Topical Microbicides to Prevent HIV: Clinical Drug Development Challenges

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Key Words

pre-exposure prophylaxis, antiretroviral drugs, adverse events, pharmacokinetics, pharmacodynamics, clinical trial simulation

Abstract

Microbicides, substances applied topically to prevent sexual HIV infection, are needed to empower receptive sexual partners with effective prevention methods. Several large microbicide trials, however, failed to demonstrate efficacy, thus motivating a reevaluation of the current microbicide development paradigm, which has been largely empirically based. Microbicide use occurs in a highly complex environment involving multi-level interactions, behavioral and biochemical, among host, virus, and drug, yet many details of these interactions remain unknown. Fundamental information regarding virus and drug distribution over time in sexually receptive body compartments that is necessary to design a microbicide able to outdistance and outlast the virus is largely absent. Recent efforts have been made to establish a simple conceptual framework for obtaining the knowledge that is likely to inform a more mechanistic, model-based development paradigm. These efforts have also advanced the development of numerous methodological approaches to obtain the knowledge needed to improve microbicide development.

CURRENT STATUS OF MICROBICIDE DEVELOPMENT

Rationale for Topical Microbicide for HIV Prevention

HIV: human immunodeficiency virus

Microbicide: drug applied topically in the vagina or rectum in a variety of formulations (gel, vaginal ring, film, enema) for the purpose of preventing sexual transmission of HIV infection in a sexually receptive partner

Pre-exposure prophylaxis (PrEP): drug administered orally or topically, including microbicides, to prevent HIV infection by any of several routes of transmission

A topical human immunodeficiency virus (HIV) microbicide is a vaginally or rectally applied substance intended to prevent sexual transmission of HIV infection. The need for HIV microbicides has grown out of the continuing HIV epidemic in which an estimated 2.5 million new HIV infections still occur yearly, with continued expansion in many areas of the world. Treatment, though highly effective, is not curative, and prospects for an HIV vaccine seem increasingly distant. Microbicides are being developed as one among an array of prevention strategies that together might significantly alter the epidemic.

Microbicides are a subset of pre-exposure prophylaxis (PrEP) strategies that include topical and oral dosing of agents to prevent HIV transmission. Microbicide development has focused on vaginal products because of the increasing feminization of the epidemic within the US and internationally. Women now comprise more than half of new infections, a result of their greater biological and social vulnerability to HIV. Accordingly, there is urgent need for a woman-controlled method to prevent sexually-related HIV infection. Recently, some attention has turned to include rectal microbicide development for both men and women who engage in receptive anal intercourse (RAI).

The rationale for drug-based HIV prevention evolved from successful biomedical HIV prevention successes as antiretroviral drugs (ARV) reduce transmission in several settings: mother-to-child, healthcare worker occupational HIV exposure, and sex partners of a treated HIV-infected partner. The topical route of microbicide dosing is supported by successful topical treatment and prevention settings (e.g., vaginal contraceptives) when a localized, accessible site of action is involved and systemic exposure is worth avoiding. The combination of a pharmaceutical intervention to augment behavioral risk reduction is well established in medicine (e.g., diabetes, cardiovascular disease, osteoporosis, and smoking cessation). To prevent malaria in travelers, prophylactic medications are commonly prescribed where behavior change—avoidance of endemic areas—is more efficacious, though arguably less enjoyable.

Sixteen candidate microbicides have entered the clinical phase of development having diverse mechanisms of action and, therefore, varied sites of action with relevance for pharmacokinetic studies (**Table 1**) (1). Four candidates that have completed efficacy trials have failed to prevent HIV infection. C31G failed to demonstrate efficacy owing to low HIV incidence in the study (2). Carraguard's failure occurred in association with less than 50% adherence (3). Cellulose sulfate demonstrated an increased risk of HIV infection (4), possibly owing to toxicity of the active ingredient (5, 6) or the hyperosmolar gel vehicle (iso-osmolar placebo) (7). Nonoxynol 9 (N9) also showed an increased risk of HIV transmission in the COL-1492 study (8). Unlike the others, N9 has a clear trail of evidence from in vitro, animal, and clinical studies showing dose-related N9 induced vaginal inflammation and epithelial disruption in some, but not all studies [reviewed by Hillier et al. (9)]. However, the clinical relevance of many of the observed changes was unclear at the time, and much of the evidence accumulated in parallel with large, empirically-based clinical trials. The clinical implication of the findings was interpreted with great clarity, in retrospect, when COL-1492 showed N9 to have a dose-dependent HIV infection risk. The challenge then, as now, in finding biomarkers with predictive clinical value is to identify the optimal balance of sensitivity for detecting toxicity and specificity to allow effective candidates through a screening program. For prevention efficacy, finding any biomarker is a special problem.

These microbicide failures occurred alongside other strategies that also failed to prevent HIV infection [herpes simplex virus type 2 suppression, female diaphragm use, and the Merck adenovirus-based vaccine candidate (10–12)]. A recent Institute of Medicine (IOM) report deals

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Table 1 Selected microbicide candidates in clinical phases of development

Class	Site of action	Mechanism of action	Candidates	Status
I	Lumen	Vaginal defense enhancer	Buffergel™	Efficacy—near completion
			Acidform™	Efficacy—ongoing
			Lime juice	Safety—not demonstrated
II	Lumen	Membrane lysis	Nonoxynol-9	Efficacy—not demonstrated[1]
			Savvy™ (C31G)	Efficacy—not demonstrated
III	Lumen	Membrane binding inhibitor	Cellulose sulfate	Efficacy—not demonstrated
			Carraguard™	Efficacy—not demonstrated
			PRO 2000™	Efficacy—near completion
			Invisible Condom™	Efficacy—planned
			Vivagel™	Expanded safety/PK
			Cellulose acetate phthalate	Safety/acceptability
IV	Tissue	Cell membrane binding inhibitor	Maraviroc	ARV licensed to IPM[2]
V	Intracellular	Reverse transcriptase inhibitor	Tenofovir	Efficacy—ongoing
			UC-781[3]	Safety/acceptability
			Dapivirine	Safety/acceptability/PK
			MIV-150[3]	Efficacy—planned[4]

[1] Studies failed to reject the null hypothesis of no difference between intervention and placebo.
[2] Antiretroviral licensed to International Partnership for Microbicides for microbicide development, currently not in clinical microbicide development.
[3] Site of action may also be intraviral for these nonnucleoside reverse transcriptase inhibitors.
[4] Clinical trial planned for combination use with Carraguard™.

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with the many methodologic challenges facing HIV prevention studies generally (13). With these failures in mind, this review focuses on methodological challenges in the early phases of clinical microbicide development, with emphasis on pharmacokinetics, safety, and proof-of-concept studies where there is room to better inform a product's development path.

Microbicide Development Contrasted with Optimal Drug Development

The microbicide development programs to date differ substantially from a desired drug development model in several critical respects unique to microbicides (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org** to **Supplemental Figure 1**). First, the local mucosal changes that occur after exposure to drug, while benign in the treatment of almost any other condition, may be devastating for HIV prevention if they increase HIV infection risk. Most of the completed phase IIb/III efficacy studies relied largely on symptom assessment and visual (sometimes colposcopically aided) inspection of vaginal tissue without the benefit of more recently developed biological, cellular, and tissue assays to assess potential for increased susceptibility to HIV infection. Continued uncertainty about the clinical importance of a given type, magnitude, and duration of mucosal change makes selection and interpretation of safety data in all phases difficult.

Second, distribution and clearance (pharmacokinetics) of candidate microbicide gels at the site of action rarely preceded large efficacy studies; hence, concentration-time data were unavailable to aid concentration-effect evaluations necessary to interpret unsuccessful trial outcomes. Third, migration of the drug target, namely cell-free or cell-associated HIV (both may be important), within the lumen of sexually receptive spaces remains largely unknown, such that the desired drug distribution and clearance characteristics are unknown. Fourth, and perhaps the most limiting,

there is no suitable biomarker established to enable a clinical proof-of-concept microbicide study to explore the impact of dose and frequency to inform pivotal clinical efficacy trial designs. Typically, only one or two dosages are tested, usually for only ambiguous, unvalidated safety markers. Data-rich exploratory learning studies (including proof-of-concept studies) to inform larger hypothesis-testing confirmatory efficacy studies in refining mutually-informing learning-confirming cycles described by Lewis Sheiner (14) are nonexistent. Without this type of information, clinical trial simulation cannot be done.

Complex Environment of Microbicide Use

The environment in which microbicides will be used is a dynamic interaction of microbicide, HIV, and the person at risk of HIV infection (**Figure 1**). Additionally, there are critically important parasexual behaviors that influence each of the other elements.

Host-virus. The interaction between HIV and host mucosal tissues is a key variable given the large difference in vaginal and rectal environments and the resulting differences in risk of transmission. The focus on vaginal microbicide development today is slowly evolving to consider the important toxicity and efficacy issues for rectal HIV exposures in men and women (discussed in detail below).

Microbicide-virus. Identification of a microbicide candidate capable of blocking productive HIV infection of susceptible cells is the starting point of development, but a mechanism of action also specifies a location of action (lumen, tissue, or cells) and a target of action (cell-free or cell-associated virus), though these are not mutually exclusive categories.

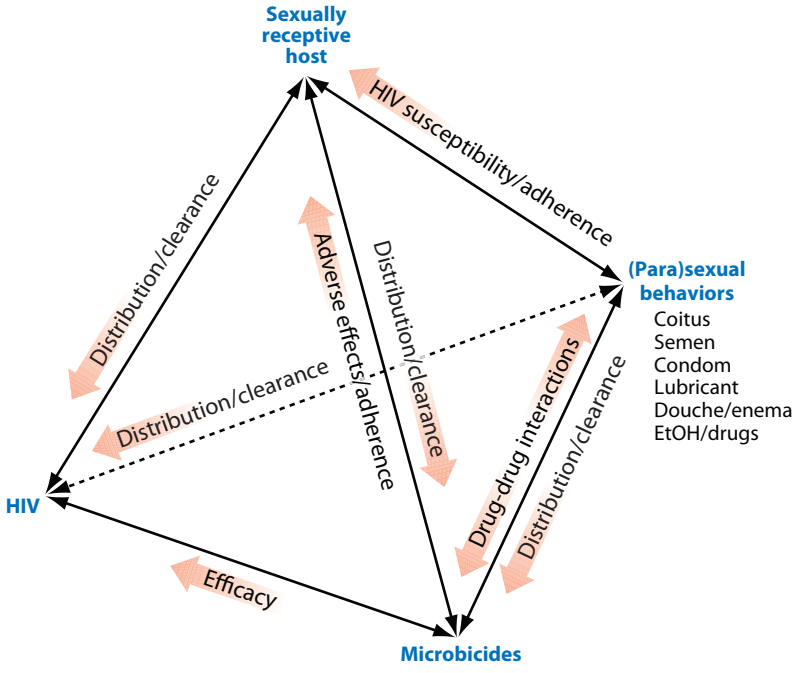

Figure 1

Complex environment in which microbicides will be developed and used.

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Microbicide-host. The host's effect on drug distribution within and clearance from the lumen (macroscopic centimeter to meter scale), adjacent interstitial tissue and cells (microscopic micron to millimeter scale), and the rest of the body compartments will vary greatly depending on the site of application, whether vagina or rectum. Understanding the potential toxicity of both the active microbicide compound and the microbicide vehicle upon sexual mucosa and within local tissues is critical given that these changes may directly impact the efficacy of HIV prevention.

RAI: receptive anal intercourse

RVI: receptive vaginal intercourse

Microbicide-(para)sexual variables. Intercourse itself may introduce microtrauma, especially with the more fragile rectal mucosa, which has the potential for increased risk of HIV infection. Dynamic coital forces may alter local distribution and clearance of topically applied products, while ejaculate dilutes and admixes with local microbicide and may alter HIV susceptibility of the local mucosa. (Para)sexual activities may include the impact of recreational drugs on microbicide adherence. Sexual lubricants may reduce friction-induced micro trauma or increase tissue susceptibility owing to product hyperosmolarity or specific ingredients potentially toxic to both columnar and stratified squamous epithelium. Vaginal drying agents may increase the risk of mucosal trauma. Some enemas very commonly used both before and after RAI disrupt mucosal integrity and are associated with increased risk of HIV infection based on epidemiologic data (15–18).

Both Vaginal and Rectal Microbicide Development Essential

Despite the fact that microbicide development today is largely focused on vaginal use, there is evidence that receptive vaginal intercourse (RVI) and RAI may represent routes of nearly equal importance for HIV infection in women. The risk of transmission by a given route, whether vaginal or anal, is a product of transmission risk per exposure and the frequency of that exposure. The log-adjusted median of estimates of transmission risk per exposure is roughly 19 times higher for RAI when compared with RVI (19–21). Reduced vaginal susceptibility is likely due to a 40-cell thick stratified squamous epithelium, hostile acidic pH, and more rapid turnover of vaginal contents through the introitus. By contrast, the distal colon is a vast expanse of fragile single columnar epithelium overlying a rich bed of susceptible CD4-bearing cells. The presence of cervical ectopy, also single columnar in nature, increases the risk for HIV transmission in women (22, 23). There is also evidence that condom use, generally poor in many parts of the world, is even worse with anal intercourse (3, 24). Data for the frequency of anal sex during most recent sex is scarce in the literature, but the log-adjusted median of a range of estimates is roughly 1 in 22 (range 1:50 to 1:6) (25, 26).

The point estimates above are simply medians taken from different studies, so any conclusions should be made cautiously. Using the numbers above, the estimated ratio of anal risk relative to vaginal risk on a population basis is 5 to 6, not far from equivalence. Despite the crude nature of these imperfect estimates, RAI clearly plays a substantial role in HIV transmission in women, with implications for microbicide trial design. For example, using the estimates above, a microbicide trial in women that limits microbicide application to the vagina (by instruction and number of unit doses) would be slightly more than 50% effective even with a perfect microbicide, perfect adherence, and prevention of every vaginal HIV exposure. Anticipated effect sizes, therefore, may need some downward adjustment if developed for a single site of application. If a vaginal microbicide is occasionally used rectally and is moderately toxic to that more vulnerable colonic tissue, these estimates can provide an explanation for increased risk of transmission in a clinical trial without any evidence of vaginal toxicity.

It seems prudent for microbicide development for women to proceed with a balanced strategy aimed at protecting both the female genital tract and rectum. Potentially, a single formulation

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could be designed for both vaginal and rectal use, but this will involve deliberate exploration of microbicide-host interactions in vagina and rectum with formulation optimization made with both locations in mind. By contrast, a clinical trial of a microbicide for men who have sex with men (MSM) will be associated with less anatomic confounding to complicate interpretations of efficacy data (one less organ of exposure) than similar trials in women. This could be an efficient path to demonstrate microbicide proof of concept and provide much needed encouragement to funding agencies and, especially, large pharmaceutical companies to expand microbicide development for men and women.

MSM: men who have sex with men

CONCEPTUALIZING A MECHANISTIC MODEL TO GUIDE MICROBICIDE DEVELOPMENT

The failure to establish proof of concept in several clinical trials and the highly complex environment for microbicide use and development motivate the search for improved understanding of fundamental processes at work. A theoretical conceptualization of host, microbicide, and virus interactions can inform the construction of a mechanistic pharmacokinetic-pharmacodynamic (PK-PD) model framework to better inform rational microbicide development. The fundamental objectives of microbicide development include identifying the drug target; that is, cell-free and cell-associated HIV following sexual exposure, developing a microbicide to both outdistance and outlast the virus, and gaining an understanding of the correlates of toxicity over space and time, primarily with regard to their impact on microbicide efficacy or subject adherence.

HIV Factors

In the minutes and hours following ejaculation, cell-free and cell-associated HIV migrate within the lumen of the vagina or distal colon and encounter natural defenses, including dilution and clearance by the natural flow of fluid within these compartments and inactivation by low vaginal pH or vaginal secretory leukocyte protease inhibitor (27). These events take place within uncertain distributions within both the female genital tract and the rectum and over an uncertain period of hours or days until HIV clearance from the lumen is complete or HIV is inactivated within a hostile luminal environment (**Supplemental Figure 1**). Once past luminal defenses, the virus must pass through a single columnar epithelium (colon, uterus, cervical canal, ectopic cervical tissue) or stratified squamous epithelium (vagina and cervix), or attach to Langerhans cells within these layers to access CD4-bearing T helper cells, macrophages, and dendritic cells in which to establish infection. In addition, HIV may interact with Langerhans cell projections within the superficial mucosal layer and dendritic cells within the lamina propria, either of which may capture HIV on their surface and present it to target cells in lymph nodes. Infected CD4+ cells are detectable in lymph nodes draining the female reproductive tract within 24 hours, and systemic infection appears at one week (reviewed in Reference 28).

Microbicide Factors

To prevent HIV interaction with susceptible cells in the mucosal tissue, microbicides are being developed with five general mechanisms of action (**Table 1**): (I) vaginal defense enhancers such as agents that maintain an acidic pH hostile to HIV; (II) nonspecific agents, like detergents, that nonspecifically disrupt membranes of cell-free HIV as well as infected donor and uninfected host cells; (III) viral membrane binding agents, commonly polyanions, that interfere with cell-virus interactions; (IV) specific host cell binding agents such as CCR5 inhibitors that prevent

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cell-virus binding with high specificity; (V) inhibitors of viral replication such as tenofovir (nucleotide analog) or UC-781 (nonnucleoside reverse transcriptase inhibitor) that act within CD4+ cells to prevent reverse transcriptase activity.

Based on mechanism of action, the microscopic site of action is restricted and relevant pharmacokinetic compartments are defined (**Supplemental Figure 2**). Luminally active drugs (Types I, II, and III), are excellent for topical administration as microbicides because they need no tissue absorption, thus minimizing systemic side effects. However, they may have local toxicity such as that associated with N9 or hyperosmolar gel vehicles that may directly diminish their preventive effectiveness by enhancing HIV infection (7, 29, 30). Tissue-active drugs (Type IV) must penetrate the mucosa, where HIV interacts with tissue CD4+ cells. Cell-active drugs (Type V) must penetrate tissue and CD4+ cells within tissue to function. It remains to be established, however, whether the oral or topical route of administration is most efficient at achieving concentrations that prevent HIV infection. The microscopic division into lumen, tissue, and cell activity is helpful, but not always exclusive as intraepithelial lymphocytes and Langerhans cells are located at the tissue-lumen interface and some drugs such as the nonnucleoside reverse transcriptase inhibitors UC781 and MIV-150 may penetrate and bind reverse transcriptase within HIV particles in the lumen and tissue (31–34).

Microbicide and HIV Distribution and Clearance

Figure 2 displays a theoretical distribution of both microbicide and HIV concentration within the vaginal or rectal lumen immediately after ejaculation. For luminally active agents, success or failure depends on microbicide achieving concentrations in excess of those required for viral inactivation or prevention of virus-mucosa interactions. For tissue-active agents, the luminal distribution is no less important because the concentration of drug in subjacent tissue is a function of luminal drug concentration. Drug and virus vary in distribution with passing time after ejaculation depending on anatomy and rheological properties of the seminal fluid and microbicide gel influenced by natural physiological forces on these fluids and their contents. HIV distribution and clearance from the lumen dictates optimal microbicide distribution and clearance within the lumen for luminally active drugs as vehicle formulation is modified iteratively to achieve a distribution surface to

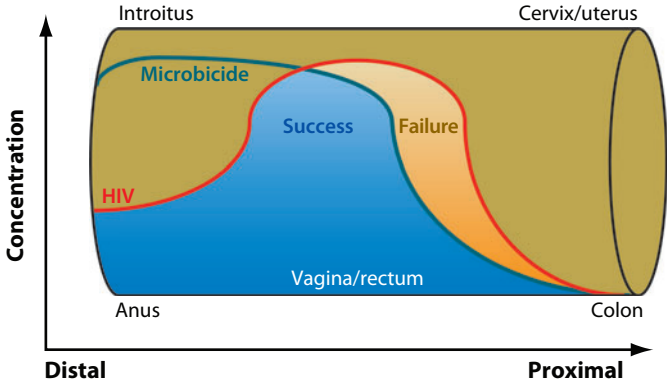

Figure 2

Microbicide and HIV distribute in the lumen of the female genital tract and distal colon following intercourse. Success or failure depends on the microbicide concentrations relative to virus throughout the distribution of HIV.

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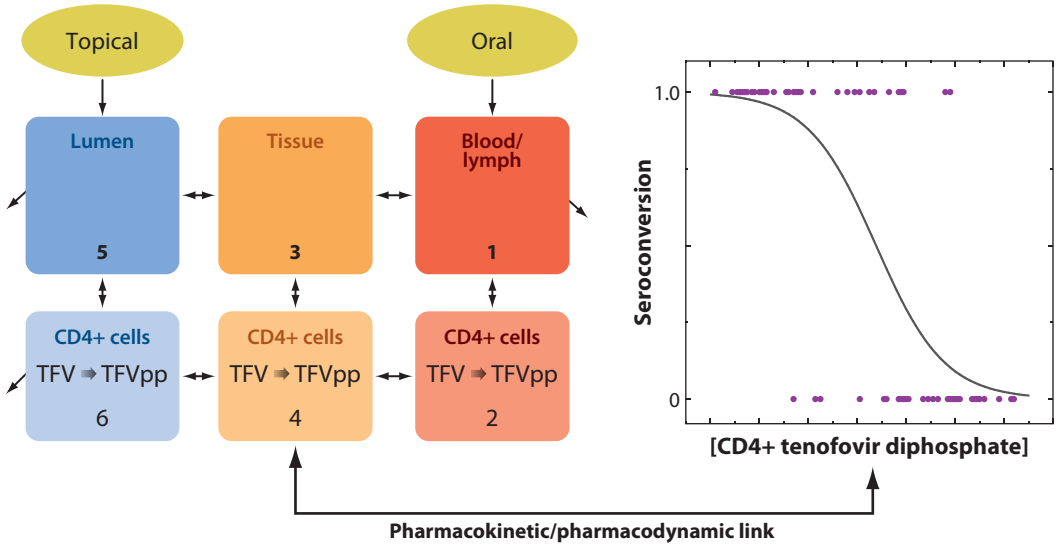

Figure 3

Pharmacokinetic-pharmacodynamic interaction model for tenofovir pre-exposure prophylaxis. Left panel shows full six-compartment model of drug kinetics with fluid and cell compartments within each of the three larger compartments—lumen, tissue, and blood/lymph—generalized with both topical and oral dose inputs. Within cells, nucleoside/nucleotide analogs undergo anabolism to the fully phosphorylated active moiety (TFV → TFVpp indicates tenofovir to tenofovir diphosphate anabolism). The rate constants of drug movement between compartments, indicated by directional arrows, can be statistically estimated given sufficient data from adjacent compartments. Right panel shows a theoretical inhibitory sigmoid concentration-response curve for dichotomous seroconversion outcomes. The PK and PD models are linked by intracellular tenofovir diphosphate concentration (TFVpp) in the CD4+ cells in tissue with the drug concentration axis on the concentration-response curve. The relevant pharmacokinetic compartment best predicting seroconversion outcomes should depend upon the site of action of the active drug.

outdistance and outlast the virus (**Supplemental Figure 3**). Luminal distribution for tissue and cell-active drugs is more complex as tissue and cell kinetics are also critical.

Compartmental Pharmacokinetic Model

Enlarging the perspective beyond the luminal distribution, one can construct a conceptual model with six compartments in which microbicide and virus distribute fluid and cellular subcompartments within the lumen (vaginal and rectal), tissue, and blood compartments (**Figure 3**). Within the cellular compartment, there are additional transformations (and rate constants) of nucleoside analogs to active phosphorylated moieties. The relevant sites of action have been discussed according to mechanism of action—lumen, tissue interstium, or cells. The utility of the other five nonactive compartments lies in their contribution to a complete compartmental model. If the concentration in multiple other compartments has a consistent, predictable relationship to the concentration in the site of action, then concentration data from more accessible sampling locations (blood and lumen) may enable imputation of values in less accessible (tissue and cellular) sites of action. The ability to impute these values becomes critical as one moves from small, intensive, exploratory clinical studies in which all six compartments can be sampled to large confirmatory clinical trials where sparse sampling of a few compartments, at best, is possible. The challenge to microbicide development is to collect sufficient information to populate this six-compartment model sufficiently well in early clinical phases, despite not having pharmacodynamic

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(seroconversion) outcomes, to maximize the value of more limited data (blood, blood cells, luminal sampling) from large studies that will be rich with seroconversion outcomes.

Pharmacokinetic-Pharmacodynamic Model

The ultimate outcome of interest in microbicide trials is HIV infection. Linkage of dichotomous infection outcomes to continuous pharmacokinetic measures, for example, intracellular phosphorylated drug levels for nucleoside analogs or luminal drug concentration for luminally active drugs, could be built (**Figure 3**). Well before seroconversion data from large pivotal trials is available, useful continuous variables of toxicity and markers of efficacy can be explored to inform development decisions and optimize clinical trial design. The concentration-response relationship with toxicity outcomes (e.g., cytokine expression, inflammatory cell infiltration, mucosal disruption) would be quite valuable earlier in the development process. Optimally, predictive biomarkers for seroconversion would be valuable in proof-of-concept studies. For example, if methods were available to quantitatively measure viral entry into sexual mucosa in animal models or human studies, then a range of doses and regimens could be explored early in development for their ability to diminish the magnitude of viral entry.

Toxicity Over Time

For microbicide development, toxicity and efficacy are two sides of the same coin. A microbicide candidate may provide a near maximal degree of desired activity through inactivation of the virus in the lumen (nonoxynol-9), prevention of virus-mucosal interaction (cellulose sulfate), or inhibition of intracellular reverse transcriptase. However, if the active ingredient or vehicle causes a (*a*) breach of existing defenses through breach of the mucosal barrier (detergents' mechanism of action, hyperosmolar vehicle), (*b*) stimulation of local inflammation (increased cytokines, loosening of tight junctions, recruitment of CD4-bearing target cells, increased cell surface CD4 receptor density), or (*c*) alteration of the beneficial vaginal microflora, this effect may sufficiently reduce native defenses so as to outweigh any viral protective effects. Potentially differing from desired drug-related antiviral effects, the time course of toxic effects depends on the rapidity of onset of toxic effects (time-dependent cytokine expression and cell migration compared with more immediate direct mucosal damage) and recovery (cytokine clearance, cell egress, and mucosal repair) (**Figure 4**). If these toxic effects are of sufficiently small magnitude and short duration that they are counterbalanced sufficiently by viral inhibitory effects after a single exposure, then single or occasional dosing may result in a net preventive benefit to the user. However, with sufficiently frequent dosing, there is a danger that the toxic effects may have a cumulative effect over time depending on the time lag for recovery from damage. Preclinical studies provide supporting evidence of this temporally dependent efficacy window (35).

Full Mechanistic Model

In the final conceptualization step, the essential elements of microbicide and virus, each interacting within the host, are linked in a series of processes from exposure to HIV infection (**Figure 5**). The behaviorally-determined input functions of both drug and virus provide key information, working with drug and viral kinetics within the host to model drug and viral fluctuations prior to HIV infection. Adherence is used here to summarize the variables of dose, route, and frequency of use. The viral behavior parallel, HIV exposure, also includes HIV dose, route, and frequency variables related to sexual exposure. Kinetics then describes the movement of particles, drug, and virus in

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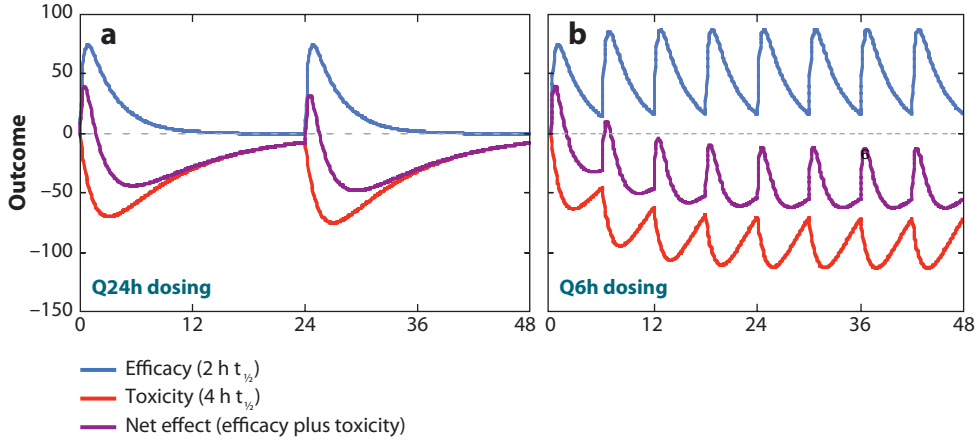

Figure 4

A microbicide's concentration-related antiviral effect (*blue*) and toxic effects (*red*) vary with time and contribute to the net effect on HIV prevention (*violet*) that varies with dosage frequency. This simulation assumes both additive antiviral and toxic effects that are measurable on the same outcome scale (y-axis) representing net HIV prevention effect, and concentration-related efficacy and toxicity, though toxicity was modeled to be subsequent to drug concentration changes with slower onset of action (toxicity) and slower resolution (restitution) than desired drug-related efficacy effects. With daily or less frequent coitally dependent dosing (*left panel*), there is a period of net preventive efficacy during which a viral exposure will result in a decreased risk of infection below the background risk. This is followed by a period of increased vulnerability to infection. In the frequent dosing scenario (*right panel*), after the second dose, the host is always more vulnerable to infection.

space and time. What is often represented as a single pharmacodynamic process is represented here as three parallel dynamic processes of drug, host, and virus. Here, pharmacodynamics is limited to drug-virus interactions in which prevention of virus interaction with the susceptible cells is the objective. Toxicodynamics, influenced by drug concentration, is added as a distinct process to capture alteration of gut or vaginal mucosa that may enhance HIV infection (for example, reduced mucosal integrity or increased target receptors on target cells) independent of drug-mediated antiviral effects. Viral dynamics involves virus interacting with cells, leading to viral replication. The HIV infection event by which microbicide efficacy is determined is the result of the interaction of these three processes. Adherence, HIV exposure, and toxicodynamics deserve special

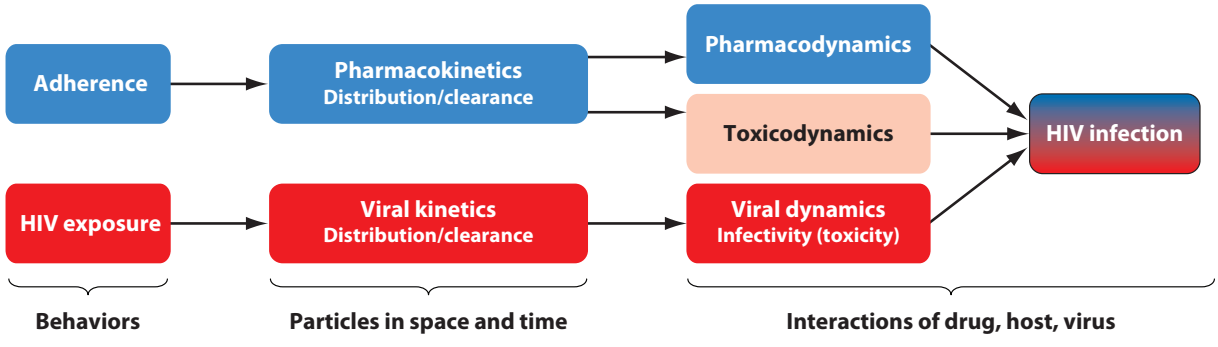

Figure 5

Linkage of human (*blue*) and viral (*red*) processes, moving from left to right, influencing HIV infection in a microbicide clinical trial.

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consideration given the failure of previous clinical trials to demonstrate microbicide efficacy, possibly due to microbicide or vehicle toxicity, poor adherence, and the likelihood of risk reallocation with microbicide use (for example, reduction in condom use). Data-driven linkage of these processes in a mechanistic model will powerfully inform microbicide development and enable clinical trial simulation to optimize pivotal clinical trial design.

EVOLVING METHODS TO INFORM MICROBICIDE DEVELOPMENT

Using the mechanistic model as an organizing framework, one can explore the variety of evolving quantitative methods needed to improve the informational content of early pharmacokinetic, safety, and proof-of-concept studies and, in turn, develop better designs for pivotal efficacy studies.

Quantifying Behaviors Affecting Microbicide and HIV Exposure

Because both adherence behaviors (microbicide dose, route, and frequency) and viral exposure–related behaviors (frequency of vaginal or anal receptive sex) can have dramatic impact on drug and viral exposures and subsequent seroconversion events, quantitative assessment provides necessary input to drive PK-PD models of microbicide effect. For example, rich time-of-dose adherence data in combination with individual pharmacokinetic parameter estimates could be used to reconstruct concentration-time data throughout a clinical study. With sporadic and definable exposures to HIV, this type of data could dramatically improve the explanatory value of pharmacokinetics with regard to infectious events.

Unfortunately, the quantitative methods for assessing drug-taking behavior are far less precise than methods for measuring drug levels or HIV seroconversion, thus increasing uncertainty and adding variability to any model. Study participant self-report data may overestimate adherence by 20% (36, 37) and pill counts have also been shown to be unreliable (38, 39). Removing the interviewer from this data collection with audio-computer assisted self-interviewing (ACASI) methods is believed to improve the quality of self-report data (40). More objective biological tests, such as dyes indicating vaginal mucus on returned microbicide applicators, have demonstrated greater than 96% sensitivity and greater than 98% specificity (41, 42). In the Carraguard trial, study participants reported 94% usage of Carraguard, but a dye test indicated that Carraguard was only used in 43% of sex acts (3). Unfortunately, this method does not perform as well with all microbicide applicators in development (43). Microelectromechanical systems (MEMS) devices, which record date and time of pill bottle opening, provide more objective data, but don't capture pill taking. MEMS devices indicate lower adherence than self report data and better predict antiviral outcomes in HIV treatment (44, 45). MEMS may also be useful in differentiating patterns of adherence, for example, sporadic versus extended drug holidays, which could prove useful in combination with drug concentration data.

Self-report time of sex, receptive route, and condom use to define HIV exposure is at least as problematic as microbicide adherence. Inability to assess the impact of mandatory and intensive condom promotion, currently the standard in all arms of microbicide trials, is a major obstacle because it may represent a major influence on HIV exposure in both the placebo and intervention arms of the trial such that the microbicide effect in one arm may be overwhelmed by the increased use of condoms. This fear is supported by condom use estimates in clinical studies that far exceed condom use in the local population. Measuring condom migration as risk reallocation is also critical for similar reasons. Objective biologic tests such as vaginal sampling for prostate-specific antigen or Y chromosome to detect unprotected sexual exposure can be useful, but can only provide evidence of exposure in recent days.

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Quantifying Microbicide and HIV Movement in Space and Time

HPTN: HIV Prevention Trials Network

A quantitative understanding of candidate microbicide distribution and clearance relative to HIV distribution and clearance after sexual exposure should enhance microbicide development through drug and vehicle optimization. Traditional drug development deals almost exclusively with blood concentrations, implicitly though imperfectly as a surrogate for active drug at the site of action that is seldom explored directly. For microbicides, the site of action is accessible, though methods to quantitatively assess drug and HIV concentration at this anatomic site are not well developed. Methods to assess the kinetics of changing drug and virus concentration at the mucosal site of action (**Figure 5**), however, are evolving rapidly.

Tissue and blood pharmacokinetics. For microbicides with a mechanism of action requiring mucosal penetration for activity, knowledge of the drug's tissue concentration is essential to understand the concentration-effect relationship and thus aid drug development: for fusion or chemokine inhibitors, interstitial drug concentrations; for reverse transcriptase inhibitors, intracellular concentrations; for nucleotide or nucleoside analogs, phosphorylated moieties within the cell. Because most blood and tissue data available for candidate microbicides relates to tenofovir, this drug is discussed.

The oral formulation, tenofovir disoproxil fumarate, marketed for HIV treatment (Viread™), is formulated as an ester of tenofovir to mask the charged phosphate moiety of this nucleotide that limits its oral bioavailability. Orally administered tenofovir disoproxil fumarate has adequate bioavailability and a 17-hour terminal half-life and demonstrates dose proportionality as reported by Barditch-Crovo et al. (46). Mayers et al., in the HIV Prevention Trials Network (HPTN) 050, demonstrated indirectly that tenofovir is present, at least transiently, in vaginal tissue after vaginal dosing of tenofovir (not the ester) when they detected tenofovir in the blood of 14 of 25 subjects, peaking at four hours, though achieving less than 2% of the dose-adjusted area under the concentration-time curve (AUC) achieved after oral dosing (47). Demonstrating tenofovir movement in the other direction, Dumond et al. also indirectly demonstrated vaginal tissue penetration after oral tenofovir disoproxil fumarate dosing by observing the drug in vaginal fluid; the tenofovir AUC was similar in both blood and vaginal aspirates, peaking at two and six hours, respectively.

Kashuba and Schwartz et al. reported the first direct evidence of vaginal tissue concentrations after tenofovir vaginal gel dosing in the CONRAD tenofovir PK study. Their study showed a one to two log decrease in concentration moving from vaginal lumen to vaginal tissue, and a five log decrease in both tenofovir peak concentrations and AUC in vaginal tissue compared with blood levels (48, 49). These results suggest higher tissue levels than might have been predicted based on steady-state oral tenofovir dosing in which blood and vaginal fluid AUC were the same (50); these studies support measurable tissue levels of tenofovir after vaginal dosing. The missing piece required for interpretation is the question of what tissue concentration is sufficient to prevent HIV transmission. Kashuba reported that tissue concentrations after oral dosing in the most highly protective animal models were two logs higher than concentrations achieved in genital fluid in women after standard oral dosing (48).

Tissue concentrations have also been assessed for the vaginal ring formulation of dapivirine, a highly lipid soluble, nonnucleoside reverse transcriptase inhibitor. Romano et al. reported tissue-associated concentrations that were similar at the vaginal introitus and cervix and within one log of dapivirine concentrations within the ring itself, although undetectable in the blood. Tissue-associated concentrations were three to four times the in vitro HIV IC_{50} (51). Caution is warranted in interpreting the tissue-associated concentrations in studies given the potential for surface contamination of the biopsy with tenacious luminal contents that may cause an upward bias in the

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concentration, hence the term tissue-associated. If the tissue-associated concentrations represent true interstitial concentrations in these biopsy studies, then this information provides a high degree of confidence in having achieved excellent tissue penetration.

MTN: Microbicide Trials Network

Intracellular pharmacokinetics. For nucleoside and nucleotide analogs, measurement of active drug at the site of action requires intracellular concentrations of their phosphorylated moieties measured in cells susceptible to HIV infection (T helper cells, macrophages, and dendritic cells). It is arguable whether the mucosal or draining lymph node location is more relevant. This awaits correlation with clinical outcomes. In HIV-infected patients on tenofovir, Hawkins et al. reported intracellular tenofovir diphosphate kinetics in peripheral blood mononuclear cells (PBMC) to be highly variable with a long intracellular half-life of 150 hours, ranging from 60 to more than 175 hours (52), much longer than the intracellular half-life reported in resting lymphocytes from in vitro studies (53). To interpret ongoing studies that may provide intracellular phosphorylated drug concentrations, additional work is needed to determine the degree of heterogeneity in phosphorylation among cell types extracted from brushes, biopsies, or lavage fluid in comparison with relevant HIV-susceptible cells (CD4+ T cells, macrophages, and dendritic cells). For example, cervicovaginal lavage (CVL) yields mostly epithelial cells, which may be irrelevant to HIV prevention, though they may have value in understanding compartmental pharmacokinetics. Intracellular heterogeneity in phosphorylation has been noted among different cell types for some nucleoside analogs, though it has not yet been studied for tenofovir (54).

Another important limitation to intracellular phosphate assessment is assay sensitivity. Several labs have reported limits of quantitation around 25 fmol/10^6 cells when using millions of cells. In a study of mononuclear cells from the male genital tract, Vourvahis et al. reported undetectable intracellular tenofovir phosphate concentrations in six of nine study subjects taking oral tenofovir with mononuclear cell yields from 1.0×10^5 to 5.2×10^6 (55). Cervical cytobrush sampling typically yields only thousands of cells before cell sorting. If one performs cell sorting on vaginal tissue biopsies, CD4 cell yields are also in this range. Colon tissue biopsies have better yields of CD4 cells, in the range of 50,000 cells per biopsy, but this still requires a large number of biopsies to get sufficient cells to achieve the 25 fmol/10^6 cells sensitivity limit. Depending on the degree of tissue penetration, detection of CD4 cell–specific intracellular diphosphate concentration in plentiful colon tissue biopsies may be beyond the sensitivity of mass spectrometry methods. Accelerator mass spectrometry (AMS), commonly used in carbon-dating studies to detect attamole levels of ^{14}C differentiated from ^{12}C background, could provide sufficient sensitivity. However, AMS trades off several logs of its improved sensitivity when used in clinical studies because only very small and single doses of ^{14}C labeled drug can be given. Accordingly, the estimated tenfold accumulation of tenofovir diphosphate at steady-state with daily dosing, due to its long 150-hour intracellular half-life (52), cannot be directly assessed with AMS methods.

Variability in intracellular tenofovir will also limit the successful construction of complex multicompartment pharmacokinetic models. In some clinical studies, the average intracellular tenofovir diphosphate concentration ranged from 16.3 to 212 fmol/10^6 cells, a 52% coefficient of variation about a mean of 76.1 fmol/10^6 cells (56). One source of intracellular phosphorylation heterogeneity is cell activation. Robbins et al. reported significantly reduced intracellular tenofovir diphosphate half-life from 33 to 50 hours in resting lymphocytes to 12 to 15 hours in PHA and IL-2-activated lymphocytes in vitro (53). In clinical studies, endemic commensal and pathogenic organisms may increase the degree of cell activation, thus increasing heterogeneity. To evaluate the clinical impact of cell activation as an explanatory variable for intracellular half-life variation, lymphocyte surface markers associated with cell activation are currently under study in a phase II clinical study comparing oral and vaginal tenofovir kinetics [Microbicide Trials Network (MTN)-001].

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MRI: magnetic resonance imaging

Evidence also supports genetic influences on intracellular tenofovir phosphate levels (a 35% increase associated with an A to G single nucleotide polymorphism in ABCC4 that encodes the MRP4 efflux transporter) contributing another source of variability to the intracellular kinetics (56). Further, concentrations of the active diphosphate are only twice as high when compared with the monophosphate concentration, thus assays which do not differentiate these two moieties and assume all measured phosphate is diphosphate are susceptible to increased assay variation if this diphosphate/monophosphate ratio itself varies.

Because microbicide dosing in some studies is coitally dependent, the time course of intracellular phosphate formation in single-dose studies is also relevant. Robbins et al. described, in an in vitro study, time to steady-state tenofovir diphosphate concentrations of approximately eight hours after extracellular tenofovir exposure (53). Adequacy of a coitally-dependent nucleotide dosing strategy plausibly depends on how soon protective phosphorylated concentrations are achieved after dosing and the timing of dosing relative to coitus.

Vaginal lumen. In the late 1970s and early 1980s, scintigraphy was used to establish patency of the female genital tract up to the fallopian tubes using planar gamma cameras, acquiring a series of images from emissions from ^{99m}Technetium (Tc)-labeled human albumin microspheres (^{99m}Tc-HAM) dosed vaginally. Even with these large particles, distribution beyond the vagina and cervical canal was easily demonstrated, which has not been seen to date in magnetic resonance imaging (MRI) studies using small molecule markers such as gadolinium-DTPA, indicating the superior sensitivity of scintigraphy to MRI. Different vaginal dosage forms (pessary, gel, and cream) have been compared in crossover designs to evaluate product distribution and retention. These studies used either ^{99m}Tc-HAM or ^{99m}Tc-DTPA, a small molecule (492 Da) the size of many antiretroviral drugs that serves as a relevant small-molecule microbicide surrogate (types IV and V) with regard to size. These studies demonstrated very little intrasubject variation in product distribution comparison, but large interindividual differences in product retention, ranging from greater than 80% retention to less than 2% over the observation periods (6 or 24 hours).

Mauck et al. studied vaginal surface coverage and distribution of sexual lubricants (Replens and K-Y jelly) as surrogates for microbicide gels using ^{99m}Tc-labelling and compared scintigraphy to MRI and a direct optical imaging method (both described below); each was used in a separate cohort (57). Multiple scintigraphy images are captured over the first 50 minutes after dosing; a radioactive reference rod was used to calibrate distances in a predose image, and three vaginal regions of interest were used to quantify product distribution by region. With its poorer resolution, scintigraphy indicated less than 5% of the vaginal surface was not covered by gel, whereas the optical method and MRI identified 60–68% and 68–75% of the vaginal surface to be uncovered, respectively. In addition, the percent of maximal extent of the gel seen with scintigraphy was much lower compared with the other two methods, both of which were in general agreement.

Barnhart et al. pioneered the use of MRI for imaging gel distribution within the lower female genital tract, including studies of the microbicide candidates nonoxynol 9, C31G, and cellulose sulfate. In studies of Gynol II (N9 contraceptive gel), 3 mL or 5 mL volumes of gadolinium-labeled gel was used to image gel distribution up to six hours after dosing. Within 10 minutes after dosing, gel distributed throughout the vagina and into the cervical canal, though without evidence of distribution into the uterus (58, 59). Spread increased with time postdosing, larger gel volume, and simulated vaginal intercourse (30 thrusts with an artificial phallus). In later studies, the group developed more quantitative methods to assess linear spread (introitus to cervix) and vaginal surface area coverage. In a C31G study, they demonstrated 92% linear coverage and 75% surface contact coverage of the vagina within 18 minutes after dosing (60). However, six hours after dosing, there was a substantial reduction in coverage, with only 60% of maximal linear

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distance covered and 41% surface contact. By 24 hours, there was minimal coverage of the vaginal mucosa. Unlike N9 products, simulated intercourse had little effect on C31G distribution. This gel performance suggests a limited period of usefulness after dosing compared with other gels. In a study of 2.5 and 3.5 mL of 6% cellulose sulfate gel distribution over 50 minutes, the 2.5 mL volume without ambulation showed poor linear spread (53.1% of the vaginal length) and surface contact (61.7%), whereas the larger volume with ambulation improved both linear spread (84.5%) and surface contact (85.9%) (61).

ARV: antiretroviral drug

OTC: over-the-counter

Neither the scintigraphy nor MRI studies attempted to assess vaginal concentration. In a comparative study of five different N9 containing formulations, Witter et al. used a series of two 135 mL CVL to assess N9 retention; the 24-hour time course was assessed by repeating the dosing procedure on different days, but modifying the sampling time in each of the women (62). The dose retained versus time profile indicated a qualitative product retention gradient: foam > cream > film > suppository. One limitation of lavage methods is the lack of a method to correct for the dilutional effect of the lavage due to uncertainty in the percent recovery of lavage fluid. More recent CVL sampling methods use far smaller volumes (~10 mL) and sometimes an intrinsic biomarker is used to roughly correct the dilutional effect of the lavage. To reduce variability due to dilutional effects to improve the data quality in PK modeling, a safe chemical marker could be added to lavage fluid to allow simple and quantitative correction of measured compounds of interest. Kashuba et al. completely avoided the dilutional problem by sampling using a self-administered aspiration procedure that yields ~300 microliters for analysis. Using this aspiration method, Kashuba demonstrated the vaginal concentrations of a variety of ARV drugs (3TC, ZDV, FTC, and TDF) that achieved female genital tract concentrations at or above blood plasma concentrations (50). Spatial information can be achieved by sampling with adsorptive materials at discrete sites along the vaginal wall, cervical canal, and uterus.

Katz et al. developed an optical imaging system to measure gel distribution and thickness along the surface of the vagina using the optics of a rigid endoscope within a transparent phallic shaped tube inserted into the vagina, essentially simulating the gel distribution during intromission (63). The endoscope rotates 360° along an axis of 15 cm depth, capturing fluorescence intensity from fluorescein labeled gels within the vaginal lumen, thus creating a three-dimensional map of gel thickness along the full extent of the vaginal wall. Using this method, an over-the-counter (OTC) N9-containing gel, Advantage S (polycarbophil and carbomer), was shown to have more extensive and uniform distribution within the vagina with smaller bare spots when compared with another N9 OTC gel, Conceptrol (sodium carboxymethylcellulose gel), which tended to pool around the cervix and had larger bare spots on the vaginal wall (64).

Low-coherence interferometry (LCI) is also under development to measure the thickness of microbicidal gels applied in situ. Compared with the optical method mentioned above, LCI measures the thickness of a formulation by analyzing the interference patterns generated by differential reflection from the front and back surface of gel on a mucosal surface (65). Gel thickness assessments should be useful to understand not only completeness of surface coverage by the active microbicide, plausibly correlated with prevention efficacy, but also because there is theoretical evidence that the gel itself may provide protection as a physical barrier (66).

Each method described has strengths and limitations such that the specific quantitative objective determines the modality chosen (**Supplemental Table 1**). Pharmacokinetic data yielded by these studies, linear distribution and surface coverage, concentration-time profiles, and spatial mapping of gel thickness, should be useful in selecting among options for microbicide vehicle candidates. Selecting the optimal vehicle, however, would be even better informed by quantitative assessment of HIV distribution and clearance, as well, to better define the desired gel distribution required to match the HIV target.

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SPECT: single photon–emitted computed tomography

Rectal lumen. Gastrointestinal distribution of rectally applied drugs began with scintigraphic studies (^{99m}Tc-sulfur colloid or ^{99m}Tc- hydroxymethyldiphosphonate) of suppository, enema, or foam containing hydrocortisone or 5-ASA. Semiquantitative region-of-interest distribution data, sometimes aided by plain X-ray studies, showed foams and enemas migrating retrograde as far as the splenic flexure, whereas suppositories were typically limited to the rectum. In one study, pretreatment with an enema facilitated distribution of the treatment enema throughout the colon (67), a finding with potential application to rectal microbicide dosing given the common use of enemas prior to receptive anal intercourse.

Hendrix et al. directly compared scintigraphic methods with MRI and a direct endoscopic sampling method in the same subjects following the same dose (68). They used a simulated intercourse model based on parameters established in MSM focus groups to replicate the mechanical and physiological effects that coitus could have on gel distribution and gut motility. The microbicide gel surrogate (OTC sexual lubricants) labeled with gadolinium for MRI and either ^{99m}Tc or ^{111}Indium chelated to DTPA served as a surrogate for a small-molecule microbicide. Simulated ejaculation through the artificial phallus, using viscosity-adjusted OTC sexual lubricants or autologous semen radiolabeled with HIV-sized sulfur colloid, allowed study of surrogates of microbicide and HIV-infected semen interactions in situ. Single photon–emitted computed tomography (SPECT) coupled with computed tomography (CT) followed immediately by MRI and repeated up to four times within the 24-hour postdose period allowed nearly simultaneous comparison of the two methods in the same individual.

MRI provided high resolution detail of gel distribution in the rectum and lower sigmoid, but gel migration more proximally resulted in a large concentration decrease in the upper sigmoid with degradation of signal-to-noise ratio. SPECT/CT provided superior signal sensitivity, though poorer spatial resolution, allowing visualization of gel migration as far, in one case, as the cecum. SPECT showed signal migrating proximally through the rectosigmoid, mostly within four hours after dosing, with distribution to the descending colon and splenic flexure in 25 and 12% of subjects, respectively (**Figure 6**). To provide concentration data, a novel three-dimensional curve-fitting algorithm was developed based on the principal curve algorithm of Hastie and Stuetzle (69) that incorporates operator-defined constraints and image intensity into the fitting; signal intensity (mass) and voxels with signal (volume) are used to estimate concentration along the path of the colon (70). Plotting concentration, distance, and time values describes a microbicide surface for each condition studied. New pharmacokinetic parameters are needed to incorporate distance into concentration and time parameters to allow quantitative assessments of gel distribution as it varies with formulation, coital simulation, presence of ejaculate, and preparatory enemas, and to provide variance estimates for sample-size planning in future pharmacokinetic studies.

Direct endoscopic sampling of luminal contents along the mucosal surface using endoscopic brush sampling and mucosal tissue biopsies provide information bridging macroscopic and microscopic microbicide distribution. Luminal and biopsy samples follow similar concentration profiles as expected, but luminal samples are typically one to two logs greater in concentration than the biopsies. Caution is also necessary in interpretation of the biopsy specimens because surface contamination with luminal contents, including the mucin layer, may significantly bias the results. The endoscope itself may introduce three additional distortions: first, introduction of the scope may stretch the colon in places; second, movement of the scope from distal to proximal locations may physically displace gel or fluid being assessed and thus further distort the distribution, especially for subsequent analyses; third, the scope may act as a physiological stimulus to alter gut peristaltic activity.

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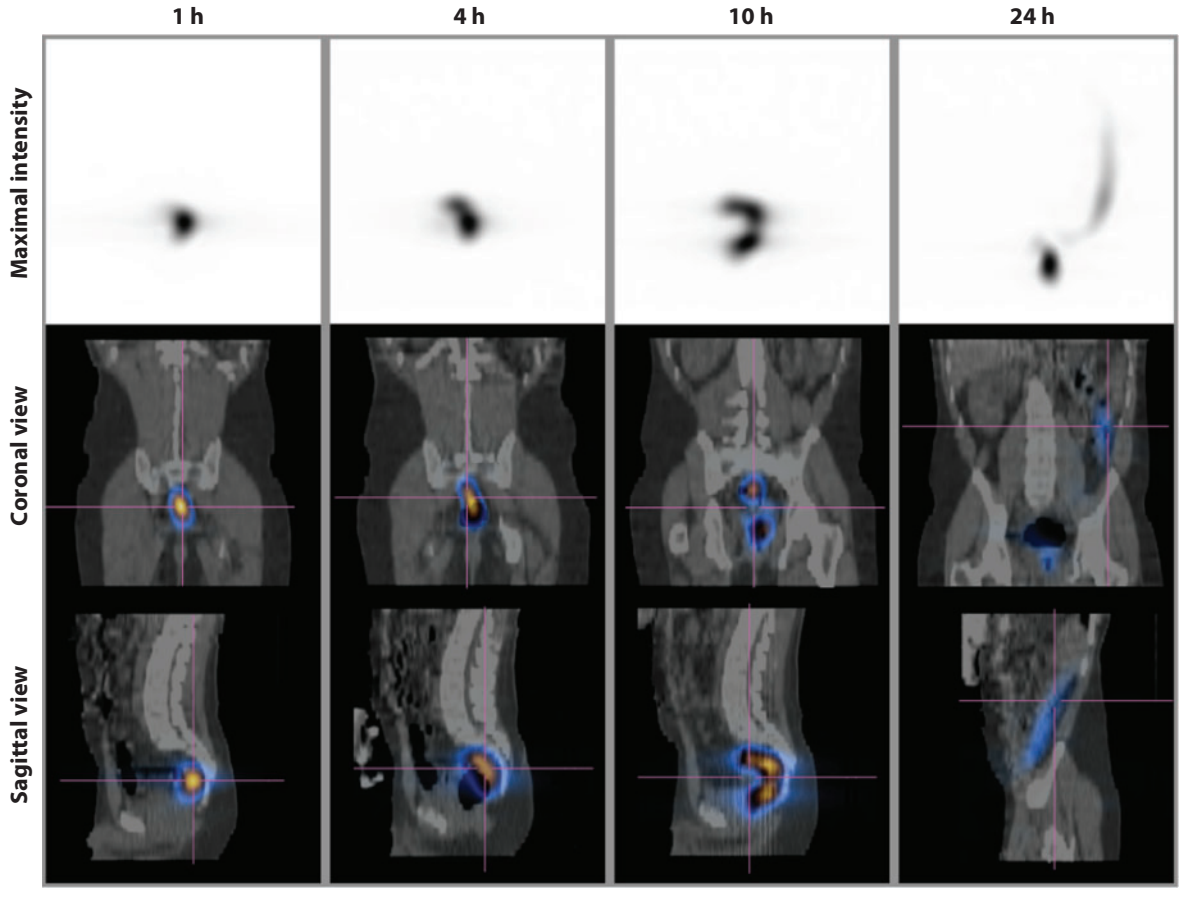

Figure 6

Fusion of single photon–emitted computed tomography (SPECT) and computed tomography (CT) images showing microbicide surrogate gel temporal migration retrograde to the splenic flexure after rectal dosing. Microbicide surrogate is an iso-osmolar mixture of over-the-counter sexual lubricants with ^{111}In-DTPA added for imaging to simulate a small-molecule (492 Da) microbicide active ingredient. Temporal sequence (*left to right*) includes 1st hour to 24th hour after dosing. Top row of images panel is grayscale maximal intensity projection of ^{111}In signal. Lower two rows of images are coronal and sagittal views of CT images (grayscale: *light gray* is bone density; *darker gray* are soft tissues; *black* is air density) with superimposed SPECT images of ^{111}In signal (*blue* through *yellow*).

Dosing forms. Although the above methods were developed using gels as drug vehicles, the methods can be used with other formulation types. The kinetics of microbicide-eluting vaginal rings can be imaged to ascertain location within the vaginal space, and direct sampling of vaginal fluid or tissue can be used to estimate tissue penetration (51, 71). Enemas could be used as drug delivery vehicles for rectal microbicides, an attractive option as enemas (douching) are commonly used prior to receptive anal intercourse (72–74). Studies of the distribution and clearance of different enema types is currently underway using SPECT/CT imaging. Luminal concentration-distance-time data for genetically modified microbicide-secreting lactobacilli spp. and *Escherichia coli* could be studied using direct vaginal or colonic sampling with selective and quantitative culture, using bacterial-secreted microbicide concentration as the relevant readout.

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Viral distribution. The same methods developed to map drug distribution in these luminal compartments can be employed to track virus. Hendrix et al. have developed a cell-free HIV surrogate comprised of ^{99m}Tc-sulfur colloid (100 nm particle similar in size to HIV) suspended in autologous seminal plasma and a cell-associated HIV surrogate comprised of autologous ^{111}In-oxine-labeled lymphocytes suspended in autologous seminal plasma. To enable comparison between cell-free and cell-associated HIV, both surrogates have been dosed simultaneously using their simulated RAI model (75) (**Supplemental Figure 4**). Because of the different energy levels of the surrogates, each surrogate distribution can be resolved separately. Studies of female genital tract distribution of radiolabeled lymphocytes are underway. In this manner, both the drug and the cell-free virus-sized particle combination and the cell-free and cell-associated virus surrogate combination have been studied. Using quantitative methods, the three-dimensional concentration-distance-time surface of HIV surrogates and drug can be mapped together (**Supplemental Figure 5**). Consistently, the concentration-distance-time profile has been largely superimposable for drug, cell-free, and cell-associated surrogates. Accordingly, the sometimes widespread migration of virus-sized particles to the splenic flexure may also be associated by the same distribution of microbicide surrogate.

Microscopic tissue distribution is being explored using simulated ejaculation of autologous ex vivo ^{111}In-labeled lymphocytes suspended in autologous semen. Gamma counting of cells extracted from postejaculate colon biopsies show from 1 to 2% of CD4 cells from the biopsy are of exogenous origin (75). Because detailed histologic evaluation has not yet been performed, it is not known whether these cells have penetrated tissue or are simply closely adherent to it within the tenacious mucin layer. Plans are underway for assessment of autologous radiolabeled HIV distribution.

Tracking of HIV and CD4 cells within the lumen and mucosal tissue has several obvious implications. First, it may extend understanding of early HIV transmission events based on in vivo human exposures of HIV. Second, defining the distribution of the microbicide target, namely HIV, will inform development of microbicide formulations designed to outdistance and outlast the virus, at least within the lumen. Third, the ability to quantitatively measure cell-free and cell-associated HIV within tissue will enable HIV challenge studies in which differences in HIV infectivity can be quantitatively assessed before and after microbicide dosing. The ability to perform proof-of-concept dose-ranging studies of microbicide candidates using relevant quantitative biological endpoints will be a powerful tool to improve microbicide development. Decisions to proceed to large phase IIb/III studies will be made with substantially more information. Dose and frequency selection can also be optimized as it is in traditional drug development. Clinical trial simulation informed by the luminal distribution, compartmental modeling, and proof-of-concept studies described above becomes much more feasible, thus enabling comparison of competing study designs for large registration trials with seroconversion endpoints.

Dynamics—Interactions of Drug, Virus, and Host

Preventive efficacy of microbicides will be the net result of interactions between active drug, virus, and host tissues. Perhaps, more than in any other therapeutic area, toxicity (undesired drug-tissue interactions and undesired drug-virus interactions such as resistance) and antiviral effect (desired drug-virus interactions) are opposite sides of the same coin, the sum of which determines preventive efficacy (**Figure 5**). A quantitative undestanding of the magnitude and time course of these interactions is key to selecting optimized candidate microbicides to go forward in development, planning pivotal clinical trials, and interpreting clinical trial outcomes.

Integrating PK and seroconversion data. On the basis of the methods described above, the data required to fully populate the comprehensive six-compartment pharmacokinetic model,

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conceptualized in the previous section, is gradually being gathered, but logistical limitations preclude its collection in all subjects of large efficacy studies with seroconversion endpoints. Increasingly, early clinical studies are including comprehensive collection of data from multiple anatomic locations for drug analysis. Parameter estimates thus generated will be used to both (*a*) inform future confirmatory trial design through clinical trial simulation and (*b*) provide estimates for imputation of pharmacokinetic parameters of interest such as intracellular phosphates in the mucosal CD4+ cells based on only blood or luminal sampling (**Figure 5**). Larger clinical trials with seroconversion endpoints are also including some degree of pharmacokinetic information to inform PK-PD model development.

One strategy employed in large seroconversion endpoint studies, as in the ongoing CDC PrEP studies, has been to include nested small substudies with more intensive collection of data from many compartments to provide initial parameter estimates. The remainder of the study population undergoes more sparse sampling, but the larger number of subjects provides informative covariates that can be modeled with population pharmacokinetic methods (**Supplemental Figure 6**). In several ongoing tenofovir PrEP and microbicide effectiveness studies, integration of logistic regression with sigmoid I_{max} models are planned to link the HIV infection rate to intracellular tenofovir diphosphate concentration, through both direct and imputed drug concentration assessment. A modified Cox-proportional hazards model will be attempted to link the tenofovir diphosphate concentration to the time-to-HIV infection data (**Figure 3**). Success of these modeling attempts depends on the richness of data used to build the model and the ability to reduce variability through inclusion of informative covariates such as renal function and cellular activation, described above. Increasingly, clinical studies are incorporating this type of rich pharmacokinetic data in their designs (**Supplemental Table 2**).

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Selecting biomarkers for toxicity. Identification of drug-induced toxicity as early as possible in any drug development program is essential, and is perhaps even more critical for microbicides. Subtle clinically inapparent local toxicity, without impact for many drug programs, directly erodes prevention efficacy for microbicides, even increasing the risk for HIV infection as seen with N9 (8). With the surge of toxicity assessments being used to screen microbicide candidates in many labs, the real challenge remains in selecting assays to detect changes that predict a clinically manifest increase in susceptibility to HIV infection, hence a focus on the type of changes seen after N9 exposure.

Current toxicity assessments go well beyond the histology and visual inspection for tissue disruption and inflammation. Mechanism-based in vitro studies of N9 show epithelial disruption secondary to loss of tight junction and adherens junction proteins (76); similar observations have been made with cellulose sulfate, another candidate that failed in phase III trials (5, 6). Animal studies show that N9-related inflammatory cell infiltration, epithelial disruption, and increased susceptibility to viral infection can be detected by a variety of methods, including visual and colposcopy inspection, histopathology, measurement of transepithelial electrical resistance, and HSV-2 challenge (9). Although clinical observation indicated increased symptoms of vaginal irritation in some but not all studies, there has been consistent objective evidence of inflammation (epithelial disruption, inflammatory infiltrate, and increased inflammatory cytokine expression), altered vaginal microflora, and increased susceptibility to ex vivo HIV infection in human cervical and colonic explant tissue (9, 77, 78). Lackman-Smith et al. and McGowan et al. recently put forward evaluations of panels of toxicity assessments in the vagina and rectum, respectively, along with a critical evaluation of the methods proposed (79, 80).

Measuring toxicity over time. Awareness of the temporal fluctuations in toxicity is essential to the timing of toxicity assessment during development. For example, using histologic methods, single-dose rectal administration of N9 demonstrated dramatic epithelial exfoliation 15 minutes after application, plausibly increasing the risk of HIV infection but with substantial reversal of these changes by 2 hours and microscopically normal epithelium after 24 hours. (29, 30, 81). The time course seen in these clinical studies is consistent with in vitro epithelial damage models (82), but there also may be late changes that take place over several hours or days. In progestin-treated mice, Cone et al. recently demonstrated a single vaginal exposure of N9 followed by HSV challenge resulted in brief partial protection, but then up to a 30-fold increase in susceptibility to infection that peaked 12 hours following a single-dose exposure (35).

The time course of toxicity and recovery (and sampling schedules to assess it) likely differs with direct chemical and mechanical stresses in contrast to immunologically mediated damage. Therefore, an optimal time of assessment for one type of toxicity (if it could be correctly chosen) may differ from the optimal time of assessment for another. Spatial variation in tissue type and drug exposure create spatial heterogeneity in toxicity as well. Further, invasive sampling can distort the conditions intended for observation. Accordingly, the ideal biomarker of toxicity can provide multiple assessments over time, noninvasively, and at differing locations with both the female genital or rectal compartments. Two such candidates under development are optical coherence tomography (OCT) and systemic permeability assessment.

OCT, using low coherence interferometry, creates an image based on differential reflection of light waves at changes in tissue layers. This has been used noninvasively in clinical assessment of skin conditions. Vincent et al. used OCT in macaques and detected vaginal tissue changes following chemical (N9) and mechanical stress (83). Colonic permeability has been used in the past to assess absorption abnormalities and loss of mucosal integrity in inflammatory bowel disease (84). Fuchs et al. administered ^{99m}Tc-DTPA, a radiolabeled small molecule (492 Da), per rectum and measured radioactivity in the urine and blood following chemical stress (N9), mechanical stress (coital simulation and 30 colonic biopsies), and control conditions. Their method detected a 22-fold increase in colonic mucosal permeability within 1 hour after administration that persisted for 18 hours following a single dose of N9; mucosal biopsies with coital simulation caused a transient 1.4-fold increase in permeability at 1 hour, which was not detectable at 4 hours (85).

Vehicle and (para)sexual toxicity. Interpretation of microbicide toxicity results requires consideration of confounding sources of toxicities, including the microbicide vehicle and (para)sexual activities to avoid incorrect attribution to the active microbicide ingredient. Coitally dependent use of microbicide gel vehicles and sexual lubricants are of particular concern because application occurs prior to and during coitus. Sexual lubricants have been associated with rectal epithelial surface disruption within 1.5 hours of application, and vaginal application of hyperosmolar gels results in leakage from the introitus in a dose-dependent manner (7, 86). Transudation of fluid owing to hyperosmolar gels will dilute microbicide concentration, potentially reducing effectiveness. Some common formulation excipients enhance mucosal permeability, sometimes resulting in relaxation of tight junctions and the observation of rectal epithelial disruption in human subjects (87, 88).

Epidemiologic evidence suggests both vaginal douching and rectal douching (with enemas) increase HIV risk (15, 17, 18). Several studies (72, 73) have established that approximately two thirds of MSM who practice RAI use rectal enemas in preparation for sex, most commonly with tap water (hypo-osmolar) and Fleet^{TM}-type enemas (hyperosmolar). Others have shown that hyperosmolar and hypertonic insult to the colonic mucosa results in cell degeneration and sloughing of epithelia, net intraluminal fluid and mucus secretion, and altered permeability (89–91).

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Rectally-administered hypertonic solutions (Fleet Enema™) and tap water also cause sloughing of the rectal epithelia (92, 93). These studies show a temporal relationship between epithelial injury and exfoliation that are similar to the results obtained by Phillips with N9.

Seminal fluid itself may increase HIV transmission as seen in explant studies (94, 95). Exposure of perfused rat colon to human seminal plasma results in mucosal cytolysis and increased paracellular permeability three hours following colonic exposure (96). Studies in rhesus monkeys have shown that Prostaglandin E_2, a marker found in seminal plasma, reaches peak concentration as early as 20 minutes following insemination, suggesting rapid alteration of both rectal and vaginal mucosal permeability (97).

Resistance. Use of ARV treatment drugs such as tenofovir as microbicides raises concern about the development of resistance. Resistance develops through the combination of (*a*) spontaneous viral genetic mutation due to low fidelity reverse transcription, occasionally resulting in viable drug-resistant clones, and (*b*) the less than fully suppressive concentrations of ARV drugs that select for drug-resistant variants. For microbicides, there is certain risk of resistance evolving in the local population of HIV-infected persons, thus reducing local microbicide efficacy. Less clear is the frequency of resistance due to selection of sporadic mutations in the susceptible host. This seems to be a far less likely event than resistance emerging in the HIV-treated population because the microbicide must fail to prevent an infection and allow some degree of replication for sufficient time in the presence of low ARV concentrations to select a resistant clone. Surveillance for resistance, therefore, both in the HIV-infected population within a community region and in susceptible persons in microbicide studies is essential. Combinations of active compounds in a microbicide formulation should further reduce the resistance risk, but it will significantly complicate development of the informative PK-PD models described, and the small anticipated number of resistance events may put modeling out of reach.

SUMMARY POINTS

1. The need for topical HIV microbicides to empower receptive sexual partners with effective HIV prevention methods is acute, but early clinical trials of microbicides have failed to prevent HIV infection.
2. Microbicide efficacy is exquisitely sensitive to local changes in sexual mucosal surfaces due to visually inapparent disruption of mucosal integrity and inflammation, which increase susceptibility to HIV infection. Parasexual variables also increase host susceptibility to HIV infection, making microbicide development more complex.
3. Anal sex is nearly as important as vaginal sex with regard to risk of HIV transmission in women (and tremendously important for MSM), which may significantly diminish the effect size in vaginal microbicide trials in which either (*a*) only the vagina is dosed or (*b*) the rectum is dosed with a drug untested for rectal safety.
4. Rational microbicide development will depend on employing more information-rich study designs before execution of pivotal clinical trials to better inform both which candidates to progress and optimal design of pivotal clinical trials.
5. Novel methods are being developed and increasingly implemented with new generations of microbicides to provide rich datasets on drug adherence, pharmacokinetics, and toxicology to better inform clinical development.

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6. A variety of noninvasive quantitative imaging methods provide needed concentration-distance-time data to guide formulation development and regimen selection. Invasive sampling is being used to provide rich data in subsets of intensively sampled study participants to understand tissue and cellular concentrations of microbicides to build multicompartment pharmacokinetic models.
7. Proof-of-concept designs are desperately needed to provide evidence of biological activity, and to explore dosing regimens to bridge the large gap between phase I and phase III studies.
8. Mechanistic modeling methods are being applied to link pharmacokinetic data with seroconversion outcomes to (*a*) identify desired target concentrations at the site of microbicide action and (*b*) provide parameter estimates to enable clinical trial simulation to improve pivotal clinical trial design.

FUTURE ISSUES

1. What are the best, most efficient surrogate measures for microbicide toxicity in early clinical studies to optimize candidate selection measures?
2. What proof-of-concept study design with relevant biomarkers of preventive effect will be needed to provide the necessary information at a key development decision point?
3. How can behavioral methods to enhance product adherence, pharmacokinetic data collection to guide dose selection and build predictive models, and seroconversion data be integrated in large clinical trial settings?
4. As a critical route of transmission with need for prevention in both women and men, when will rectal microbicide development achieve equal footing with vaginal microbicide development in science and funding?

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The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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