Recommendations for the clinical development of topical microbicides: an update

Christine Mauck[a], Zeda Rosenberg[b] and Lut Van Damme[c] for the International Working Group on Microbicides*

Topical microbicides are products that are being developed to prevent HIV infection and other sexually transmitted diseases (STD) through topical application to the genital and rectal epithelial surfaces. This paper is an update of the clinical section of a general guidance for the development and evaluation of microbicidal products that was first published by the International Working Group on Microbicides (IWGM) in 1996. (The preclinical section of that document will be updated separately later.)

All topical microbicides should be clinically evaluated in humans for safety and effectiveness. Safety studies are necessary to evaluate the potential for systemic absorption and toxicity as well as local toxic effects, such as irritation, ulceration, burning, and itching. Reported symptoms of burning and itching are relevant to future product use and acceptability. Irritation and ulceration of the vaginal, cervical, penile, or rectal epithelium have the potential to result in an increased transmission of HIV and other STD. Effectiveness studies to assess the prevention of HIV infection or STD, depending upon the product indication, are subsequently conducted. These trials need to be large enough to detect clinically meaningful levels of protection. For spermicidal microbicides, additional contraceptive effectiveness studies are also needed.

AIDS 2001, **15**:857–868

Keywords: Clinical trials, guidelines, microbicides, vaginal

Introduction

Heterosexual transmission of HIV is a serious public health concern, as it accounts for the vast majority of all HIV-1 infections worldwide. As we enter the new millennium, the Joint United Nations Programme on HIV/AIDS (UNAIDS) estimate that some 33.6 million men, women, and children are HIV infected. During 1999, 5.6 million people were newly infected with HIV and 2.6 million people died from HIV/AIDS. More than 95% of HIV-infected individuals live in developing countries, and approximately half of them are women [1].

Although condoms, when used consistently and correctly, are effective in preventing the sexual spread of HIV, there is an urgent need for methods a woman can use for HIV prophylaxis, such as topical microbicides [2]. Topical microbicides are designed for genital or rectal administration to prevent HIV infection or other sexually transmitted diseases (STD). An ideal microbicide would be safe, effective, acceptable to potential users, affordable, stable in most climates, and efficacious *in situ* for a reasonable period of time. Currently available detergent-based spermicides, such as nonoxynol-9, octoxynol-9, and benzalkonium chloride, are being clinically evaluated for the prevention of infec-

From the [a]Contraceptive Research and Development (CONRAD) Program, Arlington, VA, USA; [b]Family Health International, Arlington, VA, USA; and [c]Institute of Tropical Medicine, Antwerp, Belgium. *For the remaining members of the International Working Group on Microbicides see Appendix.

Disclaimer: This paper was endorsed by the International Working Group on Microbicies, but does not necessarily reflect the policies of individual members or their respective agencies and/or organisations.

Correspondence and reprints requests to: Christine Mauck, CONRAD, 1611 North Kent Street, Suite 806, Arlington, VA 22209, USA.

Tel: +1 703 276 3912; fax: +1 703 524 4770; e-mail: cmauck@conrad.org

Received: 19 July 2000; revised: 30 November 2000; accepted: 20 December 2000.

tion because they have been shown to have antiviral and antibacterial activity *in vitro*. Among the new classes of microbicidal compounds, e.g. sulphated polysaccharides and inhibitors of virus attachment, fusion, or replication, some appear not to be spermicidal and would permit the design of non-contraceptive microbicides [3–9].

This paper outlines the current deliberations of the International Working Group on Microbicides (IWGM) on the evaluation of topical microbicides. The IWGM was formed in November 1993 at a meeting held at WHO, Geneva, Switzerland [4]. The role of the IWGM is to facilitate the development and approval of safe and effective, affordable and acceptable, self-administered topical preparations to prevent the sexual transmission of HIV and other STD. Its members are experts in relevant scientific areas from governmental and non-governmental organizations. They meet regularly to facilitate communication, consultation and collaboration on scientific and technical, social and ethical, and industrial issues relevant to microbicide development. The group also works to establish a consensus on important matters such as recommendations for pre-clinical and clinical investigations and criteria for selecting promising leads for evaluation. The clinical considerations for microbicide development detailed below represent an effort to make safe and effective products available as rapidly as possible to women and men at risk of acquiring HIV infection through sexual contact.

In 1996, the IWGM published a document entitled 'Recommendations for the development of vaginal microbicides', which outlined the guidelines the IWGM had developed at that time for the evaluation of new vaginal microbicides [10]. Since 1996, however, microbicide research and development have grown rapidly, as measured by the increase in the number of products undergoing pre-clinical and clinical testing [11–13]. This preclinical and clinical experience has, in turn, raised new scientific, practical, and ethical issues that have necessitated updating the guidelines.

Important among the issues raised in the implementation of several phase I/II safety studies were those related to technological approaches for measuring microbicide effects on cervical and vaginal epithelium. To address these, the IWGM, together with the Contraceptive Research and Development Program (CONRAD), sponsored a workshop entitled 'The role of colposcopy in assessing vaginal irritation in research', held in January 1999 in Washington, DC [14]. This workshop made recommendations regarding the type of colposcopy to be used in vaginal product research, alternatives to colposcopy, and areas requiring additional study, and are incorporated into the material that follows.

The first phase III trials of microbicides raised other issues, largely related to study design, which will be critical to the planning and implementing of future trials. Results from a phase III study of the effect of vaginal contraceptive film (VCF) on the acquisition of HIV and other STD in Cameroon [15] highlighted problems in the measurement of compliance with study product use. In addition, the high level of reported male condom use during the study complicated the interpretation of microbicide effectiveness, as did the fact that the study participants were sex workers whose rate of condom use with their clients was higher than the rate of condom use with their primary partners. Similar concerns arose during the phase II/III effectiveness study of Advantage-S N-9 gel in sex workers in Kenya [16]. Finally, both studies experienced less than optimal participant retention rates because of the generally high mobility of sex worker populations. Indeed, poor participant retention was a principal factor in the early closure of the Kenya study.

As a result of the urgency of the present situation, discussions about ways to streamline the development and evaluation of topical microbicides have taken place in numerous workshops and conferences over the past few years and have involved microbicide researchers, regulatory agencies, sponsoring organizations, and community groups. On the basis of these discussions, it was decided to issue an update to the 1996 recommendations. The clinical update is presented below; an update on the preclinical recommendations will be issued at a later date.

Balancing considerations of efficiency and ethics in microbicide clinical trials

The following text should serve as a guide to the clinical testing of microbicide products that is both expeditious and ethical. It proceeds from two basic premises. The first is that, because of the urgent need for additional means of preventing HIV transmission, all phases of microbicide development should be carried out as expeditiously as possible. The clinical phase of development may be streamlined by: (i) running different studies on a given product in parallel whenever possible; (ii) testing multiple products (including products from different sponsoring agencies) in a single trial, thereby reducing the number of women enrolled in control arms; and (iii) using a phase II/III 'run-in' study whenever possible. These are discussed in more detail below.

The second premise is that all trials should be conducted in accordance with the current version of the Declaration of Helsinki [17] and the clinical practice guidelines applicable in the country where the trial will

take place. Studies must be ethically sound, at the same time taking into account the reality that standards of care may vary by locality. It is critical that discussions with the local institutional (ethical) review board, regulatory agency, and community groups take place early in the planning of microbicide trials.

Other ethical considerations must be integrated into the planning of microbicide trials, both by investigators and all concerned institutional review boards. The report of a conference convened in 1997 by Women's Health Advocates on Microbicides and the Population Council provides in-depth discussion of these considerations and presents the consensus reached at that time [18]. However, understanding of these issues continues to evolve and will require continuing attention. One approach to ensuring that such attention occurs early in trial design has been taken by the HIV Prevention Trials Network, which has incorporated specific ethical guidance into each of its study protocols. Among the most crucial elements of that guidance are matters of informed consent, responsibilities to the community, condom counselling, use of placebo, and STD/HIV treatment.

Informed consent

Each participant must be competent to grant consent in an entirely voluntary manner after all relevant information has been provided in a language and style that the participant can understand. Informed consent should be viewed as an ongoing process across the entire course of the study.

Responsibilities to the community

Sponsors must recognize that they have numerous responsibilities to the communities in which their studies are carried out. Community-based organizations should be involved as much as possible in the study design and implementation, especially with regard to feasibility, acceptability, and the establishment and maintenance of the appropriate clinical and social infrastructure. Trial results should be shared with the community, and a decision about the availability of the drug, if it is shown to be effective, should be made before the trial starts, recognizing that drug approval may occur several years after the trial is concluded.

Condom counselling

Because the correct and consistent use of the male latex condom during vaginal intercourse prevents HIV infections and reduces the risk of other STD, condom use must be recommended and condoms made available to all study participants. It is recognized that, despite condom counselling and condom availability, some women are unable to have their partners use condoms correctly and consistently [19]. An experimental study design using a 'condom promotion run-in phase' is being tested in HIVNET/HIV Prevention Trials Network sites in Zimbabwe and Malawi. In this approach, women are first recruited into a study for condom use assessment and are followed for 2–3 months. Women who successfully adopt consistent condom use are provided with a supply of condoms and referred to a continuing source. Women who are 'unsuccessful', that is, women with partners who use condoms at a less than predetermined frequency, will be offered enrolment in a future microbicide study. Although condom use is encouraged throughout the trial and condoms are supplied, the expectation is that this group of study participants will, nevertheless, represent a population unlikely to use condoms consistently, in which the study product can be directly compared with placebo without compromising fundamental ethics. There may be alternative ways to select for a population unlikely to use condoms using other study designs [20].

Use of placebo

The advantages and disadvantages of enrolling placebo groups are discussed in more detail below. However, it should be pointed out at the outset that the use of a placebo can only be considered as long as no microbicide known to be effective is available.

Treatment of sexually transmitted diseases and HIV acquired during a study

There is no debate about the treatment of STD acquired during a study, because some form of treatment is available in most areas. However, because the acquisition of an STD by a study participant may lower the infectious dose of HIV required for seroconversion, STD treatment may itself lower HIV incidence in the study population and require a corresponding increase in sample size [21].

There is, however, no clear consensus regarding the provision of antiretroviral therapy to study participants who seroconvert during a trial. The Women's Health Advocates on Microbicides report [18] recommends that a package of services that is likely to be 'appropriate, feasible, and replicable' in the given setting should be offered.

Design and implementation of microbicide clinical trials

The current recommendations of the IWGM for the design and implementation of microbicide trials are presented below, with the caveat that this is an evolving field of study, requiring that investigators and sponsors stay abreast of developments in both the scientific and ethical aspects of microbicide trials. Recommendations are presented by the phase of product development, phase I meaning initial safety and acceptability studies, phase II studies aimed at

providing supplementary information on safety and acceptability, and phase III studies of clinical effectiveness. The recommendations for each phase are summarized in Table 1, Table 2 and Table 3.

Phase I studies

Study objectives

The main objective of phase I trials (Table 1) is the determination of product safety in human subjects, gathering initial data on: (i) the incidence and extent of local and systemic adverse effects after administration of the product; (ii) the pharmacokinetics of the product; (iii) preliminary information on acceptability of the product; and (iv) the selection of dose and formulation for phase II/III.

Study design options

The simplest study design is an open-label trial in which each participant serves as her own control; in other words, there is no control group. For the initial safety study, the number of participants per group should be small (e.g. 10–20). Product application should occur at least once a day for 7 days between monthly menses. Longer exposure can be considered, and may have the advantage of detecting signs of irritation that might otherwise not become apparent until later studies. Exposure for 14 days is probably the longest feasible time-span that avoids product application during menses. To determine the range of product safety, multiple doses and frequencies of application can be tested in a sequential fashion (dose escalation) in different cohorts of participants, an example of which is outlined here: low dose once per day for 7 days, followed by low dose twice per day for 7 days, followed by high dose once per day for 7 days, followed by high dose twice per day for 7 days.

Alternatively, women can participate in such a study in a crossover fashion, by each woman testing the low-dose product once per day for 7 days, then undergoing a 'wash-out' period if required, testing the low dose twice per day, undergoing another wash-out period, and so on. This approach may, however, result in a bias towards the selection of women most tolerant of adverse events in the highest dose/frequency cohort, because women reporting adverse experiences may have discontinued at a lower dose.

In a population in which genital lesions are common in the absence of product use, the interpretation of study results may be difficult if a control group is not included. Other phase I study design options therefore include: the addition of a separate placebo or vehicle control group (when feasible), or the addition of a comparison arm using a product for which previous safety data are available; and a randomized cross-over design in which each participant uses one product or placebo, followed by a wash-out period, followed by the use of a second product or placebo. When such alternative study designs are used, the study should be randomized and, whenever possible, double-blinded, recognizing the difficulty in blinding a volunteer to the

Table 1. Phase I studies: summary.

Objectives:	Safety, local and systemic Pharmacokinetics Acceptability Dose and formulation section	
Study designs:	(a)	Open-label No control group Once then twice daily application for 7 days, escalating doses Parallel or crossover design
	(b)	Inactive or active control group Parallel or crossover design
Population:	10–20 per treatment group HIV-uninfected, in good health Not at risk of pregnancy or STD Sexually abstinent, then sexually active with condom, then sexually active without condoms: parallel or crossover design HIV-infected women may be studied in later phase I trials	
Site:	First trial or cohort: country of product origin	
Endpoints:	Local safety: genital symptoms, gross exam, colposcopic exam, vaginal microflora Systemic absorption: blood levels of the product or its active metabolites Systemic safety: appropriate lab tests Acceptability: interview, focus groups, questionnaires	
Ancillary studies:	Vaginal lavage for viral load Spreading and bioadhesiveness	

STD, Sexually transmitted diseases.
Note: This table must be viewed in the context of details presented in the text.

Table 2. Phase II studies: summary.

Objectives:	Local safety Acceptability If conducted as run-in to phase III, will have eventual objective of effectiveness against HIV/STD
Study designs:	(a) Stand-alone study Randomized, double-blind comparison with inactive control Parallel or crossover design Monthly visits for 2–6 months (b) Run-in to phase III ('phase II/III') Preferred over stand-alone design due to urgency of need for microbicide Interim analysis to determine whether study can be expanded to phase III Should include condom promotion run-in phase whenever possible
Population:	Several hundred participants HIV-uninfected Representative of target population for product Sexually active; condoms encouraged (condom promotion run-in phase to be utilized whenever possible) Parallel design
Endpoints:	Local safety: genital symptoms, gross exam, colposcopic exam on subset Acceptability: questionnaires
Ancillary studies:	Additional pharmacokinetic studies Use in anal intercourse

STD, Sexually transmitted diseases.
Note: This table must be viewed in the context of details presented in the text.

Table 3. Phase III studies: summary.

Objectives:	Evaluate effectiveness in preventing HIV/STD Local safety Acceptability and compliance
Study designs:	(a) Stand-alone study (b) Follow-on to phase II ('phase II/III') Preferred over stand-alone design due to urgency of need for microbicide Interim analysis to determine whether phase II study can be expanded to phase III In either case: Should include condom promotion run-in phase whenever possible Randomized, double-blind, inactive control(s) Inactive controls include placebo and no-treatment arm. Latter to be used if placebo may reduce HIV/STD incidence, although behavioral differences in no-treatment group should be evaluated May test several products against one inactive control Multicenter whenever possible Follow-up about every 3 months for 12 months
Population:	Several hundred to several thousand participants HIV-uninfected (although confidentiality issues may preclude exclusion of HIV-infected women) Representative of target population for product Sexually active; condoms encouraged (condom promotion run-in phase to be utilized whenever possible) Parallel design
Endpoints:	HIV/STD incidence Local safety: genital symptoms, gross exam, possibly colposcopic exam on subset Acceptability: questionnaires Compliance: product use for coital acts closest in time to study visits

STD, Sexually transmitted diseases.
Note: This table must be viewed in the context of details presented in the text.

identity of two products. The use of a vehicle placebo (i.e. the product formulation without its active ingredient) group enables an assessment of the safety characteristics of the vehicle, information that may be important to future studies. For example, if irritation is observed in the study product arm, results from the vehicle control arm can help distinguish between adverse effects caused by the active ingredient and those caused by the vehicle. If adverse effects can be attributed to the vehicle, the active agent can be reformulated in a less irritating

formulation. Without a vehicle control, otherwise promising candidates may be unnecessarily discarded.

Study populations

Phase I microbicide study populations generally consist of healthy, HIV-uninfected women volunteers who are 18–45 years of age and not at risk of pregnancy or STD. Women who are pregnant or nursing should be excluded, as well as women with: (i) abnormal liver or renal function if the product may be absorbed; (ii) a history of genital problems that might interfere with the interpretation of results; (iii) colposcopically observed abnormalities of the epithelium felt to be of possible clinical significance, e.g. full-thickness disruption; and (iv) unwillingness to suspend the use during the study period of other vaginal products such as douches, tampons, spermicides, or herbal preparations. Preclinical data and regulatory requirements should ultimately guide the approach to populations to be studied.

Phase I studies may be designed to enrol either sexually abstinent or sexually active women, although it should be recognized that self-reports of sexual activity or the absence of it cannot be validated. If sexually active women are enrolled, they should be in mutually monogamous relationships to the best of their knowledge to reduce the chance of exposure to STD. Allowing sexual activity in the phase I study presents several challenges: accounting for the mechanical effect of intercourse on epithelial integrity, the need to prevent conception until the level of systemic absorption and potential contraceptive/reproductive effects of the product are known, and the need to control for condom use. In the first cohort that includes sexually active women, male partners should thus use condoms in order to: (i) prevent exposure to STD/HIV pathogens until the level of epithelial disruption from the product used with intercourse is characterized; (ii) protect the male partner from the effects of the product until the level of female and male epithelial disruption is better known; and (iii) prevent pregnancy, although it is expected that women will be relying for contraception on something other than whatever contraceptive effect the microbicide may have, if any.

The product may then be tested in a second cohort of women who are sexually active, monogamous, and not using condoms, in order to assess the effect in both partners of product use in the presence of intercourse without condoms (for the man, this includes an assessment of penile irritation). Alternatively, the same couples may participate in three stages: product use plus abstinence, followed by product use plus sexual activity with a condom, followed by product use plus sexual activity without a condom. The possibility of bias arising from allowing the same couples to participate in different stages, and thereby selecting for the most tolerant couples to be included in the later stages should be recognized.

If the product appears to be safe and acceptable in sexually active, HIV-uninfected women, the study may be extended to include HIV-infected, sexually abstinent and then sexually active women. The purpose of enrolling HIV-infected women in early studies is to assess whether the safety profile in HIV-infected women differs from that of uninfected women. Safety data in an HIV-infected population in phase I are needed because: (i) during phase II/III trials, some participants will become infected with HIV and yet continue to use the product; and (ii) once a product is marketed, it may be used by women who may not know their infection status.

Study site

For ethical reasons, the first exposure of women to a product in initial safety studies should take place in the country in which the product was developed.

Study endpoints

Phase I study endpoints for evaluating product safety include local adverse effects, systemic absorption, and systemic adverse effects.

Local adverse effects: These safety endpoints can be assessed by participant report of subjective findings (symptoms of burning, itching, pain, fullness, urinary frequency, etc.). However, objective changes in the genital epithelium may occur independently of symptoms, so that it is necessary to evaluate the vaginal and cervical epithelium for disruption and inflammation by objective methods, such as vaginal speculum examination and colposcopy [22–25]. Recent research suggests that consideration be given to making such assessments both as soon as possible after product administration (within an hour) in order to detect any transient but potentially harmful effects of the product, and at a later time (8–24 h). Consideration should be given to determining whether changes seen represent simple chemical irritation of the epithelium versus a contact allergy, although making this distinction will be difficult. Changes in vaginal pH and microflora, including lactobacilli (hydrogen peroxide producers and non-producers), should be tracked, and the detection of STD and other genital infections is also recommended so as to exclude them as confounders in analysis of the data. Information on penile irritation should be collected when stable couples are enrolled or by a separate penile irritation study enrolling men only.

It is important to note here that there is significant controversy regarding the utility of colposcopy in assessing the local adverse effects of microbicides. In 1993, WHO convened a meeting to devise a standar-

dized procedure for colposcopy to be used in vaginal product development and, in 1995, a manual presenting that procedure was published [26]. In January 1999, the CONRAD/IWGM colposcopy workshop developed suggestions for simplifying the procedure for use in microbicide trials [14].

The present controversy regards the clinical significance of colposcopic findings, that is, their importance in determining susceptibility to infection with HIV or other STD. It seems reasonable to assume that disruptions in the integrity of the genital epithelium are findings of concern, as are obvious inflammatory or vascular changes. What is presently unclear is whether colposcopy enhances the likelihood of detecting disruptions or changes in the genital epithelium that are really of clinical importance. A related question is whether colposcopic lesions can be attributed to the study product or vehicle, particularly in the absence of a no-treatment arm and particularly in areas with a high background rate of lesions. Studies assessing the degree of agreement between colposcopically visualized erythema and vaginal inflammation, as measured by biopsy of colposcopically normal-appearing areas, found little correlation [24]. Our ability to resolve this controversy is currently limited by the lack of data, so that the continued use of a simplified colposcopic procedure in phase I clinical trials continues to be recommended here. As more data on colposcopy become available, recommendations regarding its use will be revised.

Systemic absorption: Clinical studies of vaginal absorption will be required unless the chemical characteristics of the product and the results of animal testing have produced strong evidence against systemic absorption. In the absence of such evidence, drug pharmacokinetics should be assessed by measuring blood levels of the study product or its active metabolites. Quantification of the study product remaining in the genital tract (e.g. by vaginal lavage) or physically expelled (e.g. collection on panty liners) may aid in determining actual product exposure. The presence or absence of vaginal epithelial breaks may affect absorption and should be assessed. Penile absorption studies should also be carried out in men if absorption is detected in women, provided that the level seen in women does not preclude further product development.

Systemic adverse effects: Standard systemic effects may be assessed by appropriate laboratory tests at enrolment and at the end of each observation period. Coagulation parameters should be tested when a product has shown anti-clotting effects in preclinical studies.

Acceptability: phase I trials offer the opportunity to obtain early information about the acceptability of both the product and the method of its administration. The small size of the trials also permits the use of key informant and in-depth interviewing; focus group techniques, with both participants and (possibly) with their partners; and what is, in effect, pretesting of acceptability instruments for later-stage trials.

Selection of dose and formulation: The selection of dose(s) and formulation(s) to be carried into phase II will be based on the three categories of safety data described above: adverse events, pharmacokinetic/pharmacodynamic results, and acceptability.

Ancillary phase I studies

At least two types of studies may be considered as ancillary to more standard phase I studies. One is the measurement of viral load in vaginal lavage specimens from HIV-infected women in phase I studies, which may allow a crude estimate of product activity against HIV or other viral pathogens. However, the amount of background variability in such measurements is likely to make the interpretation of results very difficult. That said, if other methods become available, it should be kept in mind that looking for markers of activity early in development may help distinguish more promising from less promising products.

Another category of potential ancillary study looks to evaluate the spreading and bioadhesiveness of products within the vagina through techniques that include fiberoptic probes, gamma scintigraphy, and magnetic resonance imaging. Information on the rate of spread from the point of initial placement of the product, extent and uniformity of coverage of the cervix and vagina, rate of leakage from the vagina, and the effects of intercourse are all of interest. Any disadvantages of bioadhesiveness, such as accumulation of the product, increased irritation from close apposition of the active ingredient to the epithelium, and difficulty in removing the product in case of irritation should also be evaluated.

Phase II studies

Study objectives

There are two options for initiating a phase II study (Table 2), each with slightly different objectives:

A stand-alone study preceding phase III: In this option, the objective is to gather data on: (i) the incidence and extent of local adverse effects after the administration of a specific dose, formulation, and frequency regimen of the product; and (ii) quantified data on product acceptability. In many cases, sufficient information of this type has been obtained in phase I testing or can be obtained from a phase II trial that is designed as a run-in to a phase III trial, making a separate, stand-alone phase II trial unnecessary.

A run-in study to phase III ('phase II/III' study): In this scenario, an interim assessment of safety is performed to determine whether enrolment can be expanded and the study considered phase III with the additional objective of assessing effectiveness against HIV/STD. The urgent need for microbicides recommends that phase II trials be designed as run-in studies to phase III trials whenever possible, rather than as more time-consuming stand-alone studies. Beginning the phase II/III study with a condom promotion run-in phase should be seriously considered.

Study design

Phase II trials should be randomized, double-blind comparisons of the study product to an inactive control. A placebo arm may enrol fewer participants than the test product arms if sufficient information about the placebo was obtained in phase I. The use of a no-treatment group should also be considered. A cross-over design, in which the product(s), placebo, and non-use would be tested sequentially by each participant, would also be acceptable, although participant loss to follow-up and the possible need to include a wash-out period to decrease carryover effects are limitations of this design and should be weighed carefully.

In phase II studies, participants use the study product over a longer time period than in phase I trials, ranging from 2 to 6 months, and they should be observed at monthly intervals.

Study population

Phase II microbicide studies are generally conducted in several hundred healthy, sexually active, non-pregnant women who are representative of the target population for the product under study. Women who are pregnant or nursing must be excluded. The study site should provide condoms that are either non-lubricated or lubricated with something without a microbicidal effect, and care should be taken to ensure that the supply of condoms is adequate for the duration of the study.

Study endpoints

Phase II endpoints must include:

Local adverse effects: Methods to determine local adverse effects are described above (see phase I study endpoints). There is, however, some variance with respect to assessment methodology. Whereas there is consensus that symptomatic and gross clinical findings should be documented for all participants in all trial phases, there is substantial debate concerning the need for colposcopy in phase II trials. One approach is to perform colposcopy on a subset of women enrolled in phase II or later studies. Until some resolution of this question is achieved, sponsors and researchers are advised to request input from the appropriate regulatory agency.

Acceptability: Participants in phase II microbicide trials should be more representative than phase I enrollees of the populations that will be recruited into later effectiveness trials and, to some extent, of microbicide end-users. Phase II measurements of acceptability will thus be critical not only in assessing product compliance in later studies, but in the design of future products and their introduction into user populations. As in phase I, acceptability data should be collected and analysed with an eye towards optimizing the design of the questionnaire to be used in phase III for the purpose of accumulating a large body of quantitative data.

Ancillary phase II studies

Pharmacokinetics (absorption) may be further studied in a subset of phase II volunteers early in their participation. In addition, recognizing that anal intercourse is more common among women than previously thought, and recognizing that approved microbicides will be used for anal intercourse by both men and women regardless of labelling, evaluation of the local and systemic safety of a product in anal use should be considered in phase II product testing.

Phase III studies

Study objectives

The main objectives of phase III microbicide trials (Table 3) are to determine: (i) the effectiveness of the study product in preventing the acquisition of HIV or other STD; (ii) the safety of exposure to the study product over prolonged usage (e.g. 12 months); and (iii) the compliance with and acceptability of product use.

Study design

Whenever possible, the phase III trial should be a follow-on to the phase II trial. Ideally, what is in effect a 'phase II/III trial' should begin with a condom promotion run-in phase. There are several study design variants.

The randomized, double-blind, placebo-controlled design remains the gold standard for microbicide effectiveness studies, and should be integral to the design of phase III or phase II/III trials. Depending on the availability of study products, multi-arm trials can be constructed to test several microbicides simultaneously. A major advantage to this approach is the use of a single placebo arm for multiple products, which reduces the number of participants required to demonstrate effectiveness, and thus contains time and costs overall. At the same time, if two products that are obviously different in appearance or other characteristics are to be tested, it may be necessary to employ two placebos, one for each product, within a single control arm.

One alternative is an open-label design, in which the study product is compared with a no-treatment arm and a vehicle-only arm. This design has been em-

ployed in circumstances in which the vehicle alone can reasonably be expected to have a preventive effect on HIV/STD transmission because of inherent barrier, lubricating, or other properties, and may also permit an assessment of any harmful effects of the vehicle. It may be prudent to power such studies to assume that a low level of both beneficial and harmful (as opposed to no) effects could be seen in the vehicle group. It should be recognized that, if this design is used, the behavior of participants in the no-treatment arm may differ from that of participants in the other arms, thus introducing potential bias in risk exposure. For example, women who are not using a product may try to minimize their risk of HIV by having intercourse less frequently, by more carefully selecting partners, or by using condoms more frequently. Concerns have also been raised about women in the no-treatment arm receiving products from women who did receive treatment. The US Food and Drug Administration does not generally recommend the use of a no-treatment arm in phase III studies. It should also be noted that inactive controls of any kind are recommended only as long as microbicides proved to be effective remain unavailable.

Multi-center studies should be carried out whenever feasible; this will allow assessment of the drug in diverse populations in both developing and developed countries. This approach will be more informative of the efficacy of the agents under study, and provide 'bridging' data that may be required for regulatory approval. In addition, multi-center trials make it possible for participants to be enrolled faster, and study endpoints are reached over a shorter time-frame. At the same time, multi-center studies present challenges in terms of the standardization of procedures, language, and consent, as well as higher monitoring costs.

As microbicide HIV effectiveness trials may require thousands of participants (see below), phase III study follow-up can be simplified to include visits at enrolment and, possibly, every 3 months until the end of the study. More frequent visits will be needed if bacterial STD are being studied, because they are both curable and occur with higher frequency than HIV. Whatever the design, both the study protocol and follow-up schedule should be structured to minimize the number of participants lost to follow-up, and should strike a balance between the need to estimate the time of seroconversion and the need to simplify clinic visits in terms of blood draws, transportation, and child-care issues.

As in the development of all new drugs, post-market studies will be advisable, if not essential for those vaginal microbicides that will be available, at least initially, only by prescription. Even over-the-counter microbicides will, again at least initially, require post-market monitoring of some sort, although it is premature to speculate on the characteristics of such studies at this time.

Study population

Microbicide effectiveness trials should be conducted in HIV-negative, sexually active women at risk of HIV/STD infection. The baseline incidence of study endpoints (notably HIV/STD incidence), the incidence of high-risk behaviors, the likely use of condoms, and the likely use–effectiveness of the test product(s) must be known at the outset in order to power the study adequately. Numbers sufficient to show the desired level of effectiveness will probably be in the hundreds or thousands. Even more so than in phase II and II/III studies, phase III study populations should represent the intended target population for the product. Enrolling discordant couples in which the male partner is infected may allow a reduced sample size, but condom counselling will be of critical importance in this population.

Women who are pregnant or nursing may be included in the study if the product has been shown not to be absorbed, and teratogenicity studies have been completed. Women using steroidal contraception should be permitted to enrol. Although the effect of steroids on HIV acquisition is unknown, any effect should be equally distributed among the study groups. Women presenting with clinically apparent genital infections or lesions at enrolment should be treated before inclusion in order to classify infections seen later in follow-up as incident infections. When resources permit, laboratory screening for baseline STD should be carried out. Because intravenous or rectal HIV transmission would be obvious confounders, it is preferable to exclude women who report intravenous drug use or anal intercourse, if possible (see Ancillary phase II studies above).

In some settings, it may be unethical to exclude HIV-infected women because the fact of exclusion may, in effect, reveal their infected status and thus compromise the ethical requirements for confidentiality. Furthermore, if such women use the test products, they can provide additional information on product safety and acceptability, as well as effects on other STD and HIV shedding. The management of women who seroconvert or become pregnant during the trial should be decided before it begins.

Study endpoints

There are three primary phase III endpoints: HIV/STD incidence; safety; and acceptability and compliance.

HIV/STD incidence: The long-term effectiveness of microbicides will be measured ultimately by rates of HIV or STD acquisition. Infection with herpes simplex virus and human papillomavirus are not presently recommended as endpoints for several reasons: both are

usually seen in high prevalence in target populations; the diagnosis of pre-existing infection is not straightforward; and the site of infection includes the perineum, which is not the site of product application.

State-of-the-art laboratory methods should be used to screen for incident HIV and other sexually-transmitted infections, and laboratory methods should be standardized across sites. Tests based on saliva or urine may be considered. Other than HIV, the most common STD presently measured in microbicide trials are gonorrhea and chlamydia. Trichomonas may now be included because there are new methods of detection that are more accurate than wet mounts. All diagnostic assays to be used in the study should be evaluated for adequacy of performance in the presence of both the active agent and placebo.

Safety: Long-term safety is determined by the presence or absence of local adverse effects, as diagnosed by examination and reported symptoms. As was noted in connection with phase II trials, the performance of colposcopy in a subset of women may be indicated if the results of earlier studies suggest that more data would be informative. In the current regulatory climate in the USA, it should be expected that colposcopy in a subset of phase III participants will be required. Phase III 'real use' data may produce findings and raise safety issues not found in earlier studies.

Acceptability and compliance: Estimates of the measurement of compliance with the use of the study product (defined as the estimated percentage of coital acts in which the product was used) and of study condoms are critical to an interpretation of study outcomes. A product may be shown to be ineffective in preventing HIV/STD because it was rarely or inconsistently used by most of the study population. At the same time, it may have been highly effective among women who used it consistently. If compliance levels are not documented with reasonable certitude, further development of what may have been an intrinsically efficacious product may be curtailed for the wrong reason. Experience with the use of coital diaries in clinical trials of vaginal products has generally been negative for a variety of reasons. An alternative approach to such diaries is the documentation of product use for the coital acts occurring closest in time to study visits. In phase III trials, quantitative acceptability data should be obtained from all participants and validated as much as possible. Issues related to accurately measuring compliance need more thought and further research.

Phase III statistical and quality assurance considerations

Every phase II/III and phase III study should be carried out according to the applicable regulatory guidelines and monitored to ensure the completeness and accuracy of study data. A standardized approach to collecting all clinical data, administering subject interviews, and analysing data should be used. Laboratory tests should be conducted in laboratories with proper quality assurance procedures or with accredited supervision. The sensitivity and specificity of various diagnostic tests for STD detection are not known, especially in the context of microbicide trials. Whenever possible, the trial should include the testing of performance characteristics as well as validation studies of diagnostic tests.

A Data and Safety Monitoring Board (DSMB) should oversee the conduct of all phase III trials and, when deemed appropriate, phase II trials as well. The DSMB should include at least one physician, trialist and/or statistician, and a representative from each participating country. Inclusion of an ethicist should be strongly considered, even if not required.

The study sample size will depend on a number of factors, most importantly the expected incidence of the endpoint, the level of product effectiveness being sought, rates of expected drop-out and loss to follow-up, and the planned duration of follow-up. Sample size estimates should also be based on detecting a clinically meaningful difference between the inactive control and the test product. Data accumulated from previous trials should inform decisions about plausible effect sizes in a given population.

Although some investigators suggest that studies should be powered to measure only very high product effectiveness, others suggest that even products with relatively low effectiveness have the potential to provide benefit at both an individual and a population level, especially if the product is highly acceptable, affordable, and consistently used [27]. The theory has been advanced that a product found to have a low but demonstrable level of effectiveness in the trial context may be used more consistently after the trial results are known and the product becomes available, thus increasing its effectiveness in actual use. If this were the case, failure to use a sufficiently large sample size could result in type II errors; that is, the failure to detect small but meaningful effects. Furthermore, if product effectiveness is modestly estimated from the outset, the study may prove to have more power than expected to show a difference, or it may be possible to end the trial sooner. For these reasons, trials recently completed or being planned have used effectiveness levels in the 30–50% range. Nevertheless, an increasing number of studies have indicated that product efficacy will be a critical determinant of compliance and, thereby, of ultimate product effectiveness.

Analyses of phase III data should include covariate adjustment by important baseline factors (after the

unadjusted analyses have been performed), and estimates of effect size should be accompanied by confidence intervals. Subgroups of interest based on key prognostic factors need to be pre-defined and strictly limited in number. When testing for a significant treatment effect within the subgroup, the complementary group should also be tested. Studies should be powered to assess whether there is evidence of a treatment–subgroup interaction of major interest. Results of subgroup analyses not defined *a priori* should be used only for generating new hypotheses.

Stopping rules should be clearly set out in the protocol. The DSMB has the responsibility for reviewing the data and making recommendations regarding stopping or modifying a study. As a general matter, only in very exceptional circumstances should a study be stopped for reasons other than serious drug toxicity, and stopping rules for toxicity cannot be pre-specified. In contrast, because relatively small effects are of interest, as noted in the preceding paragraph, only rarely can there be sufficient power to stop a trial for futility; this should only occur when there are convincing data from other studies demonstrating no effect. Similarly, stopping for benefit should be rare unless the effect is very large. Interim analyses of safety data should be made available to the DSMB every 4–6 months. Given the stopping rules recommended here, it would be inappropriate to perform more than a very limited number of interim efficacy analyses. One interim analysis performed approximately two-thirds of the way through a study should generally be sufficient.

If a phase II trial is designed as a run-in to a phase III trial, it will be important to specify how many women should be assessed in detail and for what period of time, and what level of serious adverse events are acceptable within that phase. In this case, it is recommended that safety data be assessed by the DSMB at the completion of phase II, in order that appropriate recommendations can be made and stop–go decisions can be made accordingly.

Conclusion

The development of topical microbicides for the prevention of HIV/STD infection is urgently needed to help stem the rapid growth of the AIDS epidemic throughout the world. These guidelines are intended to provide a strategic framework for the efficient clinical evaluation of candidate microbicide products. These guidelines, however, as well as the preclinical guidelines that will be forthcoming, must be seen as a work in progress in this critical area of reproductive health. Questions that deserve further thought and research are listed in Table 4. As development continues and these questions are addressed, these guidelines will almost certainly require further updating in the future.

Terminology

Throughout this paper, the term 'sexually transmitted disease' or 'STD' is used to refer to a study endpoint consisting of signs and symptoms of infection by sexually transmitted pathogens. However, it is recognized that infections may exist in the absence of a disease syndrome and that if laboratory results are an endpoint of a study as opposed to symptoms and signs, the proper term is 'sexually transmitted infection'. In addition, some conditions predispose to STD and sexually transmitted infection but are not sexually transmitted, such as bacterial vaginosis. These condi-

Table 4. Areas requiring further thought and research.

1.	Selecting populations unlikely to use condoms consistently: Does the condom promotion run-in phase work? Are there alternative mechanisms that should be tried?
2.	The role of colposcopy: Do epithelial changes predispose to HIV/STD? Are these changes better detected by colposcopy than by other methods? Is it possible to attribute such changes to microbicides in the setting of a high background incidence of changes and in the absence of a no-treatment arm?
3.	Vaginal lavage for viral load: Can this methodology be made useful given the high level of background variability?
4.	Testing for STD: Are current diagnostic tests affected by the presence of a microbicide?
5.	Assessing spreading and bioadhesiveness: What are the best method(s)?
6.	Assessing compliance: What are feasible means for accurately following compliance?
7.	Understanding evolving ethical issues: Requires constant attention and reassessment

STD, Sexually transmitted diseases.

tions are properly referred to as 'reproductive tract infections'.

References

1. *AIDS epidemic update*. Geneva, Switzerland: UNAIDS Joint United Programme on HIV/AIDS; December 1999.
2. Stein ZA. **HIV prevention: the need for methods women can use.** *Am J Public Health* 1990; **80**:460–462.
3. Elias C, Heise L. *The development of microbicides: a new method of HIV prevention for women*. Program Division Working Papers, No. 6. New York: The Population Council; 1993.
4. Global Programme on AIDS. World Health Organization. *Report on a meeting on the development of vaginal microbicides for the prevention of heterosexual transmission of HIV.* Geneva, 11–13 November, 1993 [Document WHO/GPA/RID/CRD/94.1].
5. Hitchcock P, Claypool L. **HIV and other STDs Working Group Report.** In: *Barrier contraceptives*. Mauck C, Cordero M, Gabelnick H, Spieler JM, Rivera R (editors). New York: Wiley-Liss; 1994. pp. 353–362.
6. Stone AB, Hitchcock P**. Vaginal microbicides for preventing the sexual transmission of HIV.** *AIDS* 1994, **8**:5285–5293.
7. Pauwels R, De Clercq E. **Development of vaginal microbicides for the prevention of heterosexual transmission of HIV.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1996, **11**:211–221.
8. Population Council and International Family Health. *The case for microbicides: a global priority.* New York and London; 2000.
9. Roehr B. **Fashioning new tools to deter HIV transmission.** *J Int Assoc Phys AIDS Care* 2000, **June**:157–168.
10. International working group on vaginal microbicides (IWGVM). **Recommendations for the development of vaginal microbicides. UNAIDS.** *AIDS* 1996, **10**:1–6.
11. Elias CJ, Coggins C. **Female-controlled methods to prevent sexual transmission of HIV.** *AIDS* 1996, **10 (Suppl. 3)**:S43–S51.
12. Van Damme L, Rosenberg Z. **Microbicides and barrier methods in HIV prevention.** *AIDS* 1999, **13 (Suppl. A)**:S85–S92.
13. Harrision PF, Kidder GG (editors). *Microbicide products database and summary.* Silver Spring, MD: Alliance for Microbicide Development; May 2000.
14. Proceedings. **Role of colposcopy in assessing vaginal irritation in research.** *Workshop sponsored by CONRAD and IWGM*. Washington, DC, January 21–22, 1999.
15. Roddy RE, Zekeng L, Ryan KA, Weir SS, Tamoufe U, Wong E. **A randomized controlled trial of N-9 film use on male-to-female transmission of HIV-1.** International Congress of Sexually Transmitted Diseases. Seville, October, 1997 [Abstract S51].
16. Martin HL, Richardson BA, Stevens CE, Lavreys L, Ngugi E, Mandalyia K. Kreiss J. **Evaluation of a low dose nonoxynol-9 gel for the prevention of sexually transmitted diseases.** *12th World AIDS Conference*. Geneva, 1998 [Abstract 33610] (p. 29 supplement).
17. World Medical Association. *Declaration of Helsinki*. Adopted by the 18th World Medical Assembly. June 1964, amended 1975, 1983, 1989, and 2000.
18. Heise,LL, McGrory CE, Wood SY. Practical and ethical dilemmas in the clinical testing of microbicides: a report on a symposium. New York: International Women's health Coalition, 1998.
19. Van de Wijgert J, Elias C, Ellertson C, *et al*. **Thinking about vaginal microbicide testing.** *Am J Public Health* 2000, **90(7)**:1153–1154.
20. Potts M. **Thinking about vaginal microbicide testing.** *Am J Public Health* 2000, **90**:188–190.
21. Grosskurth H, Mosha F, Todd J, *et al*. **Impact of improved treatment of sexually transmitted diseases on HIV infection in rural Tanzania: randomised controlled trial.** *Lancet* 1995, **346**:530–536.
22. Roddy RE, Cordero M, Cordero C, Fortney JA. **A dosing study of nonoxynol-9 and genital irritation.** *Int J STD AIDS* 1993, **4**: 165–170.
23. Goeman J, Ndoye I, Sakho LM, *et al*. **Frequent use of menfegol spermicidal vaginal foaming tablets associated with a high incidence of genital lesions.** *J Infect Dis* 1995, **171**:1611–1614.
24. Stafford MK, Ward H, Flanagan A, *et al*. **Safety study of nonoxynol-9 as a vaginal microbicide: evidence of adverse effects.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1998, **17**:327–331.
25. Stafford MK, Cain D, Rosenstein I, *et al*. **A placebo-controlled double-blind prospective study in healthy female volunteers of dextrin sulfate gel.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1997, **14**:213–218.
26. World Health Organization. Manual for the standardization of colposcopy for the evaluation of vaginally administered products. Geneva, Switzerland: WHO; October 1995.
27. Watts C, Thompson W, Heise LL. **The effectiveness of microbicides for HIV prevention.** *12th World AIDS Conference*. Geneva, June, 1998 [Abstract 33161].

Appendix

Members of the International Working Group on Microbicides

Debra Birnkrant, Food and Drug Administration, Rockville, MD, USA; Dan Davis, Food and Drug Administration, Rockville, MD, USA; Ann Duerr, Centers for Disease Control and Prevention, Atlanta, GA, USA; L. Fransen, European Commission, Brussels, Belgium; Henry Gabelnick, CONRAD Program, Arlington, VA, USA; David Griffin, WHO, Geneva, Switzerland; Polly Harrison, Alliance for Microbicide Development, Silver Spring, MD, USA; P J Hitchcock, National Institutes of Health, Bethesda, MD, USA; Charles Lacey, Imperial College School of Medicine, London, UK; Salimata Niang, Society for Women and AIDS in Africa, Senegal; Andrew Nunn, MRC Clinical Trials Unit, London, UK; Joseph Perriens, UNAIDS, Geneva, Switzerland; Michel Pletschette, Commission of the European Communities Health Research for Development, Brussels, Belgium; Patricia Reichelderfer, National Institutes of Health, Rockville, MD, USA; Jeffrey Spieler, US Agency for International Development, Washington, DC, USA; Alan Stone, Medical Research Consultant, London, UK; Janneke van de Wijgert, Population Council, New York, NY, USA.

Disclaimer: This paper was endorsed by the International Working Group on Microbicides, but does not necessarily reflect the policies of individual members or their respective agencies or organizations.