission computed tomography (SPECT)/CT imaging after simulated sex (31). For further dose optimization, biomarkers of prevention efficacy were developed, using preclinical models of rectal and vaginal viral challenge in macaques and humanized mice. In addition, HIV challenge of cervicovaginal and colorectal tissue explants ex vivo, following in vivo microbicide dosing, was also developed for use in clinical studies (32, 33).

Informed by acceptability challenges in trials of vaginal gel microbicide trials, most ongoing rectal microbicide programs include intensive quantitative behavioral assessments as early as the vehicle development stage to assure acceptability of candidates at the earliest development stages (34, 35). Some development programs included take-home doses to assess essential coital experiences after product dosing. Acceptability outcomes have been integrated in parallel with safety and pharmacokinetics outcomes in most ongoing rectal microbicide studies. Finally, while MSM have been the subject of every rectal microbicide product developed, there has been increasing inclusion of transgender women early in development as a key population with especially high HIV transmission risk and, potentially, different behavioral requirements for a rectal microbicide.

Rectal Microbicide Formulations in Development

A number of novel rectal microbicide products are progressing through early clinical development. These include gels as well as new microbicide modalities.

Gel products. The first 15 years of rectal microbicide development have been dominated by gel formulations applied intrarectally using an applicator. The longest development effort focused on tenofovir due to concurrent development of the vaginal tenofovir gel and tenofovir's long intracellular half-life in its active phosphorylated form. The first rectal microbicide study of tenofovir, RMP-02/MTN-006, used the same tenofovir 1% vaginal formulation—a highly hyperosmolar gel—dosed with the vaginal applicator (36). Mild lower gastrointestinal tract symptoms attributed to the hyperosmolar formulation filling the colon with fluid ended further development of the vaginal formulation for rectal use. A reduced-glycerin formulation advanced to the six-month extended safety study, MTN-017, in which on-demand gel dosing prior to sex was preferred to daily gel dosing (37–39). Importantly, the applicators used in that study, originally designed for vaginal use, were not acceptable for rectal application. Finally, a nearly iso-osmolar formulation explicitly designed for rectal use achieved better colorectal tissue cell tenofovir diphosphate concentrations, lower systemic concentrations, and slightly better rectal explant suppression of HIV than the reduced-glycerin formulation; however, lacking a suitable method of gel delivery, this product has not advanced (40, 41).

Beyond tenofovir, the CCR5 inhibitor maraviroc in gel form applied with a vaginal applicator demonstrated an excellent safety and acceptability profile, and colorectal tissue concentrations of maraviroc after rectal gel dosing exceeded concentrations after oral dosing in a phase I study (42). Notably, the drug failed to suppress HIV replication in the explant model, but this has been a consistent problem for maraviroc, as tissue culture conditions rapidly deplete the drug from explants; macaque and humanized mouse challenge models have shown viral protection. Phase I testing is planned for a recombinant protein, OB-002H (5P12-RANTES), a more potent CCR5 antagonist than maraviroc.

Dual dosing site products. Cisgender women and transgender women and men who engage in both receptive (neo)vaginal sex and anal sex have unique microbicide product development needs. A single product that protects both the (neo)vagina and rectum after dosing in only one mucosal location remains a challenge for topical agents, in contrast to systemically dosed FTC/TDF PrEP. Tenofovir poorly penetrates from rectum to vagina and vice versa; however, FTC and maraviroc dosed via an intravaginal ring achieve rectal fluid concentrations in the range of the in vitro IC_{90}, indicating potential for dual-compartment protection for some drugs (43–45). A simpler development approach, although more complex in clinical use, would be a product that could be applied in whichever sexual compartment is at risk. Three nonnucleoside reverse transcriptase inhibitors

are now in development for both rectal and vaginal dosing. A gel formulation of the agent IQP-0528 just completed phase I rectal dose studies; a suppository formulation of IQP-0528 is also in development. Two pharmacokinetic and safety studies (MTN-026 and MTN-033) of a dapivirine rectal gel have also recently been completed. MTN-033 directly compared applicator dosing with manual dosing as an anal lubricant, in part to evaluate an alternative to applicator-based rectal gel dosing (see below). Finally, a combination of the nonnucleoside reverse transcriptase inhibitor MIV-150 plus zinc acetate and carrageenan (called PC-1005 gel), formulated for rectal and vaginal use and with potential activity against HIV, HPV (human papillomavirus), and HSV-2 (herpes simplex virus type 2) is being developed; a clinical safety, pharmacokinetic, and explant pharmacodynamic study of rectal PC-1005 was recently completed and is in data analysis.

Fast-dissolving insert. One solution to the challenge of rectal gel applicators may be a fast-dissolving insert that can be placed into the rectum or vagina digitally, after which the tablet dissolves. A phase I study (MTN-039) recently began enrolling to evaluate two rectal doses of a fixed-dose combination of tenofovir alafenamide with elvitegravir for safety, acceptability, and pharmacokinetic readouts; it is hoped that this drug combination will provide the rapid tissue uptake and high barrier to resistance of elvitegravir with the long pharmacologic duration of tenofovir.

Medicated anal lubricants and douches. A universal challenge with PrEP strategies to date, both pills and topicals, has been that their use entails behaviors separate from, and sometimes antagonistic to, behaviors surrounding sexual activity. The consequence is a reduction in acceptability and adherence. Lubricants and cleansing douches are routinely used by a high percentage of MSM and transgender women practicing receptive anal sex; thus, medicating such products with antiretrovirals could be behaviorally congruent. Indeed, web-based surveys in the United States show 95–98% of respondents indicate high likelihood of using such a product, if proven effective, regardless of their current douche practices (46).

The feasibility of dosing a microbicide as a sexual lubricant was directly compared to a gel applicator and douche using SPECT/CT imaging of radiolabeled formulations. Imaging indicated that only 3% of the radiolabeled gel applied as a sexual lubricant reached the rectal mucosa, although the rectosigmoid distribution corresponded to semen distribution after simulated anal sex (47). By contrast, 97% and 60% of the applicator gel and douche, respectively, were retained within the lower gastrointestinal tract. MTN-033, described above, will report quantitative tissue concentrations of dapivirine to provide additional evidence regarding the feasibility of dosing a rectal microbicide as anal lubricant. A phase I dose escalation study of a tenofovir douche demonstrated excellent acceptability, no toxicity, full explant suppression, and colorectal tissue cell concentrations of tenofovir diphosphate from 1 h through 72 h after a single dose that exceeded concentrations achieved with the on-demand douche or oral TDF/FTC (48, 49). Macaque studies indicate superior SHIV (simian/human immunodeficiency virus) protection with a single weekly tenofovir douche when compared to daily oral TDF/FTC dosing (50). The PREVENT Program has recently begun phase I studies of a douche formulation of griffithsin, a red algae–derived lectin that is a highly potent viral entry inhibitor with broad activity against HIV, HSV-2, HCV (hepatitis C virus), and other enveloped viruses.

BIOLOGIC CHALLENGES IN THE DEVELOPMENT OF TOPICAL MICROBICIDES

The development of all topical microbicide products, whether delivered vaginally or rectally, rests on substantial product development science. Attention has focused recently on two key areas: pharmacologic assessments and potential modifiers of topical microbicide PrEP efficacy.

Pharmacologic Challenges

Pharmacologic studies of antiretroviral concentrations in multiple anatomic compartments—including blood, cervicovaginal and colorectal tissue, and vaginal and colorectal luminal fluid—have been used to better understand the relationship between antiretroviral PrEP use and HIV protection (51–53). In addition, PrEP drug concentrations in blood, blood cells, dried blood spots, hair, and urine after directly observed dosing have all been used to establish drug adherence benchmarks (54–57). These matrices, plus residual intravaginal ring drug concentrations, have been used for semiquantitative adherence assessments in clinical trials (14, 16). In some cases, drug concentrations as adherence surrogates were used in the context of clinical trials to target adherence enhancement efforts (58, 59). As multicompartment pharmacokinetic-pharmacodynamic models are generated and combined with viral dynamic models, clinical trial simulation becomes more feasible to help optimize trial design for future randomized microbicide trials (27, 30, 60).

The rational development of topical microbicides requires application of the accumulated knowledge of PrEP drug pharmacology—both systemically and locally in mucosal tissues. Absent significant systemic concentration, mucosal tissue concentrations following topical microbicide dosing are essential for understanding PrEP concentration–response relationships. Therefore, these sites best inform extrapolation from in vitro, preclinical, and proven clinical studies of oral or systemic agents to identify concentration targets for topical microbicide development. Protection may require both systemic and mucosal concentrations in a combination that differs between oral and topical dosing. Fixed-dose combination oral FTC/TDF provides an example of the complexity of using oral dosing pharmacokinetic-pharmacodynamic data to identify topical dosing concentration targets. The treatment dose of 200 mg FTC/300 mg TDF was used in PrEP trials, which were launched without prior understanding of preclinical animal model protection or mucosal tissue pharmacology. Vaginal dosing of tenofovir achieves local levels of drug 1,000-fold higher than with oral TDF dosing, while plasma concentrations are 100-fold lower; vaginal tenofovir levels have been associated with HIV protection in one tenofovir gel study (26, 52, 61). There are no efficacy studies of rectal microbicides to see if this estimated protective mucosal concentration difference is also found in colorectal tissue, but rectal tenofovir microbicide products in development are targeting colorectal concentrations higher than those associated with protection after oral dosing.

Potential Biologic Modifiers of Microbicide Efficacy

Whether biologic factors—of individuals or of their microbiologic environment—influence the effectiveness of microbicides is a topic of recent scientific inquiry.

Vaginal dysbiosis. Vaginal dysbiosis has been associated with increased HIV risk. A combination of sequencing and quantitative PCR has been used to identify vaginal microbiota associated with an increased risk of HIV acquisition in an East African cohort (62) and the VOICE study (12, 63) with remarkably similar results. Concentrations of bacteria that were significantly associated with increased risk for HIV acquisition included *Gemella asaccharolytica*, *Sneathia*, *Mycoplasma hominis*, *Prevotella bivia*, *Eggerthella* Type 1, *Megasphaera* Type 2, and *Parvimonas* Type 2, all of which are associated with a dysbiotic vaginal microbiome. Women having the highest concentrations of *Lactobacillus crispatus* had a decreased HIV acquisition risk in VOICE. Similarly, in another cohort of young women from South Africa, both dysbiotic microbiota and inflammation were linked with increased risk of HIV (64); in that study, none of the women having a *L. crispatus* dominant vaginal microbiome acquired HIV (but, of note, HIV incidence in women having a vaginal microbiome dominated by *L. iners* was similar to that in women with dysbiotic microbiota). A retrospective

analysis from the CAPRISA004 trial of tenofovir gel found that product efficacy was much higher among women having a *Lactobacillus*-dominant vaginal microbiome than in women with vaginal dysbiosis (65). Although this analysis was limited because the methods used were insufficient to distinguish *Lactobacillus* communities dominated by beneficial species such as *L. crispatus* versus less beneficial species such as *L. iners*, it raised concerns that tenofovir-based microbicides could be undermined by microorganisms such as *Gardnerella vaginalis* or *Prevotella* species, which could effectively reduce the level of drug available locally. A secondary analysis of a phase I study of plasma and genital tissue drug levels among women using tenofovir vaginal gel or tenofovir vaginal film with timed application of drug product found that women with higher concentrations of *G. vaginalis* or *Atopobium vaginae*, or a diagnosis of bacterial vaginosis, had reduced genital tissue concentrations of tenofovir and the active metabolite, tenofovir diphosphate (66, 67).

In vitro studies have evaluated the mechanisms by which vaginal microbiota could modify the concentrations of drug in genital tissues, potentially reducing the efficacy of topical microbicides. One hypothesis is that *G. vaginalis* metabolizes tenofovir to adenine (65); another is that *G. vaginalis* secretes adenine, which is consumed by *A. vaginae*, creating bacterial synergy between these species in the vaginal ecosystem (68). Although these authors found no evidence that *G. vaginalis* directly metabolizes tenofovir, they did report that *L. crispatus* could metabolize tenofovir and act as a sink by trapping tenofovir as tenofovir diphosphate for several days, which could lengthen persistence of the active metabolite. Whatever the mechanism governing the interactions between tenofovir applied vaginally and the vaginal microbiome, any reductions in drug levels could be overcome by increasing the amount of drug delivered or through use of continuous-release drug delivery platforms, such as a tenofovir vaginal ring. Support for this comes from a study that found no difference in tenofovir diphosphate levels in women using a tenofovir vaginal ring when stratified by *Lactobacillus*-dominant versus dysbiotic vaginal community states (69).

Concerns have been raised that the vaginal microbiome could also impact the effectiveness of oral FTC/TDF as PrEP. However, a retrospective analysis of the Partners PrEP Study did not find significant differences in efficacy based on Nugent score, or the bacterial morphotypes including *Lactobacillus* identified (70); adherence to FTC/TDF in that trial was generally high, potentially compensating for any changes in drug exposure related to microbiome. Cervicovaginal fluid-to-plasma ratios of antiretrovirals have indicated a bimodal relationship between tenofovir penetration and alpha diversity of the vaginal microbiome (71). The issue of how the vaginal and gut microbiome could impact tissue pharmacokinetics, or topically and orally administered antiretrovirals, is of increasing interest and in the early stages of careful evaluation (68).

Recent studies have also raised concerns about the effect of the vaginal microbiome on dapivirine (65, 68). Dapivirine is a hydrophobic nonnucleoside reverse transcriptase inhibitor that binds to all cells, including bacteria. In in vitro studies, dapivirine was reported to bind irreversibly to bacteria, which could impact the availability of the drug to diffuse into the genital tract tissues. However, in the ASPIRE trial, there was no modification of dapivirine ring efficacy for women with bacterial vaginosis, as defined by the Nugent criteria, compared to those without bacterial vaginosis (72).

Age, hormonal status, and contraceptive use. Although the recent ECHO (Evidence for Contraceptive Options and HIV Outcomes) study found no significant differences in HIV acquisition between women using contraceptive implants, injectable depot medroxyprogesterone acetate (DMPA), or the copper intrauterine device (2), there is some evidence that age, hormonal status, and some contraceptive usage could be modifiers of tenofovir and its metabolites in genital tissues. In a recent study evaluating the impact of the microbiome and use of DMPA on local drug levels in the genital tract, increasing age was associated with decreased exposure of tenofovir metabolites

and endogenous nucleotides (73). This effect was specific to the female genital tract, as there was no correlation between age and peripheral blood mononuclear cell drug concentrations. The age effect on tissue levels of drug is not fully understood but is unlikely to be due simply to hormones since the women in the study were of reproductive age, and age remained a significant predictor of drug concentrations even after adjusting for hormone use. This group of researchers also reported significantly lower ex vivo conversion of tenofovir and emtricitabine to their phosphorylated metabolites in cervical tissue from postmenopausal women (74). In a study evaluating the impact of endogenous and exogenous hormones on tissue levels of tenofovir, there was a lower mean tenofovir tissue concentration in the follicular phase than in the luteal phase of the menstrual cycle (75), and mean genital tissue tenofovir diphosphate levels increased after DMPA to levels higher than in the follicular and luteal phases. This study reported that the conversion of tenofovir into tenofovir diphosphate was more effective in DMPA users (molecular ratio of 2.02 versus 0.65 luteal phase). Other antiretroviral drugs that do not require phosphorylation, such as dapivirine, could be less impacted by age-related changes in endogenous nucleotides as well.

PREVENTION CHOICE AND THE FUTURE OF TOPICAL MICROBICIDES

Choice

Adherence issues in microbicide trials brought to the fore the need for robust behavioral and social science to optimize product development and use. Indeed, very low adherence in the VOICE trial of tenofovir vaginal gel (12) both refocused the field and propelled a rapid evolution in product design, clinical trial evaluation, and thinking about optimized PrEP options generally, not simply for topical agents. Work post-VOICE highlighted numerous factors associated with product nonuse: lack of appeal of a daily vaginal gel, stigma from partners and others in women's lives about using an HIV prevention product that could not be easily hidden, concern about using a product that had not yet been proven to be safe and effective, and desire for a range of product options (76–79). Recent studies have explored product preferences, sometimes using placebo products so that the only assessment is of the delivery approach itself rather than a specific (and unproven) entity. Preferences have varied across users—emphasizing that there is no one specific HIV prevention product, topical or systemic, that will appeal to all individuals. The newly launched DESIRE Study (**https://mtnstopshiv.org/research/studies/mtn-035**) is directly assessing three placebo rectal microbicide formulations—suppository, douche, and fast-dissolving insert—to gather key safety, acceptability, tolerability, and adherence data when each is used on demand (i.e., just before sex) in four-week period cross-overs. More and more, the analogy to contraception—where availability of multiple products, with different delivery approaches, optimizes coverage and prevention benefits (80, 81)—seems likely to apply to systemic and topical PrEP as well. With such personalized options, topical microbicides could have potential advantages that appeal broadly (**Figure 2**).

Multipurpose Technologies

Multipurpose technologies (MPTs) are drug delivery platforms that offer a single agent or a combination for prevention of HIV with additional effects such as prevention of unintended pregnancy or one or more sexually transmitted infections. Combining antiretroviral drugs and contraceptive hormones into single prevention products could potentially reduce the HIV-related stigma sometimes associated with PrEP products that dampens uptake and use by offering two choices at once. The integration of family planning into HIV prevention products could increase convenience for

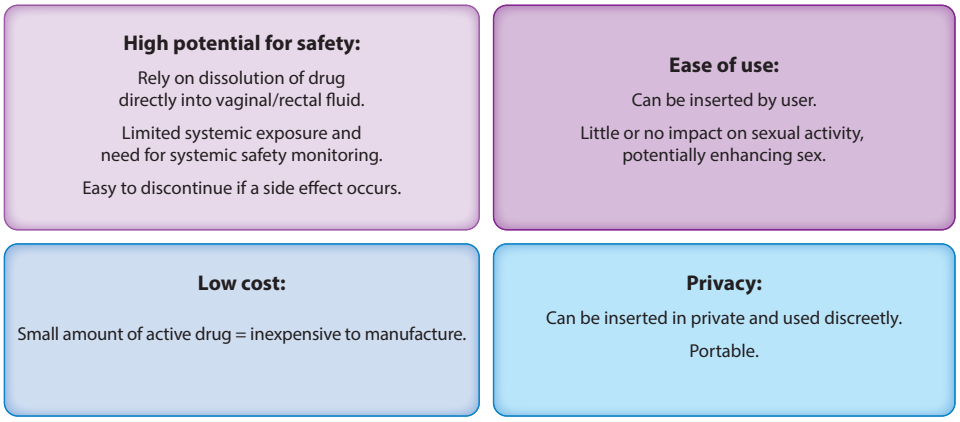

Figure 2

Topical microbicides as a choice.

women while being potentially more cost-effective to programs by reducing product streams (82). The MPT development pipeline has grown substantially over the past decade, but MPT development is still in an early phase. Indeed, of the 27 projects described in a comprehensive database (**http://mpts101.org/mpt-database**), only one is a phase III clinical trial; all other activities are preclinical and phase I.

Four phase I studies evaluating tenofovir, including in combination with levonorgestrel in a vaginal ring that could be worn for 90 days at a time, have been completed or are in progress (83). Although it is unclear if the concentration of tenofovir diphosphate, the active moiety for antiretroviral activity, from such rings in relevant target cells (i.e., immune cells for HIV and epithelial cells/keratinocytes for HSV-2) will be sufficient for protection from HIV and HSV-2 in women, data thus far have shown levels exceeding protective concentrations in monkeys, in both cervicovaginal fluids and tissues. These rings will be evaluated in expanded safety and efficacy trials for prevention of HIV, HSV-2, and pregnancy. A combined dapivirine/levonorgestrel ring has also been evaluated in two phase I studies for up to 90 days (84); the trials found the dual-purpose ring to be safe and well tolerated.

CONCLUSIONS

Three decades after the proposal that a receptive partner in a sexual relationship could have a discreet prevention tool under her or his own control, proof of concept has been demonstrated for topical microbicides to prevent HIV. A robust pipeline exists of microbicide products for potential use by both men and women, across their life cycles. The promise remains for topical microbicides to be an important, safe, effective, and used prevention choice for some of the most vulnerable at-risk populations globally.

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