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Jeremy Nuttall *Editor*

Microbicides for Prevention of HIV Infection

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Microbicides for Prevention of HIV Infection

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Preface

More than 30 years after the first cases were reported, the HIV/AIDS pandemic remains a global health priority. Heterosexual transmission of HIV accounts for 70–80 % of infections worldwide, and women are disproportionately affected by this disease, especially in sub-Saharan Africa where they account for approximately 60 % of infections. In developing countries, factors such as low acceptance of condoms, patterns of sexual behavior, social attitude, viral loads in the semen of the HIV-positive partner, local viral subtypes, and coinfection with other sexually transmitted disease mean that exposure to an infected partner is much more likely to result in transmission than in developed countries, with women again the most affected. There are also anatomical and physiological factors that put women at a greater risk of infection than men. In addition, social, legal, and economic disadvantages make women more likely to become HIV infected.

In order to change the course of the HIV pandemic, effective strategies for prevention of HIV transmission are critical. Abstinence, reducing the number of sexual partners and concurrent sexual relationships, and correct, consistent condom use has been found to be effective in reducing the probability of HIV infection. However, these methods have proven to be insufficient to control infection rates. More HIV prevention strategies are required that provide options for those populations who need protection the most. Thus, safe and effective HIV prevention methods, particularly those that are female-controlled, could play a major role in reducing the incidence of HIV-1 transmission.

In recent years, a number of biomedical interventions have shown promise in HIV prevention. These include pre-exposure prophylaxis (PrEP), treatment as prevention, and microbicides. The concept of microbicides as an HIV prevention strategy was first introduced in an article titled ‘HIV Prevention: The Need for Methods Women Can Use’ published in April 1990. Microbicides are topical products that can be applied vaginally or rectally to protect the user from acquiring HIV and, possibly, other STIs.

Early microbicides were nonspecific compounds that worked by either disrupting the viral envelope (e.g., surfactants), electrostatically blocking the virus from interacting with target cells in the vagina (e.g., polyanions), or by maintaining the low pH of the vagina, making it inhospitable to HIV. All of these microbicides were formulated as gels that were intended to be applied vaginally just prior to sex. Clinical trials of these early candidates all failed to demonstrate efficacy, which led to an increased focus on products based on more potent and highly specific antiretroviral compounds. These microbicides are able to be formulated in a wider range of dosage forms, including formulations for daily use and sustained release products for use over a month or more, and can incorporate combinations of active ingredients that may improve their effectiveness further. In 2010, the microbicide field achieved its first proof of concept when a vaginal gel containing 1 % tenofovir was shown to protect women by 39 %.

The development of microbicides is a long and complicated process, with many challenges in product design, in the conduct and design of clinical trials, and in obtaining licensure for a new class of products intended for use almost exclusively in developing countries. This edition of Current Topics in Microbiology and Immunology is entirely dedicated to the field of microbicides. It is intended to cover all the critical areas associated with the development of microbicides, from the selection of appropriate candidate molecules, their formulation, preclinical and clinical testing for safety and efficacy, strategies for product registration and finally, issues associated with product launch, distribution, and access.

The authors were all selected because of their expertise in the development of microbicides. Consequently, much of the information is derived directly from their personal experience over years of product development, and is supplemented with knowledge gained from the experience of colleagues in the field. Since no microbicide product has yet been brought to market, some of the information presented represents what is believed rather than proven to be best practice, but reflects the current state of the art and the techniques, models, procedures, and processes available. However, researchers, developers, advocates, and regulators are together learning more about the transmission process and how to prevent it, determining what will be necessary to get these products to market, and how this can best be achieved.

My hope is that this edition will prove valuable to both workers in the microbicide field and others who are interested in learning more about this promising intervention that has the potential to significantly impact the future of the devastating HIV/AIDS epidemic. Whilst it is not possible to cover every aspect of microbicide development in detail, efforts have been made to point the reader toward other sources of information that may be helpful in filling any gaps and providing a more comprehensive understanding of the issues associated with microbicide development.

I would like to thank all the authors for the high quality of their contributions, and their patience and support in putting this edition together. I would also like to acknowledge the team at the International Partnership for Microbicides, and colleagues in many other organizations whose collaborations have helped move the field forward to the point where we now have products that are in the final stages of development, and are moving ever closer to getting them into the hands of those that so desperately need them.

Jeremy Nuttall

Contents

Candidate Microbicides and Their Mechanisms of Action

Carolina Herrera and Robin J. Shattock

Abstract The development of prevention strategies against sexual transmission of human immunodeficiency-1 virus (HIV-1) is essential to curb the rate of new infections. New prevention options include microbicides, many of which are based on antiretroviral (ARV) drugs targeting different stages of the viral replication cycle including: viral entry and fusion; reverse transcription; integration; and viral maturation through proteolytic clevage. In this review, we discuss current and new potential candidate microbicides designed to prevent mucosal HIV acquisition. Preclinical methods, including cellular, tissular, and animal models, used to assess candidate microbicides and evaluate their prioritization for progress through the product development pipeline are reviewed in context with a rapidly evolving clinical landscape.

Contents

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1 Introduction

An effective microbicide should be a safe, acceptable, and affordable product delivered topically as a gel, tablet, film or intravaginal ring at the genital (vaginal, penile) and/or gastrointestinal (colorectal) mucosa to prevent, or at least significantly reduce, sexual transmission of HIV, and possibly other sexually transmitted infections. Affordability and broad spectrum of action were the primary considerations for in the first generations of microbicides that included: surfactants able to disrupt the viral membrane (Malkovsky et al. 1998); acid-buffering gels that could inactivate HIV by decreasing pH (Mayer et al. 2001; O'Connor et al. 1995); and long chain polyanionic compounds that would inhibit non-specifically viral entry into target cells (Fletcher and Shattock 2008). However, these products showed lack of potency (Ramjee et al. 2010) or lack of safety in the case of the surfactants, that affected the epithelial barrier leading to higher rates of infection when used frequently (Hillier et al. 2005; Van Damme et al. 2002). For the next generation of microbicides, safety and specific anti-HIV activity became the primary criteria for candidate selection in the microbicide pipeline. Selection of drugs meeting these criteria benefited from the experience acquired in the field of HIV treatment with the development of highly active antiretroviral therapy (HAART). Thus, potent anti-retroviral (Shattock and Rosenberg 2012) drugs that inhibited specific steps of HIV replication cycle became lead candidates for microbicide development.

Here we discuss the mechanism of action of microbicides that specifically inhibit different stages in the HIV-1 replication cycle (Fig. 1) within the context of sexual transmission, new candidate microbicides, and review preclinical models currently used for product advancement in the microbicide pipeline.

2 Targeting the HIV-1 Replication Cycle

2.1 Viral Entry and Fusion

Viral entry within a target cell occurs following sequential binding of the virion to the primary cellular receptor CD4 and co-receptors CCR5 or CXCR4 by viral envelope spikes (Envs) that comprise trimers of non-covalently linked heterodimers of glycoproteins (gp), gp120, and gp41. Binding of Env to CD4 and CCR5 or

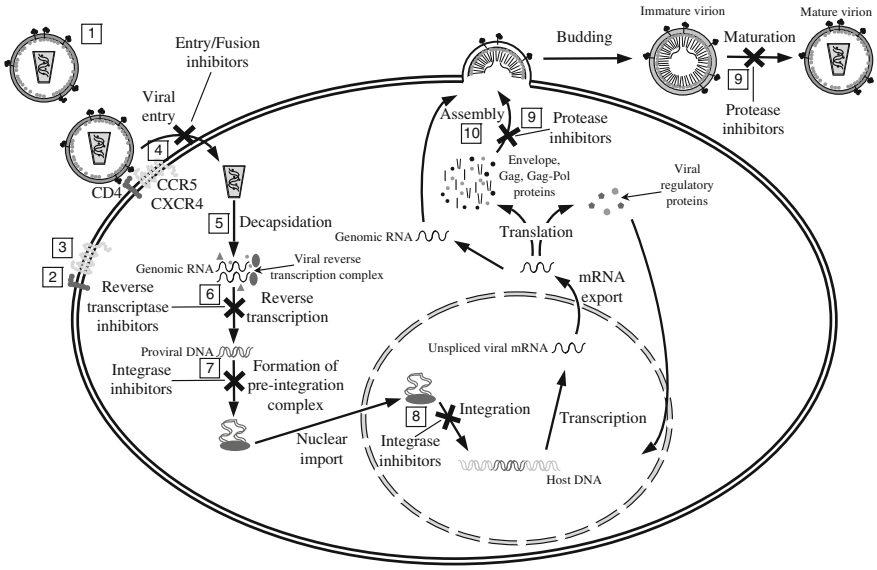

Fig. 1 Overview of the HIV-1 replication cycle. After entry and decapsidation, uncoated genomic RNA is reverse transcribed into proviral DNA by the viral reverse transcription complex. Proviral DNA is integrated within the host chromosomal DNA in the nucleus and then transcribed to unspliced messenger RNA (mRNA). mRNA is spliced and/or translated into viral proteins in the cytosolic compartment before transport to the surface membrane for assembly of the virion. The virus then buds as an immature virion and proteolytic cleavage of Gag and Gag-Pol polyproteins allows maturation of the virus. *Numbers* represent intervention points during the viral replication cycle. Adapted from Herrera and Shattock (2012)

CXCR4 triggers a series of sequential conformational changes within Env that ultimately elicits fusion of viral and cellular membranes allowing formation of pores and entry of viral genetic material (RNA) into the cell (Fig. 1). The first conformational change is induced by the binding of gp120 to CD4 leading to exposure of the gp120 binding site for CCR5/CXCR4. Engagement of the co-receptor leads to a reorganization of gp41 forming a six-helix hairpin structure, a process referred to as six-helix bundle formation that allows apposition and fusion of the viral and host cell membranes.

The first target for potential microbicide intervention is the binding of viral gp120 to CD4 (Fig. 1, intervention point 1). Inhibiting this step can be achieved by blocking the CD4-binding site within gp120. Small proteins that mimic CD4 have been identified showing high potency against HIV in vitro (Van Herrewege et al. 2008) and one of them, M48-U1 has shown promise in macaque challenge studies (Dereuddre-Bosquet et al. 2012). An alternative strategy is the use of drugs that bind to gp120 and rather than preventing CD4 binding, block the conformational change in gp120 induced by CD4 and required for fusion (Si et al. 2004) (Fig. 1, intervention point 1). Compounds with this mechanism of action are part of a family of small-molecules including BMS-378806, which is active in vitro

(Si et al. 2004) and against vaginal challenge in non-human primates (NHPs) (Veazey et al. 2005); and a derivative of this first drug, DS003 (BMS-599793). A final approach is the use of anionic dendrimers to provide a charge-based inhibition of gp120-CD4 interaction, currently designed as an aqueous gel (SPL7013 3 % gel or VivGel) (McGowan et al. 2011; Telwatte et al. 2011).

Binding of Env to cellular receptors can also be inhibited by targeting Env with neutralizing antibodies (NAbs) (Fig. 1, intervention point 1). Proof of principle that NAbs could be used as a microbicide was obtained with the demonstration that vaginal application of b12 (NAb elicited against the CD4 binding site in gp120) protected NHPs against vaginal challenge (Veazey et al. 2003). Anti-gp41 NAbs, 2F5, and 4E10, and the anti-gp120 NAb 2G12 (that recognizes mannose moieties on *N*-linked glycans) have also shown protective activity when used in combination systemically (Stiegler et al. 2002; Trkola et al. 2005) and the combination has been shown to be safe in a phase I clinical trial when administered as a vaginal gel (Morris et al. 2010). More recently the potent broadly NAb, VRC01 (Zhou et al. 2010), has shown potent efficacy against HIV-1 vaginal challenge in a humanized mouse model (Veselinovic et al. 2012) (see Sect. 4). A twist on the antibody theme is the use of an engineered CD4-immunoglobulin G2 chimeric molecule, CD4-IgG2 (PRO-542), which has very similar properties to NAb b12 recognizing the CD4-binding site in gp120 (O'Hara and Olson 2002; Trkola et al. 1995). Despite rapid advances in the identification of broadly NAbs with greater potency and breadth (Walker et al. 2011), large-scale production will still need to be addressed to ensure cost effectiveness. Potential alternatives include single domain NAbs with potent in vitro neutralizing activity (Forsman et al. 2008; Koh et al. 2010), exceptional stability, and ease of manufacture in yeast (Gorlani et al. 2012).

Another group of compounds that can prevent Env binding to CD4 are lectin-like proteins that recognize mannose moieties on *N*-linked glycans in gp120. Two lectin candidates are cyanovirin-*N* (CV-N) and griffithsin (GRFT). CV-N has shown anti-viral activity in vitro (Sexton et al. 2009) and in NHP models against both vaginal and rectal challenges (Tsai et al. 2003, 2004). GRFT also has proven potent anti-viral activity in several preclinical microbicide studies (Ferir et al. 2012; O'Keefe et al. 2009) and can be manufactured to scale in plants (O'Keefe et al. 2009).

A novel strategy for preventing gp120-CD4 interaction is the direct targeting of cellular CD4 by either inducing its down-modulation using cyclotriazadisulfonamide (CADA) (Vermeire et al. 2008) or by knock-down of the CD4 gene expression with CD4 aptamer-siRNA chimeras (Wheeler et al. 2011) (Fig. 1, intervention point 2). These strategies aim to reduce CD4 expression on mucosal T cells thereby reducing the number of susceptible target cells. Proof of concept studies are awaited in animal challenge models.

A second point of intervention in the viral replication cycle is the binding of Env to co-receptors, CCR5 or CXCR4 (Fig. 1, intervention point 3). Epidemiological and genetic studies show that >90 % of sexually transmitted infections worldwide are due to viruses using CCR5 as co-receptor (known as R5-tropic HIV) and in few instances R5X4 dual-tropic isolates are transmitted (Keele and

Derdeyn 2009; Salazar-Gonzalez et al. 2009; van't Wout et al. 1994; Zhu et al. 1993). CCR5 specific inhibitors in the microbicide pipeline have shown activity in vitro and in NHPs, and include modified RANTES chemokines and small molecule CCR5 inhibitors. RANTES protein analogs such as PSC-RANTES and 5P12-RANTES (Gaertner et al. 2008; Kawamura et al. 2000; Lederman et al. 2004; Veazey et al. 2009) have been generated by modification of the N-terminus of RANTES while retaining their binding properties to CCR5 (Arenzana-Seisdedos et al. 1996). These RANTES derivatives bind to the extracellular domain of CCR5 and inhibit Env-CCR5 interaction either by inducing CCR5 internalization or by blocking the co-receptor preventing gp120 binding (Hartley et al. 2004; Pastore et al. 2003). In contrast to PSC-RANTES, 5P12-RANTES lacks chemotactic and leukocyte activation capacities to prevent potential induction of mucosal inflammation (Gaertner et al. 2008). Small molecule CCR5 antagonists include CMPD167 (Veazey et al. 2005) and maraviroc (Dorr et al. 2005; Veazey et al. 2010). These antagonists are thought to inhibit gp120-CCR5 engagement by binding to a predominantly hydrophobic pocket within the transmembrane domains of CCR5 and modifying its extracellular domains, which are recognized by gp120 (Dragic et al. 2000; Garcia-Perez et al. 2011; Hu et al. 2010; Kondru et al. 2008; Maeda et al. 2006). Maraviroc is the only CCR5 inhibitor currently proven to be safe and effective in humans when used orally in HAART (Emmelkamp and Rockstroh 2008), making it an attractive candidate microbicide. Anti-CCR5 monoclonal Abs, such as PRO140, can also block CCR5 showing potent activity (Trkola et al. 2001). The small molecule CXCR4 inhibitor AMD3100 (Donzella et al. 1998) has the potential for being used in combination with a CCR5 inhibitor to prevent possible transmission of R5X4 viruses.

A third target during viral entry into the host cell is the formation of the six-helix bundle by gp41 that allows fusion between viral and cellular membranes (Fig. 1, intervention point 4). This six-helix hairpin structure is formed by folding in an anti-parallel manner of the C-terminal region (HR2) and the N-terminal domain (HR1) of each gp41 in the Env trimer. Peptide analogs to gp41 HR1 or HR2, inhibit fusion by binding to HR2 or HR1 domains, respectively, and hence, block the formation of the six-helix bundle. Several fusion inhibitors (FIs) based on the sequence of HR2, mimicked by the peptide C34, have been examined for their potential as microbicides. The first FI to be evaluated in microbicide models was T20 (enfuvirtide/Fuzeon) currently used in HAART. Other HR2-based peptides were later developed demonstrating higher potency in vitro and efficacy in NHPs models, including C52L (Veazey et al. 2005) and T1249 (Veazey et al. 2008). The most recent FI, L'644, is a cholesterol-derivatized version of C34 (Ingallinella et al. 2009). L'644 may be a more effective FI candidate microbicide due to the presence of the cholesterol tag that acts as an anchor for L'644 to the cell membrane showing increased anti-viral potency, longer half-life and persistent activity in the presence of biological fluids (Harman et al. 2012; Ingallinella et al. 2009). Furthermore, FIs as a class have broad activity across HIV-1 clades (Veazey et al. 2008).

2.2 Reverse Transcription

Following fusion of viral and target cell membranes and formation of pores, the viral capsid is released into the cellular cytoplasm. Decapsidation or uncoating is followed by formation of the viral reverse transcription complex that produces proviral double-stranded DNA from viral genomic RNA by the HIV-1 enzyme reverse transcriptase (RT) (Fig. 1, intervention point 6). This step in the viral replication cycle can be inhibited by targeting either the substrate of the enzyme or RT directly.

Nucleoside and nucleotide RT inhibitors (NRTIs) are analogs of nucleosides or nucleotide 5′-monophosphates that lack the 3′hydroxyl group necessary for the formation of a phosphodiester linkage with the next incoming nucleoside. NRTIs require metabolic activation by phosphorylation to become triphosphorylated as the natural deoxyribonucleotide triphosphates (dNTPs) and inhibit reverse transcription by substrate competition (Mehellou and De Clercq 2010). Their potency derives from their ability to act as chain terminators of the polymerization chain reaction. NRTIs were the first class of drugs developed for HIV therapy (Broder 2010) and have shown efficacy in post-exposure prophylaxis and in prevention of mother-to-child transmission (Wiznia et al. 1996).

Tenofovir (9-[(R)-2-(phosphonomethoxy) propyl] adenine monohydrate/PMPA) is the first NRTI that was formulated as a microbicide gel (1 % tenofovir) and showed efficacy in NHPs when used either as a rectal microbicide (Cranage et al. 2008) or as a vaginal product (Parikh et al. 2009). In addition to these two studies, proof of concept was obtained in a clinical efficacy trial, CAPRISA 004, of 1 % tenofovir gel (Abdool Karim et al. 2010) when used in a pericoital fashion (applied before and after intercourse). HIV infection was reduced by 39 % in the active arm after 30 months of follow up. Interestingly, at 12 months the efficiency was higher with a reduction of 50 %, and in high adherers (gel used with >80 % compliance to the protocol) it was reduced by 54 %. However, in another clinical trial performed by the Microbicide Trial Network (MTN) (MTN-003/VOICE), with daily dosing independent of the timing of intercourse, showed no protection (MTN 2011; NIAID 2011). A placebo-controlled trial, FACTS 001, finishing in 2014 has been set up to confirm and extend the CAPRISA 004 results (CONRAD 2011). Tenofovir gel has also been tested for safety and acceptability as a rectal microbicide (Anton et al. 2011a). Furthermore, initial studies in NHPs have shown a certain level of protection even 3 days after vaginal application of the 1 % gel (Dobard et al. 2012), reflecting the long intracellular half-life of active tenofovir. These data support coitus-independent dosing. However, disappointing results from the MTN-003 trial (VOICE) evaluating daily dosing with 1 % tenofovir gel suggest efficacy in humans likely reflects suboptimal product adherence that may be compounded by differences in drug concentration/turnover at the site of initial mucosal infection (van der Straten et al. 2012).

Another NRTI in the microbicide pipeline, emtricitabine (FTC), has been co-formulated in combination with tenofovir (Truvada gel) to increase potential

efficacy. Truvada gel has demonstrated full protection of NHPs (Parikh et al. 2009) and is in preclinical evaluation.

Non-nucleoside RT inhibitors (NNRTIs) represent a different class of structurally diverse non-competitive inhibitors that do not require metabolic activation and that block proviral DNA elongation by binding directly to the enzyme and inducing conformational changes of the active site of RT. The NNRTIs being developed as candidate microbicides are currently not used in HAART and include: dapivirine (TMC120), UC781, MIV-150, MIV-160, MIV-170, MC1220, and IQP-0528. Dapivirine has shown high activity in a range of preclinical studies (Fletcher et al. 2009; Herrera et al. 2009) and when tested in animal models (rabbits and NHPs) accumulation of drug was detected in the first layers of cervicovaginal tissue (Nuttall et al. 2008) even 48 h post-administration. Dapivirine has been formulated as a gel, and in an intravaginal ring, and both dosage forms have been tested in phase I and phase II pharmacokinetic and safety trials (Nel et al. 2009a, b; Romano et al. 2009), and the ring has now progressed into phase III trials (IPM 027, MTN 020). UC781 has also proven activity in cellular and tissular preclinical models (Fletcher et al. 2005; Herrera et al. 2009) and has been formulated as a gel. Safety, acceptability, and preliminary efficacy data have been obtained for rectal use in a phase I clinical trial (Anton et al. 2011b). MIV-150, MIV170, and MC1220 have shown efficacy in NHP challenge studies (Caron et al. 2010; Crostarosa et al. 2009; Kenney et al. 2011; Singer et al. 2011), although no clinical data are currently available for these candidates (Elinder et al. 2010). As alternatives to these compounds, new NNRTIs are being assessed in preclinical studies (Venkatraj et al. 2011). One, IQP-0528 belonging to the pyrimidinedione family, is potent against HIV-1 (Buckheit et al. 2001), and in contrast to most NNRTIs is also active against HIV-2 (De Clercq 1998). It has been formulated as a vaginal gel; and tested successfully in in vitro and ex vivo preclinical studies (Mahalingam et al. 2011).

2.3 Viral Integration

Shortly after proviral DNA is synthesized in the cytoplasm, the enzyme HIV-1 integrase (IN) removes the 3′dinucleotide ends of both DNA strands and catalyzes the transfer and integration of the pro-viral DNA into the cellular DNA by attachment of the 3′end of the viral DNA to the 5′end of the genomic DNA in the cellular nucleus (Engelman et al. 1991) (Fig. 1, intervention point 8). A few IN inhibitors (INI) have been developed to inhibit the different steps within the integration process (Crucitti et al. 2012; Engelman et al. 1991; McElrath et al. 2010; Terrazas-Aranda et al. 2008). Raltegravir (MK-0518), which inhibits the strand transfer by binding to the enzyme active site, and is currently used in therapy under the trade name Isentress® (Powderly 2010), is now being considered as a candidate microbicide. Another INI blocking strand transfer, L-870,812 has shown activity in a pilot NHP study (Dobard et al. 2010). Elvitegravir, which also

inhibits strand transfer, is in early development as a microbicide candidate (Crucitti et al. 2012). One potential benefit of INIs is that by acting later in the viral cycle, there might be a wider window within which to get the drug to the initial site of infection within mucosal tissue. Although yet to be tested in animal models this might even allow for postcoital dosing (Crucitti et al. 2012).

2.4 Viral Maturation

Once integrated, viral DNA is transcribed into messenger RNA, which is then translated into HIV proteins. Viral proteins together with two copies of HIV RNA are assembled and new virions bud out of the infected cell (Fig. 1). However, the assembled virion is immature and requires post-translational processing of precursor viral polyproteins ($Pr55^{Gag}$ and $Pr160^{GagPol}$) into functional proteins for the budded virion to be infectious (Kohl et al. 1988). HIV protease (PR) is responsible for this processing that allows yielding of the viral structural proteins matrix (MA), capsid (CA), nucleocapsid (NC), and viral enzymes PR, RT, IN. Due to their delayed antiviral activity (inhibiting infection once viral DNA has been integrated), protease inhibitors (PIs) (Fig. 1, intervention point 9) have only recently been considered as candidate microbicides. Supporting the potential importance of PIs as microbicides are the fact that PIs have shown efficacy even as monotherapy (Knechten et al. 2011) and recent data suggest that more potent anti-HIV activity is associated with late-acting drugs (Donahue et al. 2010). Furthermore, the late activity of PIs, means PI-based microbicides may have a wider window for protection with potential for pre and/or postcoital application (Herrera and Shattock 2012; Stefanidou et al. 2012).

Out of the ten PIs currently approved for clinical oral use (Mehellou and De Clercq 2010), at least four (saquinavir (SQV), darunavir (DRV), lopinavir (LPV) and ritonavir (RTV)) are being considered in preclinical studies as potential candidate microbicides (Herrera and Shattock 2012). LPV and RTV have shown limited activity in preclinical assays (Evans et al. 2010) compared to SQV (Stefanidou et al. 2012) and DRV (Evans et al. 2010). SQV and DRV are being further assessed in preclinical in vitro models and in NHP studies.

2.5 Targeting Multiple Stages Within the Viral Replication Cycle

Multiple compounds designed to inhibit specific targets within HIV-1 replication cycle have been shown to be capable of inhibiting other steps involved in viral replication. Among them several NNRTIs have multiple inhibitory mechanisms. IQP-0528 belongs to a family of NNRTIs that have been shown to inhibit reverse transcription (Fig. 1, intervention point 6) and viral entry by an unknown mechanism targeting a conformational epitope formed after binding of Env to CD4 and

co-receptor, but prior to fusion of the viral and cellular membranes (Buckheit et al. 2008; Mahalingam et al. 2011). Dapivirine has been shown to inhibit post-integration stages by interfering with HIV-1 Gag-Pol polyproteins processing resulting in a decrease in viral production (Figueiredo et al. 2006) (Fig. 1, intervention point 8). It has also been suggested that PIs may inhibit decapsidation of the viral core and the formation of the pre-integration complex (Fig. 1, intervention points 5 and 7, respectively), providing a potential inhibitory mechanism early in the viral replication cycle (Nisole and Saib 2004; Tozser et al. 2003).

Another class of candidate microbicide targets multiple stages of viral replication because they inhibit a protein, the nucleocapsid protein (NCp7), with essential functions throughout the replication cycle. NCp7 is a small protein important for reverse transcription and integration, and is required for dimerization and packaging of the viral genome late in the viral replication cycle (Buckman et al. 2003) (Fig. 1, intervention points 6, 8, 10). NCp7 contains two zinc-binding domains, which form tight rigid loops and therefore, nucleocapsid inhibitors are also known as zinc finger inhibitors (ZFIs). *S*-acyl-2-mercaptobenzamide thioesters (SAMT) (Miller Jenkins et al. 2010) are the main family of ZFIs. Four SAMT (SAMT-8, SAMT-19, SAMT-89 and SAMT-247) have shown promising results in cellular and tissular preclinical studies (Herrera et al. 2012; Sexton et al. 2009; Wallace et al. 2009) and one of them has even shown activity against vaginal challenge when used topically in NHPs (Cheng-Mayer et al. 2011; Wallace et al. 2009).

A novel approach is to design chimeric compounds, for example, of an entry inhibitor and a fusion inhibitor, that show enhanced inhibitory activity (Zhao et al. 2011) (Fig. 1, intervention points 3 and 4).

Microbicides can also target multiple steps of the viral replication cycle by including combinations of ARVs. This strategy has been inspired by the development and application of HAART where multiple drug combinations are used in conventional treatment regimens (Jordan et al. 2002). The first combinations tested in preclinical studies as candidate microbicides were based on RTIs (Cost et al. 2012; Herrera et al. 2009, 2011). Currently combinations of drugs targeting different steps of viral replication, such as entry/fusion, reverse transcription and maturation, have progressed in the microbicide pipeline. These have been tested in NHPs studies (Veazey et al. 2005) and in the case of maraviroc and dapivirine have been formulated as gels (Herrera et al. 2012) and as vaginal rings, the latter being tested in a Phase I clinical trial (MTN-013/IPM 026).

3 Microbicides: When the Context is Crucial

3.1 A Short Window of Opportunity

It remains unclear whether sexual transmission is mediated by infectious HIV-1 (cell-free virus) and/or infected cells in semen or mucosal secretions (cell-associated virus). However, NHP studies with simian immunodeficiency virus

(SIV) indicate that transmission involves establishment of an initial focus of infection or "founder population" established by a single or a few viruses in the first hours after viral exposure (Li et al. 2009) (Fig. 2). Hence, in both the genital and colorectal tracts, the virus must cross the mucosae in order to establish a systemic infection and several mechanisms have been proposed (Shattock and Moore 2003). Viral amplification from the founder population is considered essential to establish irreversible acquisition of infection and takes place in the first few days (1–3 days) following infection of the initial founder population. This amplification is mediated through the onward infection of adjacent $CD4^+$T cells and is likely enhanced by interaction with Langerhans cells and dendritic cells (DC) (Hu et al. 2004; Keele and Estes 2011) and the probable influx of additional $CD4^+$T target cells in response to viral exposure (Haase 2011). Onward dissemination of virus to secondary lymphoid tissue occurs prior to the irrevocable seeding of the lymphoreticular system and associated viremia (Li et al. 2009). This period lasting about 10 days is known as the "eclipse phase", the period before HIV becomes detectable in circulation (McMichael et al. 2010). Hence, microbicides have a limited window of opportunity for blocking infection. In NHPs, a 30–60 min exposure to an infectious inoculum is sufficient to establish infection (Shattock and Moore 2003); therefore, entry/fusion inhibitors have a time frame of minutes/hours to act. As a consequence such compounds need to be maintained at inhibitory concentrations to cover the potential time of initial exposure. RTIs and INIs acting post viral entry but before integration could inhibit viral amplification within the founder population during the first hours/days post-exposure; while protease inhibitors active before release of infectious virus could block the local expansion phase taking place during the first days post-exposure, that is reliant on secondary infection of surrounding CD4 cells and occurs before viral dissemination to draining lymph nodes (Fig. 2).

3.2 Modulation of the Mucosal Environment

The efficacy of microbicides in the genital and colorectal tracts could be positively influenced by factors that may decrease the infectious dose before crossing mucosae, increase innate resistance mechanisms to infection and prevent the establishment, and/or dissemination of initial foci of infection. In contrast, the potential efficacy of microbicides could be offset by disturbance of factors that would favor mucosal infection by disturbing the normal flora, reduction of innate protective factors, or by damage or inflammation of the protective epithelium (Haase 2011). Indeed, CAPRISA 004 revealed that vaginal inflammation at baseline correlated with increased risk of infection in those using tenofovir gel (Roberts et al. 2012a, b).

Several approaches are being developed to enhance innate mucosal barrier functions, among them: anti-inflammatory agents (Fahey et al. 2011; Haase 2011); and the use of lactobacilli, commensal bacteria, to maintain a healthy mucosa and,

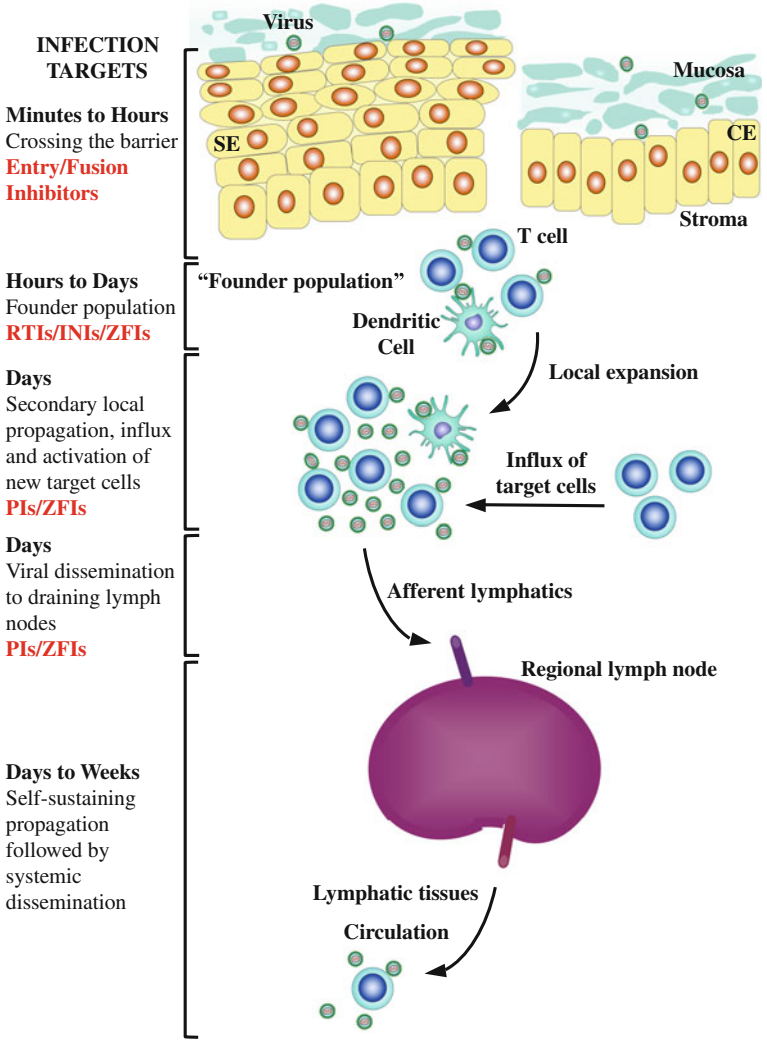

Fig. 2 Mucosal transmission of HIV-1 and timeline for inhibition. Virus present in the cervicovaginal and/or colorectal lumen crosses the epithelium and reaches the underlying stroma in the first hours post-exposure, window where entry and fusion inhibitors can block infection. After a few hours or days, a small "founder population" of infected cells is established in the stroma, where reverse transcription, integration, and maturation can be inhibited. If production of infectious virus by this focal population is not inhibited, the eclipse phase will be initiated and local expansion will occur through infection of new target cells migrating toward this initial locus. Protease inhibitors and zinc finger inhibitors could potentially inhibit this secondary expansion and dissemination of infection through transport of infected cells to draining regional lymph nodes. Infected cells and newly produced virus spread to the local draining lymph node prior to dissemination through out the lymphoreticular system and systemic circulation. The eclipse phase ends by initiation of the systemic phase of infection. Adapted from Shattock and Rosenberg (2012) and Haase (2011)

through genetic engineering, to produce antiviral peptides (Chang et al. 2003; Fahey et al. 2011; Lagenaur et al. 2011). Other approaches modulate the expression of innate intracellular HIV restriction factors such as APOBEC3G, TRIM5α, and tetherin (Eade et al. 2012; Ejima et al. 2011; Neagu et al. 2009; Vazquez et al. 2011); or naturally occurring host defense peptides (Fahey et al. 2011; Wang et al. 2010). Some of these peptides have been isolated in HIV-1 resistant cohorts (Burgener et al. 2008, 2011) and are currently being tested as candidate microbicides.

An interesting new strategy aims to transduce cervico-vaginal epithelial stem cells by adeno-associated virus (AAV) gene transfer of broadly NAb genes so that they secrete neutralizing antibodies over multiple menstrual cycles (Abdel-Motal et al. 2011).

An alternative or complementary approach to inhibit local viral expansion is to target the dissemination of virus to draining lymph nodes by migratory DCs, thought to lead to self-sustaining propagation. Binding to c-type lectins like DC-SIGN, can mediate the viral capture by DCs. Hence, DC-SIGN itself and/or other C-type lectins could be targeted to prevent lectin capture of HIV (Berzi et al. 2012). There is a lack of evidence to suggest they might be efficacious when used alone, however such approaches could supplement the activity of other candidate microbicides directly targeting different stages/receptors in the viral life cycle. Indeed, as proof of concept recent in vitro studies have shown that simultaneous targeting of both CD4 and DC-SIGN binding sites on gp120 by a novel bifunctional fusion protein represents a novel antiviral strategy (Du et al. 2012).

4 Pre-clinical Models to Assess Candidate Microbicides

Phase III clinical trials are expensive (estimated at $50–100 million), time consuming, and require a large number of participants to determine efficacy (Douville et al. 2006; Nuttall et al. 2007). Furthermore, with the introduction of prevention interventions, incidence of infection within communities will decrease, closing the window to perform placebo-controlled trials, and causing the trials to become even larger to test later-generation products. Hence, preclinical models are becoming increasingly important tools to facilitate and accelerate prioritization of candidate microbicides and their formulations for clinical testing.

Efficacy and safety testing of candidate microbicides currently utilizes a wide range of assays including: in vitro cellular models; ex vivo mucosal tissue explants; small animal models; and NHP studies. Multiple cell lines are routinely used to screen potential efficacy of compounds including the PM-1 $CD4^{+}$T cell line (Lusso et al. 1995), and the TZM-bl luciferase reporter cell line (Wei et al. 2002). Assays in PM-1 cells require at least 7 days of culture, allowing multiple rounds of viral replication, and infectivity is determined by measurement of p24 antigen content in culture supernatants with enzyme-linked immunosorbent assay (ELISA). In contrast to PM-1 cells, TZM-bl cells are a single viral cycle assay model that requires 2 days of culture before infectivity is assessed by measurement

of luciferase expression within the cell, driven by production of the viral transactivator of transcription (Tat). The extent of luciferase expression is recorded in relative light units. However, TZM-bl cells are HeLa cells expressing endogenous CXCR4 that have been stably transfected with CD4 and CCR5, and therefore, express artificially high levels of CD4 and CCR5 (Polonis et al. 2008). A recently developed cell line, Affinofile (Johnston et al. 2009), resolves this issue by expressing variable levels of CD4 and CCR5 or CXCR4 based on the amount of selection antibiotic used in culture. Primary cells such as lectin-activated peripheral blood mononuclear cells (PBMCs) as well as cells derived from PBMCs, including monocyte-derived macrophages and immature DCs, provide more physiologically relevant cellular models for assessment of anti-viral activity. These models involve longer experiments (7–14 days) allowing multiple viral replication cycles, and therefore tend to be used after initial assessment in immortalized cell lines such as those described above. Another important model is co-cultures of DCs with PM-1 cells. This model replicates the interaction between DCs and $CD4^{+}$T cells during the viral amplification of the "founder population" and subsequent viral dissemination to draining lymph nodes (Fig. 2); allowing measurement of drug efficacy against *trans* infection (Hu et al. 2004).

Safety of compounds is initially assessed in cellular models by assessing the level of potential cytotoxicity most often by measuring tetrazolium salt (MTT) cleavage into a blue-colored product (formazan) in viable cells (Slater et al. 1963) or by similar assays of cellular viability. Epithelial cell lines represent an important model to assess potential toxicity or disruption of epithelium integrity induced by the drug (Dezzutti et al. 2004). Available epithelial cell lines including urogenital epithelial cells (e.g., ME-180 (Sykes et al. 1970) and HEC-1-A (Kuramoto 1972)) and colorectal epithelial cells lines (such as Caco-2 (Fogh et al. 1977) and SW837 (Leibovitz et al. 1976)). Primary epithelial cells can also be isolated (Greenhead et al. 2000). Co-cultures of epithelial cells with primary cells, PBMCs (Dezzutti et al. 2004) or DC (Van Herrewege et al. 2007), in a dual-chamber model mimicking transepithelial migration of drugs and virus have been successfully used to assess safety and allow studies of HIV transmission and efficacy of candidate microbicides.

The next phase in microbicide evaluation often utilizes tissue models such as ex vivo culture of mucosal tissue explants (Grivel and Margolis 2009). Explants are obtained as biopsies or as surgically resected tissue, and several models have been developed for penile (Fischetti et al. 2009), cervical, vaginal, and colorectal tissues including polarized (Abner et al. 2005; Collins et al. 2000; Cummins et al. 2007) and non-polarized systems (Fletcher et al. 2006; Greenhead et al. 2000; Grivel et al. 2007; Hu et al. 2004). In cervical and penile tissue models, cells emigrating from the tissue have been described and these can be cultured separately from the explant and in the presence of $CD4^{+}$T cells, such as PM-1 cells, to assess anti-viral activity of a drug against dissemination by migratory cells (Fischetti et al. 2009; Hu et al. 2004). Despite the variety of models, it has been shown that consistent results can be obtained among different laboratories through protocol standardization (Richardson-Harman et al. 2009).

Animal models are more often used in the later stages of preclinical development due to cost and ethical considerations. Small animal models including rodents and rabbits are often used to assess preclinical safety of compounds (see Holt and Nuttall 2013, this volume), but novel strains of mice have been engineered by transplanting human fetal stem cells into specific immunodeficient strains of mice, making them susceptible to HIV-1 infection (including via vaginal and rectal transmission), and therefore can be used to test the efficacy of candidate microbicides (Veazey et al. 2012). However, NHPs remain the main animal model for studying transmission, immunology, and pathogenesis of HIV-1 infection as well as potential of candidate microbicides prior to clinical trials. The majority of NHPs used are macaques including the rhesus (*Macaca mulatta*), pigtail (*Macaca nemestrina*), and cynomologous (*Macaca fascicularis*) sub-species. The main limitation of the NHP model is that HIV-1 does not replicate efficiently in macaque cells (Stremlau et al. 2004). Hence, alternative challenge viruses need to be used such as SIV (including isolates derived from chimpanzees (SIVcpz) and macaques (SIVmac)), and SIV/HIV chimeras known as SHIVs. Most SHIVs include an HIV Env in an SIV backbone, but other HIV-1 proteins such as RT have been inserted in SIV (Veazey et al. 2012). Preclinical models have been criticized for the use of high viral challenge stocks that often exceed human exposure (Pilcher et al. 2004); however, these are routinely used to ensure all animals become infected, reproducing a single infectious event rather than recapitulating the multiple exposures that may be needed for an infectious event to occur in humans, which would be impractical to mimic fully in an animal model. Nevertheless, microbicides that prevent infection under such stringent conditions may well be more potent against lower infectious challenges in humans. However, these stringent conditions could at the same time, potentially exclude candidate microbicides that might ultimately be efficacious in humans. More recently, many investigators have adopted a repeated low dose challenge model where animals are exposed to an infectious doses that infects the majority of animals after four or more sequential challenges (0.5 animal infectious doses), thought to be more representative of a real life scenario (Veazey et al. 2012), but still potentially higher than that seen in mucosal infection of humans.

5 The Future Integration of Microbicides Within a Wider Prevention Profile

Other prevention strategies in development include the use of oral pre-exposure prophylaxis (PrEP), treatment for prevention, and vaccines (Kelesidis and Landovitz 2011; Kwong et al. 2012; Laga and Piot 2012). One scenario that is being explored is whether oral PrEP combined with microbicides might provide optimal protection delivering both systemic and local activity of ARVs (Dobard et al. 2012). Furthermore, a number of groups are considering the potential of

combining oral PrEP or microbicide use with candidate vaccines to provide a comprehensive prevention strategy (Shattock et al. 2011). Initial and ongoing studies support this idea (Barouch et al. 2012; Cheng-Mayer et al. 2011).

6 Conclusions

The use of ARVs for topical prevention provides a rich pipeline of highly potent drug candidates with prior positive clinical experience that have the potential to positively impact the epidemic. Compounds with varying mechanisms of action targeting different stages in the replication cycle of HIV are in development. However, the strengths and weakness of these different mechanisms and the most effective targets for prevention are not fully understood. Here, sustaining efforts to better define the early events in mucosal transmission and the impact of the mucosal environment on viral transmission are likely to be fundamental. Indeed, the relative merits of targeting early versus late stages of the viral life cycle may have important implications around product use. Ultimately, ARV combinations that act at different stages in the transmission process are likely to provide the greatest efficacy against HIV transmission and be more amenable to a wider range of delivery options. Nevertheless, it will not be possible to test all new compounds, combinations, and their formulations in large efficacy trials. Thus, preclinical assays remain critical to understanding the relative potential of compounds and for selecting the best candidates. However, all preclinical assays have their limitations and their value in predicting clinical efficacy has yet to be established. Hence, the process of product prioritization needs to be based on a range of criteria that include: in vitro drug potency, animal efficacy data, stage of product development, cost of goods, existing safety data, and ability to measure PK/PD parameters in clinical trials. The proof of concept for ARV use in topical prevention provided by the CAPRISA 004 trial of 1 % tenofovir gel, while encouraging, serves as an important bar to drive ongoing development of strategies and products with increasing efficacy and effectiveness.

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Microbicide Dosage Forms

L. C. Rohan, B. Devlin and H. Yang

Abstract Microbicides are topically applied, user controlled dosage forms that are being developed to prevent the transmission of HIV during coitus. Early candidates focused on coitally dependent dosage forms such as gels and creams. More recent development has focused on broadening the coitally dependent options through the introduction of films and fast dissolving tablets. Additionally, it has become important to have longer acting products to minimize the burden of user compliance and thus vaginal rings have been developed providing sustained delivery of antiretroviral drugs. This chapter discusses the history of microbicides along with a detailed description of coitally dependent products, gels, films, tablets diaphragms, as well as coitally independent dosage forms such as vaginal rings and the introduction of a new technology, electrospun fibers.

Contents

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1 Microbicide Products

Microbicides are products that can be applied vaginally or rectally to protect the user from acquiring HIV and, possibly, other STIs. Many variables can impact the in vivo effectiveness of any given microbicide product such as the anti-HIV activity (efficacy of the product), the user's willingness and ability to use the product as instructed (acceptability and adherence), the safety of the formulation, and HIV 'dose'-related variables (Morrow and Hendrix 2010). The appropriate drug-delivery strategy for each microbicide drug candidate will depend upon many variables such as the physicochemical characteristics and pharmacokinetic profile of the candidate, its mechanism of action against HIV-1 transmission, its dosing regimen, delivery route, and patient acceptability (Buckheit et al. 2010). Microbicide products can be classified as coitally dependent or non-coitally dependent. Coitally dependent microbicides are applied immediately before and/or after intercourse, whereas coitally independent products are applied and provide sustained protection over time.

During the past two decades, the majority of microbicide product development has focused on vaginally applied products. However, receptive anal intercourse (RAI) is practiced by both MSM and women around the world (Kalichman et al. 2009; Chandra et al. 2011). In fact, a US national survey found that 36 % of female responders aged 25–44 had ever had anal sex with an opposite-sex partner (Chandra et al. 2011). Development of a microbicide that is effective and safe in both mucosal compartments (vaginal and rectal) should be considered (Minces and McGowan 2010). Rectal microbicide research is currently in the early phase of clinical development. Aside from a number of studies designed to investigate the behavior of placebo products in the rectum, a few studies have evaluated rectally applied microbicide products contacting antiretrovirals. These studies are focused on the drug safety and acceptability evaluation for rectal use, such as of tenofovir gel (McGowan et al. 2013) and UC781 (Ventuneac et al. 2010).

1.1 History of Clinically Evaluated Microbicides

1.1.1 Early Generation Microbicides: Nonspecific or Moderately Specific

Initial products developed as vaginal microbicides contained agents which did not directly affect the life cycle of the HIV virus. These nonspecific antiretroviral microbicides were surfactants/detergents (Weber et al. 2005). Three surfactants were tested clinically as microbicides. These were products containing nonoxynol 9 (N-9) (gel, sponge and film), SAVVY® or C31G gel, and sodium lauryl sulfate (SLS) gel.

Acidifying agents help to restore or maintain the protective acidic pH of the vagina. Acidifying agents clinically assessed for safety and effectiveness against male-to-female HIV-1 transmission include BufferGel and Acidform. Both BufferGel and Acidform are acid-buffering bioadhesive vaginal gels. BufferGel was found to be well tolerated, but not effective for the prevention of HIV-1 vaginal transmission in a Phase II/IIb trial (Abdool Karim et al. 2011). Acidform vaginal gel was found to have favorable formulation properties (Garg et al. 2001). However, no clinical effectiveness trials pertaining to the prevention of male-to-female vaginal transmission of HIV-1 have been reported for Acidform.

The next microbicide candidates evaluated were primarily macromolecular linear anionic polymers (Balzarini and Van Damme 2007). Their anti-HIV mechanism of action centered on their ability to block viral entry into cells (Weber et al. 2005). These agents carry negative charges, which result in a charge interaction with viral envelope proteins blocking the binding of the virus to target cells. Such microbicide candidates include Carraguard® gel, cellulose sulfate gel, cellulose acetate phthalate gel, VivaGel®, and PRO2000 gel. In a Phase III study of Carraguard it was found to be safe but ineffective against vaginal transmission of HIV (Skoler-Karpoff et al. 2008). Cellulose sulfate was also found to be ineffective, while the PRO2000 showed an increased risk of HIV infection (Tao et al. 2008).

This early work in microbicide product development provided a number of lessons: nonspecific agents, e.g., surfactants, are unlikely to provide protection against HIV transmission and may increase HIV infection risk; moderately specific agents, e.g., some polyanions have little effect but are safe; and product adherence is crucial for protection. These lessons led to an emphasis in the field for evaluation of specific or antiretroviral containing microbicide products.

1.1.2 Specific Microbicide Products

Due to the clinical failure of early microbicide candidates development efforts in the field have now shifted to antiretroviral microbicide candidates that directly and specifically act against the HIV life cycle, namely specific antiretroviral products (ARVs). The two classes of ARVs being most widely evaluated are entry inhibitors

and reverse transcriptase (RT) inhibitors (Balzarini and Van Damme 2007). Evaluation of other classes such as HIV integrase inhibitors is also beginning to emerge (Reeves and Piefer 2005).

The majority of antiretrovirals under current clinical investigation are reverse transcriptase inhibitors (RTIs). Tenofovir is the most widely studied RTI microbicide candidate to date. Several Phase I studies demonstrated the safety and pharmacokinetics of tenofovir (Mayer et al. 2006; Beigi et al. 2011; Keller et al. 2011). A Phase IIb trial (CAPRISA 004) to assess the safety and effectiveness of tenofovir 1 % gel in the prevention of male-to-female HIV transmission was performed in South Africa (Abdool Karim et al. 2010). The results of this trial showed that tenofovir gel use was associated with an overall 39 % decrease in HIV-1 acquisition, which is statistically significant. Additionally, among women with high gel adherence, the tenofovir gel reduced HIV infection by 54 % in comparison to placebo gel (Abdool Karim et al. 2010). The results of CAPRISA 004 have paved the way for future vaginal microbicide trials by providing proof-of-concept for antiretrovirals as microbicides for HIV prevention. The Vaginal and Oral Interventions to Control the Epidemic (VOICE) trial is a Phase IIb five-group study examining the safety and efficacy of daily oral tenofovir disoproxil fumarate, oral Truvada® (a fixed dose combination of tenofovir disoproxil fumarate and emtricitabine), oral placebo, tenofovir 1 % vaginal gel and placebo vaginal gel in HIV-negative women in Malawi, South Africa, Uganda and Zimbabwe (also called MTN 003). This daily-use regimen of a vaginal microbicide (topical PrEP) and oral PrEP differs from the coitally dependent regimen utilized in CAPRISA 004 and earlier microbicide efficacy trials. In September 2011, it was recommended that the VOICE participants randomized to oral tenofovir be discontinued from this group due to futility. In November 2011, VOICE discontinued use of tenofovir gel as it concluded from an interim evaluation that the gel was "not effective in preventing HIV in the women enrolled in the VOICE trial."

Tenofovir is not the only antiretroviral that is being clinically tested. Formulations of UC781 and dapivirine, both non-nucleoside RTI (NNRTIs), have been clinically evaluated. Early-phase trials of these NNRTI formulations looked to assess short-term safety, tolerability, acceptability, and PK of these products (Neurath et al. 1999; Jespers et al. 2007; Schwartz et al. 2008b; Nel et al. 2009a, 2010a, b). To date, early-phase clinical trials have found RTIs to be generally safe and well tolerated. Additionally, a Phase I safety and PK trial of a dapivirine/maraviroc (NNRTI/CCR5 co-receptor antagonist) IVR was conducted (MTN-013/IPM 026). Studies involving the delivery of specific antiretroviral agents in vaginal polymeric quick-dissolving films and tablets are also planned or ongoing.

Although the potent antiviral activity of a microbicide candidate is essential, the formulation or delivery system plays a key role in developing these candidates into practical dosage forms with safety, acceptability, and effectiveness in clinical application. Different formulations are being evaluated for vaginal delivery of microbicide candidates including gels, rings, films, tablets, and suppositories. In this chapter microbicide dosage forms (gel, film, vaginal ring and tablet) will be discussed in detail.

1.2 Vaginal Drug Delivery

Vaginal drug delivery systems can be designed to achieve topical or systemic effect. The earliest record of vaginal product use was in Egypt in 1850 BC (O'Dowd 2001). Numerous records also show the application of vaginal formulations in Greece and Rome from the Middle Ages, and later Renaissance, through modern day. Modern vaginal administration of drug was fully recognized in 1918 after Macht reported drug absorption in vagina (Macht 1918). Since then, many chemicals or therapeutics have been administered vaginally, especially steroids, prostaglandins, and antimicrobials.

The major advantages of the vaginal route of administration for systemic drug delivery are the avoidance of hepatic first-pass metabolism (thus increasing drug exposure), and reducing gastrointestinal side effects, and decreasing hepatic side effects such as those seen with steroid application (Vermani and Garg 2000). The rate and extent of drug absorption and blood drug concentration after intravaginal administration are dependent on formulation factors, vaginal physiology, age, and menstrual cycle. Most drugs in vaginal formulations are indicated for the treatment of topical conditions such as vaginitis and other vaginal infections, and include antimicrobials (Bradshaw et al. 2012), labor inducers (Seeras 1995; Seeras et al. 1995), spermicides (Iyer and Poddar 2008), and sexual hormones for postmenopausal symptoms (Rad et al. 2006). Vaginal preparations are traditionally used for local treatment, however, systemic effects via the vaginal route can also be achieved (Alexander et al. 2004; Song et al. 2004). Vaginal drug delivery systems include a large variety of dosage forms such as foams, creams, gels, ointments, pessaries, tablets, capsules, films, tampons, vaginal rings, and douches. Generally, these dosage forms can be classified into solid, semisolid, and liquid formulations. For microbicide products these dosage forms can be further categorized with regard to their coital dependence for use as shown in Fig. 1.

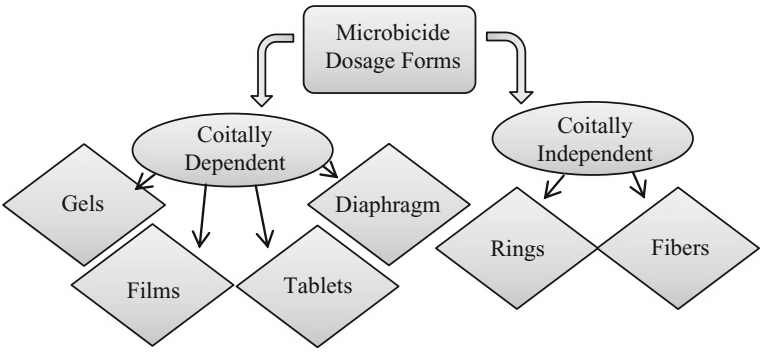

Fig. 1 Types of microbicide dosage forms

2 Coitally Dependent Dosage Forms

2.1 Gels

2.1.1 What are Gels?

Gels are defined as semisolids in which suspensions of (small) inorganic particles or (large) organic particles are interpenetrated by a liquid (aqueous or non-aqueous). The majority of vaginal products marketed fall into this category. Familiarity of users with gel dosage forms for vaginal drug delivery as well as their ability to provide lubrication during sexual intercourse is an advantage of this dosage form. Gels present a number of additional features which contribute to their wide use as vaginal preparations such as consistency, good spreadability, bioadhesion, acceptability, feasibility, and generally low manufacturing cost. The main disadvantages associated with vaginal gel products are messiness and leakage (Hussain and Ahsan 2005). The preponderance of microbicide gel products evaluated to date are hydrogels, or aqueous gels. The application of hydrogels for biomedical use dates back to 1960 when Wichterle and Lim developed a cross-linked poly (hydroxyethyl methacrylate) (pHEMA) (Wichterle and Lim 1960). Since that time the use of hydrogels in the biomedical and pharmaceutical field has been widespread.

2.1.2 Microbicide Gel Product Components

Vaginal gels being developed as microbicides most commonly employ water soluble polymers as gelling agents. Generally, gels are manufactured by the addition of polymers and other excipients to water with continuous mixing. Cellulose derivatives like hydroxyl ethyl cellulose (HEC) and poly acrylic acid derivatives like Carbopol 974P are the most commonly used polymers due to their generally recognized as safe (GRAS) status, cost effectiveness, and safety profile. The universal placebo gel used as a placebo control in a large number of microbicide clinical trials consists of 2.7 % w/w HEC as the gelling agent. Another common practice in the formulation of gel products is to include preservatives to avoid bacterial growth within the product upon storage. A combination of methyl paraben and propyl paraben is one of the most widely utilized preservative systems in microbicide products currently being evaluated. Many vaginal gels also contain cosolvents, humectants (such as glycerin, polyethylene glycol, and propylene glycol), buffering agents, and acidifying agents to improve aesthetic aspects of the product and to also aid in solution of the drug substance and stability. In addition to traditional gels, nonaqueous/lipid gels (Politz et al. 1994; Forbes et al. 2011), thermoreversible gels (Roy et al. 2001; Escobar-Chavez et al. 2006), and bioresponsive gels (Gupta et al. 2002; Chen et al. 2012) are being developed. A silicone

hydrogel preparation was developed as a microbicide product for the delivery of the CCR5 antagonist, maraviroc. This product resulted in a high concentration of maraviroc in plasma, vaginal fluids, and vaginal tissue in rhesus macaques (Forbes et al. 2011).

2.1.3 Historical Overview of Microbicide Gel Products

Gels have been historically the most common drug delivery formulation for microbicides. In clinical studies conducted to date, gel products have been applied as a single daily dose or as a single dose before and/or after sexual intercourse. Early clinical trials for nonspecific microbicide drug candidates utilized the gel formulation platform. The first gel tested as a microbicide was the marketed contraceptive product which contained N-9. Other gel products evaluated were SAVVY® (C31G gel), a sodium lauryl sulfate gel, cellulose sulfate, Carraguard®, acidifying agents (BufferGel and Acidform), VivaGel, and PRO2000. Although many of the gels were found to be safe, to date no nonspecific microbicide drug containing gel clinically tested has been found to be effective.

Gel dosage forms containing specific microbicide drug candidates have also been evaluated. A number of these candidates tested are viral replication inhibitors. A tenofovir gel product was found to be both safe and effective, resulting in an overall statistically significant 39 % decrease in HIV-1 acquisition in the CAPRISA 004 Phase IIb clinical trial. However when this gel was tested in the VOICE Phase IIb clinical trial utilizing a varied dosing regimen the gel was found not to be effective in preventing HIV. A third trial which utilizes the same dosing regimen as the CAPRISA trial is ongoing. Other non-nucleoside reverse transcriptase inhibitor microbicide drug products which have been studied include UC781 and dapivirine.

Evaluation of Microbicide Gels

There are several critical product assessment tools which are utilized to evaluate potential microbicide gel products. Of central importance are the evaluations of gel safety, anti-HIV activity, and physical and chemical characterization including stability assessments. It is known that gel characteristics can change over time and upon exposure to certain environmental conditions. These product changes may result in variation of gel product in vivo performance (Gallagher et al. 2003). Critical characteristics of gels might include API content and uniformity, pH, osmolarity, and viscosity.

Safety is essential for a microbicide gel product. The failure of clinical trials of nonoxynol-9 (N-9) and cellulose sulfate gels highlights the importance of safety considerations in microbicide gel product development. Results from clinical trials indicate that minor perturbation of the vaginal membrane could significantly increase the susceptibility of women towards HIV infection. The excipients, pH,

and the osmolality of a vaginal microbicide gel could have profound effects on the vaginal environment and epithelium which could lead to toxicity or enhanced infection (Turpin 2011). Irritation caused by gels could also compromise patient adherence to the products. In addition to consideration of the effect of a microbicide gel product on the vaginal epithelium, it is also important to consider its effect on the innate microflora. The acidic pH environment of the vagina is maintained by the microflora, specifically *Lactobacillus* species, and increased vaginal pH can result in enhanced susceptibility to HIV infection. Thus, a safe microbicide should not have any toxic effects on innate vaginal lactobacilli (Martin et al. 1999). Additionally, it has been shown that hyperosmolality of products is associated with mucosal irritation and tissue damage in the slug mucosal irritation model (Adriaens and Remon 2008). In a phase I trial unacceptable vulvo-vaginal side effects were observed with use of a hyperosmolar vaginal microbicide product (Lacey et al. 2010). Furthermore, some commonly used gel excipients such as glycerin and disodium EDTA were shown to increase the susceptibility of mice to HSV-2 infection in a dose-dependent manner. In the same mouse model, propylene glycol and polyethylene glycol-8 also increased HSV-1 susceptibly by >10 times (Moench et al. 2010). These reports indicate that the selection of excipients and their concentration in microbicide gels can have a profound effect on the safety of a microbicide product. Formulation excipients or excipient combinations may lead to not only irritation and toxicity but also enhanced infection.

Several models have been utilized to evaluate bioactivity of microbicide drug candidates as described by Shattock and Herrera (2013). Most cell-based models for activity are limited in their usefulness due to the requirement of significant dilution of the gel product because of its high viscosity. The most widely used model is an ex vivo challenge model in which vaginal, cervical, or colorectal tissue is exposed to the gel product and subsequently challenged with HIV virus.

Evaluation of gel product drug release is an important product performance and quality control assessment. A simple, reliable, and reproducible release rate method can guide formulation development and monitor manufacturing reproducibility and product stability. The FDA published SUPAC-SS guidance details release testing for active pharmaceutical ingredients from semisolid dosage forms after certain post approval changes (FDA 1997). Several in vitro models have been applied to evaluate drug release from gels such as the Franz cell diffusion system, the enhancer cell, and flow through cell apparatuses. Generally, drug release from semisolid preparation from Franz cell and Enhancer cell are the same once the data are standardized by surface area (Kumar 1993; Fares and Zatz 1995). The Franz cell diffusion system is one of the systems recommended by the FDA for evaluating drug release from topical gels for vaginal use (Shah et al. 1989; Siewert et al. 2003). The Franz cell system was utilized to compare two formulations (3.0 % hydroxyethyl cellulose (HEC) formulation and a 0.65 % Carbopol) for IQP-0528, a NNRTI being evaluated for use as a microbicide product. Diffusion-controlled release of IQP-0528 was observed for both gel formulations (Mahalingam et al. 2011).

Rheological testing is normally conducted for microbicide semisolid products because these properties may be critical to the success of the product. Specifically, the viscosity of the product can be monitored to assess product stability (Tamburic and Craig 1996) and can also impact product performance in vivo. Once a microbicide gel is applied to the vagina, these preparations will be diluted by vaginal fluid and during coitus will be further diluted with semen and exposed to shear forces through coital activity. Thus, the viscosity changes under these conditions should be evaluated. Owen et al. evaluated the rheological properties of several gel formulations using vaginal or cervical fluid simulant (Owen et al. 2003). The dilution of gels with vaginal or cervical fluid simulant may reduce the anti-HIV activity performance. Sassi et al. reported that hydrogel products of UC781 maintained greater activity against HIV-1 as compared to liposomal products after dilution with vaginal/cervical fluid simulant (Sassi et al. 2008).

Currently, most hydrogel microbicide gels are coitally dependent products, which could provide anti-HIV activity by forming a physical barrier after application. If a physical barrier to virus entry is a desired characteristic of a microbicide, then it is important that complete coating of the vaginal mucosa surface is achieved to obtain maximum barrier function. In one study it was predicted that a gel layer thickness of 150 μm was needed to reduce HIV transport in an in vitro model (Lai et al. 2010). Mahalingam et al. presented a preclinical algorithm for use in design of gel products with specific mechanical properties using biomechanical modeling of gel spreading. Their model presents relationships between gel composition and spreadability (Mahalingam et al. 2010). Furthermore, coital activity as well as dilution with fluids can impact the vaginal coverage achieved by a particular semisolid formulation.

Gel Applicators

Microbicide gels are semisolid formulations, generally with high viscosity, and an applicator is required to vaginally apply semisolid products. Several types of applicators have been utilized for microbicide administration. Applicators can be refillable or disposable. Most applicators are made of high density polyethylene/polypropylene (HDPE) or low density polyethylene/polypropylene (LDPE). Two important considerations for vaginal applicators are compatibility with the microbicide product and the potential for vaginal trauma, epithelial damage, and irritation from applicator use. Such damage could lower patient compliance or result in reduced natural barrier function, both of which could potentially lead to microbicide product failure. Therefore, it is essential that applicators are adequately evaluated to determine their suitability for use with microbicides.

2.2 Films

2.2.1 The History and Background of Polymeric Thin Films

Thin polymeric films are solid dosage forms which consist of an active agent incorporated into a polymeric matrix. When applied to a mucosal surface the film disintegrates and releases the active drug agent. Oral thin films were first introduced in the 1970s. In 2001, Pfizer introduced the Listerine® Strip to freshen breath and by 2006, nine oral thin films were marketed worldwide. The initial thin films that were marketed were for consumer or cosmetic products. In 2010, the first prescription oral thin film drug product (Zuplenz) was introduced in the United States. Over the years this dosage form has become widely used.

The first vaginal film introduced was the contraceptive vaginal film. In 1973, an article published in the journal Contraception evaluated C-Film (Lichtman et al. 1973). C-Film was a water soluble film measuring 2″ × 2″ inch which contained the nonionic spermicide nonylphenoxypolyethoxyethanol (Nonyl-9) and was designed to be placed in the vagina 30 min prior to sexual intercourse for protection from unwanted pregnancy. Vaginal film formulations of nonoxynol-9 (N-9) (Mauck et al. 1997a, b, c; Roddy et al. 1998) and some feminine hygiene products are also commercially available. Apothecus Pharmaceutical Corporation in New York, USA currently markets three vaginal film products, the Vaginal Contraceptive Film (VCF), VCF Lubricating Film, and Vaginal Scented Film.

In addition to vaginal films being used for the delivery of the contraceptive agent N-9 (Mauck et al. 1997a, b, c; Roddy et al. 1998), polystyrene sulfonate (PSS), an antimicrobial contraceptive agent, was formulated in a vaginal film (Garg et al. 2005). The PSS films were colorless, transparent, thin, soft, and tough, and found to rapidly dissolve in physiologic fluid in in vitro studies. Several other pharmacologically active agents have been developed as vaginal films. Metronidazole (Kawarkhe and Poddar 2010) and clindamycin phosphate (Dobaria and Mashru 2010) were formulated into vaginal film dosage forms as treatment for bacterial vaginosis (BV). The antifungal drugs clotrimazole (Sudeendra et al. 2010) and itraconazole (ITR) (Dobaria et al. 2009) were both formulated as vaginal films for treatment of vaginal candidiasis. A film formulation was studied which contains s-nitrosoglutathione, an endogenous NO donor, for use in female sexual arousal disorder (FSAD) (Yoo et al. 2009).

A number of drug candidates being evaluated for their use in HIV prevention have also been formulated as film dosage forms, including several with nonspecific action against HIV such as sodium dodecyl sulfate (SDS) (Yoo et al. 2006) and cellulose acetate phthalate (Neurath et al. 2001, 2003) cellulose acetate 1,2 benzenedicarboxylate (Trifonova et al. 2006) and the nucleoside reverse transcriptase inhibitor zidovudine (AZT) (Chatterjee et al. 2010), the non-nucleoside reverse transcriptase inhibitors UC781 (Yang et al. 2008), dapivirine (Akil et al. 2011), and the pyrimidinedione IQP-0528 (Ham et al. 2012) and the gp120 inhibitor RC-101 (Cole et al. 2010; Sassi et al. 2011). A number of other anti-HIV agents

are being formulated as vaginal films including small molecule drugs, proteins, peptides, monoclonal antibodies, and probiotics.

2.2.2 Vaginal Film Features

Vaginal films can be used to deliver drug candidates with a broad range of chemical attributes. Both hydrophilic and hydrophobic drug candidates can be successfully formulated into film dosage forms (Table 1). Furthermore, highly susceptible entities such as biomolecules and bacteria can be incorporated. One of the limiting factors for this dosage form is the amount of active agent which can be put into the film. Generally, the active agent does not constitute greater than 50 % of the dry weight of the film product (Vondrak and Barnhart 2008; Hariharan and Bogue 2009). Extremely low concentrations of active agents are also difficult to manufacture in this dosage form due to the difficulty in achieving content uniformity. Thin polymeric films designed for microbicide vaginal use commonly are $1'' \times 2''$ or $2'' \times 2''$ in size (Table 1).

Vaginal films can deliver drug agents to the vagina in order to achieve either topical or systemic effects. This dosage form is dependent upon the fluids in the vaginal vault for disintegration and subsequent dissolution and distribution of the drug. In order to achieve successful delivery, a number of commonly used excipients are incorporated in addition to the active agent. The primary component of a vaginal film is generally a water soluble film forming polymer. A number of polymers have been utilized in this capacity such as cellulose based polymers, poly vinyl alcohol, and Pullulan to name a few. Many film products incorporate combinations of polymers to achieve acceptable attributes. In addition to the base polymers, vaginal thin films use plasticizers to enhance flexibility and reduce brittleness. Commonly used plasticizers include glycerol, propylene glycol, phthalate derivatives, and polyethylene glycol. Other excipients that may be incorporated into the film dosage form are disintegration agents, buffers, stabilizers, fragrance, and coloring agents.

Table 1 Size and dosing level for published microbicide films

Film size (inch)	API	Percentage of w/w (dry film)	Reference
$\sim 1 \times 1.5$	IQP-0528 (pyrimidinedione)	0.05–0.1	Ham et al. (2012)
2×1	Dapivirine	0.52–1.36	Akil et al. (2011)
2×1	EFdA (4′-ethynyl-2-fluoro-2′-deoxyadenosine)	0.8–1	Zhang et al. (2012)
2×2	Tenofovir	10	Agashe et al. (2012)
1×1.3	RC-101 (retrocyclin analog)	0.04–0.8	Sassi et al. (2011)
2×2	Nonoxynol-9 (N-9)	28	Roddy et al. (1998)
1×1	Polystyrenesulfonate (PSS)	57	Garg et al. (2005)
N/A	Cellulose acetate phthalate (CAP)	40	Neurath et al. (2001)

2.2.3 Manufacturing Processes

Thin film dosage forms are manufactured using two methods. The major method used is aqueous solvent casting. This method involves casting a viscous polymer solution onto a substrate. The polymer solution is dried to a solid film and the film is removed from the substrate, cut, and packaged. Hot melt extrusion can also be used to manufacture thin films. This process has the advantage that the polymer, drug, and excipients can be processed without requiring solvent use. This results in a rapid manufacturing process which is compatible with drugs that are susceptible to hydrolytic degradation. The disadvantage of this manufacturing process is the requirement for high temperature and high shear.

2.2.4 Evaluation of Vaginal Films

Vaginal films should undergo a range of chemical, physical, and mechanical testing in order to adequately characterize them for pharmaceutical use. Chemical assessments of thin films generally include swelling index, bioadhesion properties, moisture content, disintegration time, dissolution and drug release, and drug content uniformity. Physical tests generally include film weight, size, appearance, and thickness. Mechanical testing may include tensile strength, puncture strength, elongation at break (film deformation), Young's modulus (stiffness), tear resistance, fold endurance, and bioadhesive strength. In addition to standard testing for films several additional in vitro assessments are required for vaginal microbicide products. Drug release and disintegration are critical evaluations for prediction of in vivo efficacy for film products. It is important to determine these parameters when considering biorelevant conditions such as decreased pH and low fluid volume. It is also essential that these products are nontoxic and retain bioactivity. Preliminary toxicity assessments can be conducted in either a cell based or ex vivo tissue model, but more extensive assessments are necessary to support clinical trials (see chapter by Holt and Nuttall 2013). Compatibility with the innate microflora should also be established.

Acceptability

Drug product efficacy can be directly correlated to user compliance and acceptability. The film formulation of N-9 has been evaluated for acceptability in a broad range of international and domestic studies (McGowan et al. 2013). Women who utilized the film noted a lack of lubrication provided by the film but were less likely to report "messiness" as compared to that experienced with gel products (McGowan et al. 2013). Another study evaluating foaming tablets versus N-9 film for contraception over 28 weeks found that the film compared favorably in terms of acceptability to foam tablets. Several recent studies have been conducted to assess film acceptability. The first was the Product Acceptability Study (PAS II)

conducted by International Partnership for Microbicides which compared a tablet, film, and softgel placebo product (Nel et al. 2011). The study was a marketing study in that the primary objective of the study was to assess consumer opinions of, and preferences between, the test products in order to gain insight for continuing product development. The outcome of the study showed that films would be an acceptable microbicide product dosage platform. The second study involved a focus group evaluation conducted at the University of Pittsburgh (Fan et al. 2011). In this study, questionnaires and focus groups were used to explore women's preferences for vaginal film physical characteristics. Results from this study indicated that women most frequently preferred vaginal films to be $2'' \times 2''$ square size, smooth, thin, and translucent. Driving these preferences were six major themes: ease and accuracy of use, desire for efficacy, discretion, film disintegration, intravaginal comfort and minimal impact on intravaginal homeostasis, and freedom to continue sexual activities unimpeded.

2.3 Tablets

Although tablets are primarily used for the treatment of HIV/AIDS in oral form, vaginal tablets are a dosage form of choice for some microbicide developers that encounter issues with drug delivery from rings or stability concerns with gels. A tablet is a dosage form comprising active pharmaceutical ingredient(s) and excipients, usually in powder form, that are blended and compressed into solid formed shapes. For vaginal delivery the typical shapes are double convex (Fig. 2) or ovule (Fig. 3c) to allow for easy insertion. The excipients in a vaginal tablet can include diluents, binders or granulating agents, disintegrants, glidants, and lubricants. Diluents act as a dispersant to the API to allow uniform distribution of drug throughout the granulation. The binder ensures that the adhesion of the API and other excipients is adequate to enhance compression. Disintegrants ensure the tablet will break apart once it reaches the vaginal fluid. A glidant acts as a flow aid for the granulation which allows the powder to flow freely from the bulk blend into the cavities of the tableting equipment during manufacturing. Finally, the lubricant

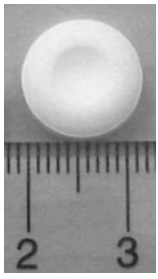

Fig. 2 Image of CONRAD bi-convex tenofovir fast dissolving tablet

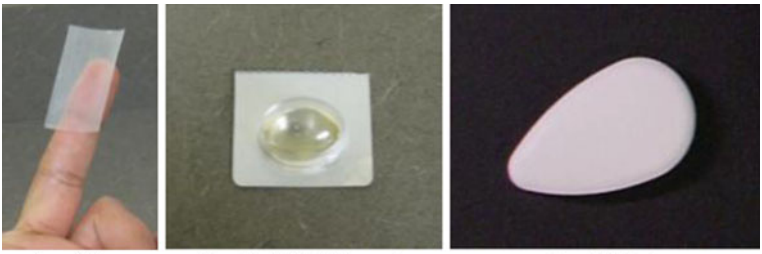

Fig. 3 Comparison of dosage forms assessed in PAS II study

coats the granulation which prevents the tablets from sticking to the tablet tooling during the compression operation. Oftentimes, a polymer coating is applied to the tablets at the end of the manufacturing process to make the tablets visually more attractive, but also to make the tablet smoother and easier to insert into the vaginal tract. The polymer coating can also control the release of the API from the tablet core and extend the tablet shelf life by protecting it from the environment.

The drug delivery from vaginal formulations can be aimed at three different areas, i.e., surface, within mucus layers, and systemic. Microbicide vaginal tablets are developed with the intention of surface and mucus layer penetration with minimal or no systemic absorption (Garg et al. 2010). As such, in vitro performance of vaginal tablets often focuses on mucoadhesive properties of the formulations and the methodologies designed to examine these effects (Baloglu et al. 2011).

The International Partnership for Microbicides (IPM) developed a combination tablet containing the NNRTI dapivirine (TMC120) and a gp120 inhibitor DS003 (Gupta et al. 2011). This formulation utilized standard solid dosage form excipients (Table 2) and direct blend methodology, followed by standard tableting techniques to produce an ovule-shaped tablet. A placebo version of this tablet

Table 2 Composition of dapivirine and DS003 vaginal tablet

Ingredients	Functionality	Percentage of composition (w/w)
Dapivirine	API	0.125
DS003	API	0.25
Polyethylene oxide	Swelling agent	7.5
Crospovidone	Disintegrant	15
Mannitol	Filler/flow aid	10
Colloidal silicon dioxide	Lubricant	2
Sodium bicarbonate	Effervescent	3.5
Tartaric acid	Acidifier	3.3
Microcrystalline cellulose	Diluent	10
Compressible lactose	Diluent	47.325
Magnesium stearate	Lubricant	1

(Fig. 3c) was then placed into a Product Acceptability Study (Nel et al. 2011) (PAS II) where it was compared against a placebo film (Fig. 3a) and softgel formulation (Fig. 3b). The PAS II was designed to be conducted in two markets within each of four countries: Burkina Faso (Bobo-Dioulasso and Ouagadougou), Zambia (Lusaka and Chipata), Mozambique (Maputo and Beira), and Tanzania (Dar es Salaam and Morogoro). The countries and locales were selected on the basis of four factors: (1) Feasibility of conducting the study in the country (i.e., approval by an ethics review committee, logistics, etc.); (2) Areas where there is a significant need for microbicide products; (3) The extent to which countries represent a diversity of cultural norms regarding the use of vaginal products and lubricants; and (4) Whether IPM was conducting, or planned to conduct, microbicide clinical trials and/or HIV incidence studies in the country. In each country, the researchers interviewed women from urban center and rural or peri-urban settings. The outcome of the study showed that the film and softgel capsule had the most potential. Targeted end-users' reactions to the tablet were comparatively negative enough to conclude that the particular formulation utilized in the study was not as good a candidate as the other options presented. Conversely, Panacea Biotec (New Delhi, India) developed a microbicide Praneem polyherbal vaginal tablet (Joshi et al. 2005) and 95 % of the women reported that the product was easy to use and did not affect sexual pleasure (Joglekar et al. 2006). This disparity in acceptability results could demonstrate that tablet composition plays an important role in user preference.

In addition to direct blend methodology for making tablets such as the dapivirine/DS003 tablet, product developers can employ a technique called dry granulation. Using this approach, APIs are blended with excipients, compressed into large pellets called slugs, and ground down to uniform particles before being blended with lubricants and compressed into tablets. For other API blends that lack flow, cohesion, or lubricating properties (Gohel and Jogani 2005), a process called wet granulation is utilized. With this approach a fluid binding solution is added to the blended API and excipients, wet mass sifted, dried, and ground to produce uniform particles. These granules are blended with a lubricant and compressed on tableting equipment. An alternate method of tablet manufacture is the production of a fast dissolving dosage form whereby drugs are trapped or dissolved within the matrix of the fast dissolving carrier material (Virley and Yarwood 1990; Seager 1998). CONRAD (Arlington, VA) is currently developing fast dissolving tablets containing tenofovir alone (Clark et al. 2011, 2012) and a combination tablet containing tenofovir and emtricitabine. These bi-convex tablets are derived from a fast dissolve oral technology developed by Aptalis Pharmaceutical Technologies (Seattle King County Department of Public Health 2000). These tablets are prepared on standard tableting equipment and comprise excipients commonly used in other vaginal products.

Tablets are the most commonly used dosage form (Sam and Fokkens 1997) outside the microbicide field due to their inexpensive manufacturing costs, ease of dosing, potential for high drug loading, and stability compared to aqueous-based dosage forms like liquids and gels. Although there has not been significant

development effort on tablets in the microbicide field until recently, they do offer the potential for an inexpensive, discreet, portable product that has no environmental waste. Among the many factors to consider, developers need to formulate to ensure the tablet adequately disintegrates upon use to avoid unwanted particulate discharge.

3 Coitally Independent Dosage Forms

3.1 Vaginal Rings

Vaginal rings (VRs) are torus-shaped polymeric drug delivery devices designed to release drugs in the vagina in a controlled fashion. The size of VRs generally ranges from 54 to 58 mm in diameter with cross-sectional diameters from 4.0 to 8.4 mm (Johansson 2000; Roumen et al. 2001). VRs do not require a healthcare professional for insertion and can be managed by the user for easy insertion and removal when needed. The concept of a vaginal ring for sustained drug administration was first described in a 1970 patent application (Duncan 1970), and since then a wide range of drugs and ring designs have been evaluated. Estring® was the first ring product to reach market in 1992, a silicone elastomer reservoir device providing constant release of 17-β-estradiol daily over 3 months for the local treatment of menopausal urogenital atrophy. Two other rings have also reached market; Organon's NuvaRing®, delivering the contraceptive steroids etonogestrel and ethinyl estradiol over 21 days, and Warner Chilcott's Femring® (Menoring® in the UK), releasing an estradiol prodrug, estradiol-3-acetate, continuously over 3 months. A number of VRs are also currently in development in the microbicide field, as single agents or in combination with other microbicides or hormonal contraceptives. Intended to be worn continuously, such coitally independent microbicide rings are being developed to maintain effective vaginal microbicide concentrations over many weeks or months, thereby overcoming issues around timing of product application, user compliance, and acceptability associated with more conventional semisolid formulations (Malcolm et al. 2010). IPM is in Phase III clinical trials with a dapivirine loaded VR. They are also developing dapivirine in combination with maraviroc, and dapivirine with a hormonal contraceptive. Population Council is developing a thermoplastic ring containing MIV-150. CONRAD is developing thermoplastic ring containing tenofovir and a combination ring containing tenofovir and levonorgestrel. This list is not exhaustive and it is important to note that multiple developers are studying microbicides or multi-prevention products in VRs.

The most basic design of the VR is a matrix formulation whereby API is dispersed throughout the polymeric matrix and typically the drug release rates are proportional to the amount of API loaded and the surface area of the VR. A second type of VR is a reservoir or core design where the API is loaded into core and over-molded with a separate polymer sheath. In this case the sheath often controls

the diffusion of API out of the reservoir. A third design is a pod insert design whereby tablets or pods containing API are inserted into VRs to improve loading and control release of drug from the VR (Moss et al. 2012). Lastly, there are novel approaches to ring designs such as multi-segmented rings and hydrogel vaginal rings (Han et al. 2007; Saxena et al. 2009). The most effective choice of polymer for a given microbicide depends on the solubility of the drug in the polymer, and on whether the drug has a low enough molecular weight to diffuse through the polymer (Fetherston et al. 2010). The latter is also affected by the degree of cross-linking within the polymer under study. The focus in this section will be on the primary polymeric systems utilized to design these different ring types, namely elastomers and thermoplastics, and the following subsections describe them in more detail.

3.1.1 Silicone Elastomer Rings

Silicone elastomers are formed by the cross-linking of functional linear siloxanes and are the most common material used in manufacturing VRs, via injection molding (Fetherston et al. 2010). A VR fabricated from this hydrophobic elastomeric polymer can be self-inserted high into the vagina, where it is held in place due to its shape and inherent elasticity (Woolfson et al. 1999). There are several kinds of curing systems but only two of which have been explored in the microbicide field to date, the platinum catalyzed cure and the tin condensation cure system.

For the platinum catalyzed cure, the elastomer is thermally cured via an addition-cure (platinum catalyzed) reaction and the resulting elastomer consists of cross-linked dimethyl and methyl-vinyl siloxane copolymers and reinforcing silica. Elastomers featuring this type of cure system are supplied as two-part kits: one part contains the catalyst, the other a silicon hydride-functional cross-linker and an inhibitor to provide working time once the two parts have been mixed. A major advantage of addition cure rubber is that the cure reaction produces no by-products (2009).

Tin condensation systems involve hydroxyl functional polymers and alkoxy-functional cross-linking compounds. The alkoxy functional cross-linker first undergoes a hydrolysis step and is left with a hydroxyl group. This hydroxyl group then participates in a condensation reaction with another hydroxyl group attached to the polymer. The reaction can proceed without the assistance of the tin catalyst, but the presence of the catalyst boosts the rate of reaction (Reilly and Bruner 2004). Condensation systems can be advantageous due to their ability to cure at room temperature which may be of benefit should an API be temperature sensitive. However, the main disadvantage of condensation systems is the long cure time which increases manufacturing time and, hence, cost. An additional complication for developers that incorporate API into a tin catalyzed silicone system is that API may migrate to the surface of the ring during the extended curing process and create a “burst effect” upon release in vitro and in vivo.

In the early development of the dapivirine VRs, IPM developed reservoir and tin-catalyzed condensation cure rings before the final platinum catalyzed ring

containing 25 mg dapivirine matrix ring. The tin catalyzed VR showed the burst effect due to the redistribution of dapivirine that was dissolved in propanol which was released during the condensation reaction and consequently deposited on the surface of the ring. As a result a platinum cure silicone was employed which was a complete cure and contained no by-products. Dapivirine is well suited for delivery via VR, as evidenced by the favorable safety and pharmacokinetic data generated to date (Nel et al. 2009b). Clinical trials have demonstrated sustained delivery of high levels of dapivirine throughout the cervicovaginal vault for up to 1 month and Phase III efficacy and safety trials (The Ring Study and ASPIRE) are underway.

3.1.2 Thermoplastic Rings

Thermoplastic elastomers have a long history in the biomedical device arena and are an important material class from which FDA approved intravaginal devices have been constructed (Creatsas et al. 2001; Thyssen et al. 2001; Foran 2003; Novak et al. 2003). The most commonly used thermoplastic elastomers used for intravaginal ring formulations are poly(ethylene vinyl acetate) (EVA) and segmented polyurethane (PU) (Malcolm et al. 2010).

NuvaRing® is one of the first thermoplastic hot melt extruded combination rings on the market (Kiser et al. 2012). It was developed by Organon, approved in Europe and USA and releases etonorgestrel (120 μg/day) and ethinyl-estradiol (15 μg/day) for 3 weeks′ continuous use followed by 1 week off (Novak et al. 2003). NuvaRing® comprises EVA which is copolymer of vinyl acetate and ethylene that varies from 10 to 40 % vinyl acetate content. NuvaRing® is a thermoplastic reservoir VR fabricated from two different grades of EVA (van Laarhoven et al. 2002a, b) and is comprised of coaxial fibers whereby the hormone is dissolved or dispersed in a core polymer. The release from these coaxial fibers depends directly on the concentration gradient over the rate limiting membrane (van Laarhoven et al. 2002a, b). Particle Sciences (Bethlehem, PA) initiated the development of a dual purpose EVA ring containing UC781 and levonorgestrel CONRAD (Loxley et al. 2011) before the development of UC781 was discontinued.

Polyurethanes are thermoplastic elastomers consisting of linear segmented block copolymers of which there are many types. They are formed by a reaction between a polymeric diol (e.g., polytetramethylene oxide or polyethylene oxide) and an aliphatic or aromatic isocyanate. Aromatic isocyanates have potential toxic side effects (Szycher 1988), therefore aliphatic isocyanates have been chosen for VR manufacturing in the microbicide field. Polyurethanes are starting to be utilized for intravaginal applications (Gupta et al. 2008; Johnson et al. 2010, 2012) for formulating microbicides such as pyrimidinediones, dapivirine, MIV-150, and tenofovir, either alone or in combination. The particular advantage of polyurethane VRs is their ability to swell in aqueous environments which enables the incorporation of hydrophilic compounds (such as tenofovir) that were previously very challenging to formulate into a VR.

4 Other Dosage Forms

There are other dosage forms such as ARV-loaded SILCS diaphragm (Schwartz et al. 2008a), hydrogel vaginal rings (Han et al. 2007; Saxena et al. 2009), and electrospun fiber meshes containing drugs that are currently under development within the microbicide field. The following section describes a small portion of these unique drug/device combinations to demonstrate some of the creative approaches taken to supply the HIV prevention field with options for novel dosage forms and multi-prevention technologies.

4.1 Electrospun Fibers

Electrospinning is a technique that applies electrostatic forces to form micro- or nanoscale polymer fibers that can be fabricated into meshes of varying geometries. The diversity and number of polymers that can be electrospun should enable a correspondingly large number of active agents to be encapsulated for sustained delivery (Pham et al. 2006; Greiner and Wendorff 2007; Mauck et al. 2009). As such, electrospinning is an elegant method to deliver combination drug therapies because polymers can be selected based on their drug compatibility as well as their degradation or dissolution rates. In addition, controlling processing parameters (applied voltage, polymer flow rate, capillary-collector distance), nozzle configuration (single, multijet, coaxial), and choice of materials (non-degradable, biodegradable, water-soluble) allows great versatility and flexibility to design topical prevention strategies. The University of Washington has begun development of an electrospun drug-eluting matrix uniquely designed for the geometry and physiology of the vagina, which provides chemical and physical barrier methods for HIV-1 prevention and contraception. This vaginal drug delivery platform could potentially be used to prevent other STIs or RTIs alone and in combination, for intravaginal delivery of nanoparticles, or be designed for rectal drug delivery.

Electrospinning has been used to fabricate controlled-release systems to deliver small molecule drugs (Jiang et al. 2005; Cui et al. 2006; Huang et al. 2006; Luong-Van et al. 2006; Stitzel et al. 2006; Taepaiboon et al. 2006; Feng et al. 2010; Okuda et al. 2010), proteins (Reilly and Bruner 2004; Wei et al. 2006; Zhang et al. 2006; Chiu et al. 2007; Jin et al. 2008; Maretschek et al. 2008; Fletcher et al. 2009; Ionescu et al. 2010), and nucleic acids (Liang et al. 2005; Cao et al. 2010; Kim and Yoo 2010; Wang et al. 2010). Drug release kinetics from the electrospun matrix can be finely tuned by controlling the nanofeatures (size, geometry, architecture) as well as physicochemical properties of the materials (polymer wetting, swelling, and dissolution as well as drug-polymer interactions). Electrospinning also presents a novel method for delivering combination drug therapies by assembling a composite matrix from component electrospun matrices that are individually

coated or embedded with a single active agent (Reilly and Bruner 2004; Kidoaki et al. 2005, 2006). Based on these qualities, electrospun nanofibers are uniquely positioned to have a significant impact on the development of contraceptive microbicides for dual-protection against sexual HIV-1 transmission and pregnancy.

In addition to the design of the drug-eluting properties, electrospun fiber meshes must also be engineered to withstand a complicated loading environment that includes anisotropic multiaxial tension, compression, and shear forces that arise during application and coitus. Randomly deposited nanofibers produce scaffolds that exhibit isotropic mechanical properties primarily reflective of their polymer composition and, given the large number of polymers that can be used for electrospinning, a wide range of mechanical properties are achievable (Duncan 1970). However, fiber alignment also significantly influences mechanical properties of nanofibrous scaffolds and can alter the stiffness or elasticity of scaffolds electrospun from otherwise identical polymer compositions (Theron et al. 2001; Ayres et al. 2006; Courtney et al. 2006; Nerurkar et al. 2007). Random scaffolds exhibit a relatively linear stress-strain response in the regime before the material yield point and then extend linearly after this point. In contrast, aligned scaffold tested in the fiber direction have a sharper increase in stress with increasing deformation, whereas the same scaffolds tested in the transverse direction exhibit a much lower stress–strain profile (Duncan 1970). Another factor that can affect the mechanical properties of nanofibrous scaffolds are the number of fiber–fiber cross-links that are introduced by controlling solvent deposition and evaporation at the grounded collector, or the use of chemical cross-linking agents (Kidoaki et al. 2006; Tan et al. 2008). Therefore, a large number of factors can be employed to impart specific mechanical properties to electrospun fiber meshes intended for topical prevention strategies. This technology has recently been applied to develop a combination anti-HIV and contraceptive product (Ball et al. 2012).

4.2 SILCS Diaphragm

Contraceptive diaphragm technology has been available since the late nineteenth century (2000). Although the device design and composition has changed significantly over time, the diaphragm still offers a female-controlled, non-hormonal, barrier method for prevention of pregnancy. PATH (Seattle, WA), along with research partner CONRAD, developed one such device called the SILCS diaphragm that improved upon earlier versions of the diaphragm by making it more comfortable and easier to use. Patent protection was sought and awarded on the first generation of this diaphragm in 1998 (Austin et al. 1998). Subsequently, it was recognized that the polymeric construction (thermoplastic spring core and overmolded silicone elastomer membrane) made this device a viable candidate for incorporation of HIV microbicides into this core. The drug loaded core can be viewed as a vaginal ring that allows permeation of drugs from the thermoplastic core through the silicone sheath layer while the diaphragm itself acts a barrier

contraceptive. Queen's University Belfast (QUB) has developed prototypes of this multi-prevention device using UC781 (Major et al. 2010) dispersed in polyoxymethylene (POM) copolymer. Additionally, QUB initiated development of dapivirine-loaded POM spring cores of the SILCS contraceptive diaphragm (Major et al. 2012). These devices could offer women a coitally dependent, non-hormonal contraceptive along with HIV prevention.

5 Conclusion

A multitude of dosage forms and devices are being evaluated for the delivery of microbicide drug candidates. These formulations offer both coitally dependent and coitally independent mechanisms for HIV prevention. A number of advantages and disadvantages exist with each product type. It is critical that any microbicide product be safe, effective, and acceptable to the user. Ultimately, it will most likely be advantageous to offer drug candidates in a variety of platforms since a user's product compliance will vary based on preference, circumstance, and economic situation.

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Preclinical Safety Evaluation

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Abstract Before pharmaceutical products are evaluated in humans, it is essential that they undergo a rigorous safety assessment using in vitro models and studies in preclinical species. Once products progress into the clinic, additional preclinical studies are needed to support further clinical testing. Although regulatory guidelines provide a good framework for the types of studies that should be performed, there are some areas where it is unclear how these should be applied to microbicides, what study designs should be used, whether certain tests are relevant or if additional assays are appropriate. In this chapter we provide an overview of the key issues for the preclinical development of microbicides, and describe the purpose of each of the tests along with the key considerations to be taken into account when designing the individual safety studies as well as the overall preclinical program.

Contents

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1 Introduction

The goals of the safety assessment program for microbicides are similar to those for other pharmaceutical products. Initially, the objective is to determine a safe starting dose for the first-time-in-human (FTIH) clinical trial (Phase 1). Subsequently, studies are conducted to provide sufficient information to support clinical trials involving higher doses or a longer duration of administration, and as the program progresses additional studies are required to address use of the product in special circumstances, such as in pregnant or breastfeeding women. Finally, further work may be necessary to address issues related to the long-term use of the product in the broader population to support marketing authorization.

However, for microbicides there are additional considerations that are not typical for other types of product. First, since microbicides are intended for topical application there is a greater emphasis on local rather than systemic effects. Second, there is the fact that the products are intended for use by otherwise healthy women to prevent HIV-infection rather than as a treatment. This affects the acceptable risk–benefit ratio relative to what would be deemed appropriate for the treatment of HIV-infected individuals. Third, there are specific concerns related to the potential for the microbicide to increase susceptibility to HIV-infection.

In general, the guidelines established by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH; www.ich.org) are applicable to the development of microbicides as well as other pharmaceuticals. However, because microbicides are vaginally or rectally administered products intended to prevent infection, there may be certain assessments that are not necessary and additional investigations not typically performed that should be included.

One other crucial factor that plays a significant role in determining the scale and scope of the preclinical safety assessment program for a microbicide product is the status of the active ingredient. Microbicides containing active ingredients that are present in pharmaceutical products that are either marketed or in an advanced stage of development can draw greatly on the safety data available for the existing product. The safety assessment program can be largely reduced to an evaluation of the local toxicity associated with the change in administration route and formulation, along with any bridging studies to relate the existing data to the new indication.

Since the development of microbicides is an emerging field, consultation with regulatory agencies is an important element of the product development process. Scientific advice from regulators will assist in establishing how the guidelines should be applied and whether any additional considerations need to be addressed in order to satisfy their expectations.

2 Preclinical Formulations

The preclinical development of pharmaceutical products intended for systemic use typically focusses on the safety assessment of the active pharmaceutical ingredient (API). Consequently, the formulations used in the preclinical studies are intended to deliver high doses of compounds in dosage forms that are stable and appropriate for the routes of administration and species used, rather than being representative of the final formulation intended for clinical use. In contrast, wherever possible it is highly advisable to use the intended clinical formulation as the dose formulation for the in vivo preclinical safety assessments for microbicides, particularly for the definitive toxicology studies. This is because of the importance of adequately characterizing the local effects associated with a microbicide product, which may be due to the API, excipients in the formulation, or an interaction between the active and inactive ingredients. While there is a wide variety of excipients that are approved for use in vaginal (Garg et al. 2001) and rectal (Rytting and Fix 2006) products, the concentrations that are safe for use have not been widely characterized. In addition, many excipients have been used in products intended for short-term use and may not be suitable for chronic application, and chemicals considered to have acceptable safety profiles for general pharmaceutical products may in fact be inappropriate for HIV prevention because of low-grade irritant effects that could enhance susceptibility to HIV. For some clinical dosage forms such as gels, the drug concentration can be varied in order to achieve safety margins in the preclinical species, but other alterations in the formulation should be avoided where possible to ensure that variables are not introduced that could alter the safety profile. For other dosage forms, some modifications may be necessary for practical reasons. Vaginal tablets/pessaries or films may not be suitable for administration to preclinical species. In these cases, a degree of pragmatism is necessary for the preclinical safety assessment program, and the use of dispersions of the dosage form in water or saline may be an appropriate approach to allow for intravaginal administration in these animal models.

It is important that any formulations used in preclinical studies are well-characterized prior to use. In those cases where the clinical formulation is used, an extensive program of work is necessary to support good manufacturing practices (GMPs) and therefore any further characterization is likely to be minimal or unnecessary. However, simply increasing a drug concentration in a formulation can significantly alter the physico-chemical properties of the formulation, and these changes alone may be sufficient to affect the outcome of a study. For

example, a higher drug load may change the state of a drug within a formulation from a solution to a suspension, or could change the pH or osmolality of the formulation, which could result in local changes to the vaginal or rectal mucosa that are unrelated to the biological activity of the active ingredient.

3 Studies Required to Support Clinical Trials and Product Registration

The program of preclinical studies necessary to support the development of a microbicide is generally similar to that for other pharmaceutical products, particularly those intended for topical use. However, for microbicides with limited systemic absorption, studies with purely systemic endpoints, such as safety pharmacology and pharmacokinetic assays, may be reduced or assessments of these endpoints incorporated into other studies. All pivotal studies, with the exception of pharmacokinetic studies, should be performed in full compliance with the principles of good laboratory practice (GLP) (toxicokinetic evaluations included in toxicology studies should also be fully compliant). Preliminary dose range finding investigations do not need to be performed to GLP.

It is also important that wherever possible the species and strains used at the laboratories conducting the preclinical studies have been widely used in similar assays with background data available, so that unusual findings can be evaluated in the context of a wider body of data than concurrent negative controls.

A general layout of the timing of the preclinical safety studies for a microbicide based on a new chemical entity (NCE) is presented in Table 1. However, it should be noted that different compounds may require alternative strategies and study timing, particularly those that have previously been developed for other indications. Microbicides containing combinations of drugs or those involving medical devices for drug delivery are not represented in Table 1, but are described later in this chapter.

3.1 Secondary and Safety Pharmacology

The pharmacological assessment of candidate microbicides includes evaluation of the primary activity, i.e., determination of the anti-HIV activity as described in the chapter by Shattock and Herrera (2013), but also investigations into the potential for other functional effects that may have safety implications. These studies are broadly divided into two categories: secondary pharmacology studies, which evaluate the pharmacodynamic activity of the compound at targets other than those associated with the HIV prevention indication, and safety pharmacology studies, which evaluate the functional effects of a drug on specific organ systems, such as

Table 1 Timing of preclinical safety assessment studies for microbicides

Study type	Stage of development			
	Prior to phase I clinical trials	Prior to phase II clinical trials	Prior to phase III clinical trials	Prior to product registration
Secondary and safety pharmacology	Receptor, ion channel and enzyme screen hERG assay Effects on central nervous system, cardiovascular system, and respiratory system[a] Effects on sperm motility and endogenous microflora[b]			
Pharmaco-kinetics	Toxicokinetics during toxicology studies	Toxicokinetics during toxicology studies Tissue distribution studies[c] In vitro metabolism studies Cytochrome P450 inhibition and induction studies[d] Excretion balance studies[c]	Toxicokinetics during toxicology studies Metabolite profiling[c]	Toxicokinetics during carcinogenicity studies
Acute toxicity	Parenteral and oral studies in mice and rats			
Repeat dose toxicity	14 or 28 day studies in rodents and non-rodents [e, f]	Studies in rodents and non-rodents ≥ planned trial duration[e]	6 month study in rodents and 9 month study in non-rodents[e]	
Genotoxicity	Bacterial reverse mutation assay Chromosomal aberrations assay in mammalian cell line	In vivo micronucleus test		
Reproductive toxicity	Segment I study in rats Segment II studies in rats and rabbits			Segment III study in rats
Carcinogenicity				2 year intravaginal study in rats
Other toxicity	Sensitization			

This table is intended to provide a general guide for the timing of safety studies for development of a new chemical entity as a microbicide. Different compounds may require alternative development strategies and study timing, particularly those that have previously been developed for other indications. This table does not apply to drug–drug or drug-device combinations. Regulatory advice should be sought throughout the development program to ensure all expectations are met

[a] May be evaluated in separate studies or as part of toxicology studies

[b] Only necessary for vaginal microbicides

[c] May not be required for poorly absorbed compounds

[d] Required for systemically absorbed compounds prior to trials that may involve co-administered medications

[e] If the intravaginalor intrarectalroute is used, additional studies may be necessary to assess systemic toxicity of compounds with low systemic absorption in animals

[f] If the rabbit is not selected as the non-rodent species, or if the intravaginal route is not used, a 10 or 14 day rabbit vaginal irritation study is required for vaginal microbicides

the central nervous system, cardiovascular system, and respiratory system, along with any other systems where there may be expected to be activity based on what is already known about the compound, or class of compounds to which it belongs. The secondary pharmacology studies typically include in vitro screens of a range of receptors, ion channels, and enzymes. Functional studies can also be conducted in isolated tissues that are known to be rich in certain receptors.

There are also several secondary pharmacology issues that relate specifically to a vaginally administered product. These include potential effects on sperm, the natural vaginal microflora and pathogens associated with sexually transmitted infections (STIs). Sperm motility can be evaluated in vitro using human sperm samples as an evaluation of the potential contraceptive effects of a microbicide (Doncel 1994; Wood et al. 2003). The normal vaginal flora plays an important role in protecting against infection. In particular, the *Lactobacillus* species naturally present in the vagina produce hydrogen peroxide, which has properties that help protect against infection by HIV and other mucosal pathogens (Lederman et al. 2006), and reductions in lactobacilli, such as in the case of bacterial vaginosis, have been associated with an increased risk of HIV transmission (Rosenstein et al. 1998). The impact of microbicides on the natural microflora can be addressed in in vitro tests to assess the effects on pH and cytotoxicity to major species of flora found in the vaginal environment (Rohan et al. 2010; Fichorova et al. 2011). The pigtailed macaque has similar genital and rectal flora to that in humans and has also been used to assess changes in normal rectal and vaginal flora following administration of various products (Patton et al. 1999, 2001), and further in vivo assessments can be included in clinical trials. There is also the possibility that a microbicide may have activity against vaginal bacterial pathogens, which can also be evaluated using in vitro assays (O'Brien and Thoms 1955).

A core battery of safety pharmacology studies is recommended in the ICH S7A (2000) guidance to evaluate the functional effects of drugs on organ systems. Given that these studies evaluate changes resulting from systemic exposure to drugs, the importance of the data may be limited for those microbicides for which absorption is expected to be low. Lard-Whitefard et al. (2004) suggest that the studies should be performed prior to clinical trials for microbicides that have been shown to be absorbed systemically, but do not make any recommendations where there is little or no absorption. Although the absorption of microbicides may be low in preclinical species, there may be inter-species differences that mean it is more extensive in humans. Therefore, it is recommended that at least a minimal package of safety pharmacology studies be conducted for all candidate microbicides. Many of the safety pharmacology endpoints can be included in toxicology or other studies, rather than in separate studies, and this practice is becoming increasingly commonplace for a variety of pharmaceutical products where the likelihood of activity at the organ systems being assessed is low (Luft and Bode 2002). More extensive evaluations may be appropriate once the extent of systemic absorption is fully understood and following consultation with regulatory authorities.

3.2 Pharmacokinetics

Although microbicides are intended to prevent infection by delivering effective drug concentrations at the potential site of infection, for most products there is also likely to be some degree of systemic absorption. In order to determine the potential for systemic toxicity, it is important that the extent of absorption is evaluated during the preclinical program. Pharmacokinetic studies enable the absorption profile to be characterized, and help to define how the drug is handled following absorption. They also help to provide validation of the species used in the preclinical investigations by confirming whether the APIs are handled in a similar way in animals and humans. If there are shown to be significant differences between these species, and particularly if systemic exposure is lower in animals than in humans, additional studies using species that more closely reflect the pharmacokinetic characteristics in humans may be necessary. A comparison of the exposures achieved in animals with those observed during clinical trials can be used to establish preclinical safety margins, and investigations into the metabolism and excretion of the APIs can help to address other safety concerns.

For microbicides that are shown to have low levels of absorption, the pharmacokinetic studies may be limited to determination of drug concentration profiles in blood plasma during toxicology studies (known as toxicokinetic assessments). Additional investigations, such as quantitative whole-body autoradiography studies may be appropriate to determine relative tissue exposures, since plasma levels may not be representative of drug levels in other tissues. Metabolite profiling studies are unlikely to be necessary since any metabolites formed will represent only a fraction of the parent drug concentration. The toxicity of those metabolites formed in the preclinical species will be evaluated during the corresponding toxicology studies, and the risk associated with the possibility of metabolites occurring in humans that are not present in the preclinical species can reasonably be assumed to be negligible because the exposure levels will be extremely low.

For microbicides that show significant systemic absorption, the pharmacokinetic evaluation should resemble that of pharmaceutical products for systemic use (ICH M3(R2) 2009). This should include toxicokinetic data from the repeat dose toxicity studies, and in vitro metabolism (in hepatocytes or hepatic microsomes prepared from liver tissue from the toxicology species and humans) and plasma protein binding data for animals and humans, prior to initiation of human clinical trials. Further absorption, distribution, metabolism, and excretion information should be available before exposing large numbers of human subjects or treating for long duration. There should also be some evaluation of the potential for cytochrome P450 induction or inhibition, since this may have implications for drug interactions with co-administered products. Metabolite characterization may be warranted prior to Phase III trials for metabolites with exposures representing greater than 10 % of total exposure to drug-related material, or which are shown to occur at significantly greater levels in humans than in the toxicity studies.

3.3 Acute Toxicology

Acute toxicity studies involving administration of single high doses are typically the first assessment of the general toxicity of a pharmaceutical, and therefore should be performed early in the development process. Data from these studies are helpful for dose level selection in subsequent repeat dose studies, and are also helpful in defining potential risks related to accidental or intentional overdosage.

The studies are generally performed in rodents (mice and rats) and guidance documents on various study designs are available (US FDA 1996; OECD 2001a, b, c). For most pharmaceuticals the recommended routes of administration are the intended therapeutic route and a route that achieves high systemic exposure. In the case of microbicides, the practical limitations concerning the type and amount of dose that can be administered via the vaginal or rectal routes mean that alternative routes are more suitable for these studies. In addition, the potential for overdosage in humans via these routes is low. Lard-Whiteford et al. (2004) suggest that these studies can be conducted via the oral route and a parenteral route, which allows for dosing at high doses in order to gain an initial read on the general toxicity, as well as representing more plausible routes for possible overdosage.

More recently, the value of acute toxicity studies has been questioned (Robinson et al. 2008), and the latest version of the ICH M3(R2) (2009) guideline suggests that information on the acute toxicity of a compound can be obtained during preliminary dose range finding studies to support the repeat dose toxicology program, and can be limited to the clinical route only. However, given the practical limitations of the intravaginal and intrarectal routes, there still may be value in acute toxicity studies for microbicides via alternative routes that achieve good systemic exposures.

3.4 Repeat Dose Toxicology

Since they are intended for chronic use, the toxicity of microbicides following repeated administration should be evaluated. Regulatory guidelines generally recommend studies in one rodent and one non-rodent species. The duration of the studies should be equal to or greater than the treatment period planned for the clinical trials (ICH M3(R2) 2009). A minimum of 2 weeks is usually necessary to support first time in human trials, but performing studies of 1 month duration will support both the initial first time in human trial and subsequent trials of up to 1 month, thus reducing; (i) the number of studies performed, (ii) the development time line, and (iii) the associated costs. In order to select appropriate dose levels, it may be necessary to conduct dose range finding studies prior to the main studies.

The rat is frequently selected as the rodent species because of its wide use in preclinical safety assessment and because intravaginal and intrarectal administration is relatively straightforward. Smaller species, such as the mouse, have

limitations both in the practicalities of dosing via these routes and difficulties in observing changes at the site of administration. The rabbit is often used as the non-rodent species because of its known sensitivity to vaginal irritants (Kaminsky et al. 1985; Costin et al. 2011).

The dose volume used should provide sufficient exposure of the local tissues to allow an adequate assessment of safety to be made. In rabbits, the recommended volume for intravaginal dosing is 1 mL, whereas in rats 0.2 mL is typically used (Gad 2008). The same volumes are suitable for intrarectal administration (Martinsson et al. 1999; ISO 2002). Dose administration via a rubber catheter attached to a syringe is recommended for rabbits, since steel cannulas or other rigid instruments could cause trauma during the dosing procedure, particularly if the animal moves unexpectedly. For rats, the dose can be administered by gently inserting the tip of a syringe into the vagina or rectum.

The study design used should be similar to that routinely used in preclinical toxicity studies and include toxicokinetic assessments, clinical sign observations, measurements of body weight and food consumption, clinical chemistry, hematology and urinalysis, and post-mortem examinations including organ weights, and macroscopic and microscopic pathology. Particular attention should be given to observations at the site of administration since these may indicate changes that could increase susceptibility to HIV infection. Additional studies to evaluate this further may be warranted as discussed later in this chapter.

Since studies of vaginal microbicides can only be conducted in females, it may be beneficial to include additional animals (e.g., 10 per group in rodents and 5 per group in non-rodents) to provide more data points.

As the clinical development program progresses, additional preclinical studies will be necessary to support longer durations of dosing in clinical trials. The maximum duration of administration for repeat dose toxicity studies required by regulatory authorities is 6 months for rodents and 9 months for non-rodents (ICH M3(R2) 2009).

The intended clinical route is the preferred route of administration (Lard-Whiteford et al. 2004); however, as discussed above the dose levels that can be achieved via the intravaginal and intrarectal routes in preclinical species are limited. It is generally expected that the high dose level should be a maximum tolerated dose (MTD) at which frank toxicity is observed (ICH M3(R2) 2009), but this may not be achievable via these routes. In addition, the extent of systemic absorption via these routes may be limited, and therefore the studies may not provide an adequate assessment of systemic toxicity. Although for some compounds it may be reasonable to predict that no, or very low, absorption will occur in humans, in most cases the degree of absorption cannot be accurately predicted prior to clinical pharmacokinetic trials and therefore it should be assumed that some absorption will occur and consequently there is the potential for systemic toxicity. In these circumstances, studies using alternative routes of administration (i.e., oral or parenteral) may be necessary, either in addition to, or instead of, studies via the intended clinical route.

3.5 Genotoxicity

It is important to understand whether a microbicide has mutagenic activity early in the development process because this may have implications for the viability of the candidate. The types of assays that should be performed are described in the ICH S2(R1) (2011) guideline, and include in vitro assessments of the potential to cause point mutations/deletions in a bacterial reverse mutation assay and chromosomal aberrations in a mammalian cell line. In addition, an in vivo mammalian (rodent) assay of clastogenicity such as the micronucleus assay should also be performed. The in vitro assays should be completed prior to Phase I clinical trials and the in vivo assay should be completed prior to Phase II (ICH M3(R2) 2009). However, since all of these studies are quick to perform and are relatively inexpensive, it is prudent to complete the package as early in development as possible.

3.6 Reproductive Toxicology

The target population for vaginal microbicides is sexually active women, including women of childbearing potential. Consequently, microbicides must be evaluated for the potential to affect the various stages of reproduction.

Although there are regional differences in the regulatory requirements for the timing of reproductive toxicity studies to support the inclusion of women of childbearing age in clinical trials (ICH M3(R2) 2009), the guidelines in all regions allow short-term trials to be conducted without the prior completion of preclinical reproductive toxicity studies, providing women agree to be on adequate contraception during the trial. However, for microbicides there has generally been an expectation that studies of fertility and reproductive performance (Segment I) and embryofetal development (Segment II) be completed prior to commencing clinical trials because of the close proximity of the site of application to the developing embryo. A peri- and post-natal development study (Segment III) needs only be performed prior to registration of the product (i.e., in parallel with Phase III trials).

International consensus on the designs of the reproductive toxicity studies has been achieved through ICH (ICH S5(R2) 1994). For vaginal microbicides, it is generally only necessary to conduct studies in females, unless significant exposure of the male partners of women using the microbicide is anticipated. The Segment I and Segment III studies are generally conducted in rats. For microbicides with good systemic absorption, the intravaginal route may provide an adequate assessment of both local and systemic effects, but for those that are poorly absorbed, an alternative route that results in good systemic exposure may be more appropriate. This is particularly important for the Segment III study since exposure of the newborn offspring to the drug is via the mother's milk, and therefore sufficient drug needs to be absorbed in order for it to be excreted in the milk. To assess effects on embryofetal development, two Segment II studies (one in rats and

another in rabbits) are standard, and in those cases where the microbicide is poorly absorbed one study may be performed intravaginally and the other via a route that results in good systemic exposure.

For rectal microbicides, reproductive toxicity studies may not be required prior to short-term Phase I trials, but Segment I and Segment II studies should be completed prior to larger trials in men and women. For trials only involving male subjects, Segment II studies are not necessary, but dosing of male animals should be included in the Segment I study.

3.7 Carcinogenicity

Microbicides are intended for chronic use, and therefore there is a need to evaluate their carcinogenic potential. The ICH guidelines (S1A (1995), S1B (1997) and S1C(R2) (2008)) provide detailed guidance on the assessment of the carcinogenic potential of pharmaceuticals for human use. However, for microbicides particular attention should be given to (1) the selection of models and the number of studies needed, (2) the route of administration, (3) the implications of using only female animals for vaginal microbicides, and (4) group sizes if only females are used.

Historically, carcinogenicity studies in two rodent species have been required by regulatory authorities. More recently, the value of this approach has been questioned and a 'weight of evidence' approach has been proposed involving the evaluation of data derived from one long-term carcinogenicity study along with other appropriate experimental investigations, which could include short or medium-term in vivo test systems that evaluate carcinogenic endpoints, such as assays using transgenic rodents (ICH S1B 1997).

Very few new chemical entities have been developed as microbicides to the point where carcinogenicity studies are required; therefore there is very little experience in the field to draw upon. Recently issued draft guidance from the US Federal Drug Administration (FDA) suggests that a single study performed intravaginally may be adequate (US FDA 2012). This is consistent with the scientific advice provided to the International Partnership for Microbicides (IPM) for the development of the dapivirine vaginal ring during meetings with the FDA, European Medicines Agency, World Health Organization, and a number of sub-Saharan African authorities. A study in transgenic animals was not recommended because of the lack of experience with such models in the evaluation of local tumors following intravaginal administration; however, others have suggested the Tg.AC transgenic mouse model for the evaluation of microbicides (Lard-Whiteford et al. 2004).

Standard carcinogenicity study group sizes (not < 50 animals) are acceptable whether both sexes or females only are used, because this provides sufficient power for each sex to give a statistically meaningful outcome individually. However, if only females are used it may be desirable to add additional animals because very few laboratories have experience of intravaginal dosing in rats for the

duration of a carcinogenicity study and it is possible that some animals could be lost due to dosing-related causes.

Carcinogenicity studies are required to support marketing applications and are therefore usually conducted during the Phase III clinical program.

3.8 Dermal Sensitization

As microbicides are topical products, they should be evaluated for their potential to cause a hypersensitivity reaction. Although a sensitization model involving intravaginal administration has been developed in the guinea pig (Newmann et al. 1983), it is generally recommended that more conventional dermal models are used because of the familiarity of regulatory bodies with these models and because they are widely established at toxicology testing laboratories.

There are two main types of sensitization assay. The first is the murine local lymph node assay and the second comprises various guinea pig models.

The mouse local lymph node assay (LLNA) measures the incorporation of ^{3}H-thymidine into the local lymph nodes following the daily application of the test material to the ears of mice for 3 days, as an indicator of proliferative activity due to a sensitization reaction (Kimber et al. 1994). One advantage of this model is the endpoint is an objective measurement, rather than the subjective visual assessment associated with the guinea pig models. It is also shorter than the guinea pig assays because the endpoint is a function of the induction phase. Unlike the guinea pig models, there is no assessment of response to a post-induction challenge, which is also a limitation of the model. The LLNA offers a number of animal welfare benefits over the guinea pig assays: fewer animals involved, the potential for reduced stress because of the lack of a challenge phase, and the fact that there is no need for the use of an adjuvant as there is in the guinea pig maximization test. However, there are still many classes of compounds for which the LLNA's activity has not been validated, with false negative findings associated with certain metals and false positive findings for certain skin irritants (National Institute of Environmental Health Sciences 1999).

There are two main variants of the guinea pig sensitization test; the guinea pig maximization test (Magnusson and Kligman 1969) and the Buehler, or occluded patch, test (Buehler 1965). The main difference between the models is the incorporation of the occluded patch for contained, repeated, dermal delivery of the test material in the Buehler test, and the use of intradermal dosing of test compound with Freund's complete adjuvant in order to maximize the induction phase in the maximization test. Both models record visual changes (oedema, erythema and inflammation) at the site of application following a challenge with test article, and are consequently somewhat subjective. The Beuhler assay is less sensitive than the maximization test, but may be viewed as more relevant for microbicides because it only involves topical administration. On the other hand, the maximization test is able to detect sensitization by chemicals administered intradermally

that might not otherwise cross the epidermis. Both models are considered validated for broad classes of chemicals and are generally accepted by regulatory authorities as predictive models of dermal sensitization.

4 Investigative Studies Specific to Microbicides

The preclinical studies described above reflect those expected by regulatory authorities to support clinical trials and marketing authorization applications, but there are additional studies that, while not strictly necessary from a regulatory point of view, may be valuable in better understanding the safety profile of a microbicide. Since these studies are investigative in nature and are therefore not considered pivotal, it may not be necessary to conduct them in full compliance with GLP. Indeed, because these studies are not routine assays it may be difficult to find GLP compliant laboratories with the capabilities to conduct such studies. Nevertheless, efforts should be made to ensure scientific rigor and integrity are maintained in the performance of these investigations and the data generated.

4.1 Assessment of Increased Susceptibility to Infection

In the wake of the nonoxynol-9 trials that showed an increased risk of HIV infection in women using the test product (Kreiss et al. 1992; Roddy et al. 1998; Van Damme et al. 2002; Beer et al. 2006), there has been a greater emphasis on the importance of identifying changes that could increase susceptibility. The first challenge has been to identify those factors that raise the likelihood of infection. Although knowledge of these factors is not complete, there are a number of conditions that we know are associated with higher infection rates. These include the presence of other sexually transmitted infections (Mayaud and McCormick 2001; Freeman et al. 2006), vaginitis (Moodley et al. 2002; Uma et al. 2005), disturbances of the vaginal flora (Gray et al. 1997; van de Wijgert et al. 2000), and lesions of the cervical vaginal mucosa (van de Wijgert et al. 2000; Galvin and Cohen 2004). Common to all of these conditions is mucosal inflammation with or without epithelial disruption and impaired innate immunity. Therefore, attempts have been made to establish preclinical models that are able to identify these types of effects in candidate microbicides.

There are several different in vitro models that can be used to provide an early read on the potential for local changes that could increase susceptibility to infection. These models range from simple cytotoxicity assays to more complex experiments involving analyses of cytokines produced by cell lines following exposure to a test compound (Mesquita et al. 2009; Dezzutti et al. 2004; Fichorova et al. 2004). These assays can be conducted using biologically relevant cell types such as ME180 cells derived from the cervix, the human vaginal epithelial cell line

VK2/E6E7, and colorectal epithelial cells lines such as Caco-2 and SW837 cells (Fichorova et al. 1997; Dezzutti et al. 2004). Transepithelial electrical resistance (TER) can also be measured to determine the integrity of the epithelial barrier as a result of both cellular health and maintenance of tight junctions between the cells (Mesquita et al. 2009). The in vitro assessments can be further expanded to include studies using surgically removed human or animal tissue explants, such as cervical and colorectal tissues (Doncel and Clark 2010; Fichorova et al. 2004, 2005; Doncel et al. 2004; Gali et al. 2010).

An in vivo slug model has been developed for rapid and quantitative screening of the irritant effects of compounds applied to mucosal surfaces (Adriaens and Remon 2002, 2008), but the standard in vivo model for vaginal microbicides is the rabbit vaginal irritation (RVI) study, in which the test product is instilled into the vagina daily for 10 or 14 days, and any macroscopic findings scored using a modification of the Draize system for assessment of skin irritation (Draize et al. 1944). Histopathological findings in the vagina are also scored according to the Eckstein criteria (Eckstein et al. 1969) or a modification of that scoring system (Auletta 1999; D'Cruz 2004). Nonoxynol-9 is typically included in the assay as a reference control because of the known irritant effects of the compound. This assessment is the only model of vaginal irritation that is required by regulatory authorities (Lard-Whiteford et al. 2004). In cases where repeat dose toxicity studies are performed in rabbits using the intravaginal route, these endpoints can be readily incorporated into the studies and therefore there is no need for a separate assessment of the vaginal irritancy. However, if there are subsequent significant changes to the product formulation, an additional RVI study may be warranted. For rectal microbicides, the rabbit has also been used to assess local toxicities applying a similar design to the RVI, but using intrarectal administration (Piret et al. 2008; Wang et al. 2011).

The RVI model has a number of limitations, including an inability to adequately characterize the type of inflammatory response that may be observed. Modifications to the assay, such as inclusion of phenotype and activation status of infiltrating leukocytes, mucosal expression of lymphocyte homing receptors, assessments of tight junctions and biomarkers of inflammation, cervicovaginal lavage for analysis of pro- and anti-inflammatory cytokines and innate factors, have been made in order to try and enhance the model (Doncel and Clark 2010). However, there are significant differences in the anatomy, histology, and physiology of the lower female reproductive tract between rabbits and humans which make the rabbit particularly sensitive to local irritancy (Barberini et al. 1991), and consequently the clinical relevance of the data obtained from these assays is unknown. The monkey has also been investigated as a safety evaluation model for both vaginal and rectal microbicides (Veazey 2008; Patton et al. 2004, 2009). The anatomy, histology, and physiology of the lower female reproductive tract in the monkey are much closer to those in humans, and this model also allows for assessments of the effects on the vaginal microflora. In addition, the types of investigations proposed in the expanded RVI model described above can be included.

Other in vivo studies have been developed specifically to evaluate increased susceptibility to infection. A mouse model has been used to evaluate effects on markers of innate immunity and measure changes in susceptibility to genital herpes as a biomarker of increased risk of HIV infection (Cone et al. 2006; Wilson et al. 2009). Macaques, which have been widely used to evaluate the potential efficacy of microbicides, can also be used to assess increases in susceptibility to infection with simian immunodeficiency virus (SIV) or a hybrid simian/human immunodeficiency virus (SHIV). This can be achieved using standard efficacy models (Veazey 2008), but challenging with a viral inoculum that results in less than 100 % infection in untreated controls (Kenney et al. 2011), so that increases rather than, or as well as, decreases in infection can be observed.

All of these models provide additional data that may provide insights into the safety profile of candidate microbicides. However, they should be considered exploratory/investigational, since the clinical relevance of the findings is not yet adequately understood.

5 Products Containing Previously Marketed Drugs

Some microbicides are based on active ingredients present in marketed products for the treatment of HIV/AIDS. For example, the vaginal gel used in the CAPRISA 004 trial that showed a 39 % reduction in HIV acquisition in women (Abdool Karim et al. 2010) contained the nucleotide reverse transcriptase inhibitor tenofovir. A prodrug of tenofovir (tenofovir disoproxil fumarate) has been marketed as Viread™ by Gilead Sciences, Inc. (Foster City, CA, USA) for the treatment of HIV/AIDS since 2001. Tenofovir disoproxil fumarate is also present in the fixed dose combination tablet Truvada™ (also Gilead Sciences, Inc.) along with the nucleoside reverse transcriptase inhibitor emtricitabine.

The preclinical development program for these types of microbicides can be very much abbreviated compared to that for a NCE because of the wealth of preclinical and clinical data already available on the active ingredient. In 2008, the US FDA issued draft guidance on the nonclinical safety evaluation of new formulations of established drug products intended for administration by an alternate route (US FDA 2008). This guideline indicates that for new vaginal or rectal formulations of previously marketed compounds, repeat dose local toxicity studies should be conducted via the proposed route of administration in one species. In the case of vaginal products, the new formulation should also be evaluated for delayed hypersensitivity. Reproductive toxicity studies are only necessary if the exposure associated with the previous route/formulation was inadequate to cover exposure via the vaginal route, and only if the previous studies did not show a developmental risk (compounds previously identified as having reproductive toxicity would be assumed to present the same risk when administered vaginally, however, such compounds are unlikely to be good candidates for microbicides).

6 Drug-Device Combinations

In recent years, a number of microbicide products have entered development that combine an antiretroviral drug with a medical device as a means of providing sustained delivery of the drug. These include vaginal rings and diaphragms (Nel et al. 2009; Mesquita et al. 2012; Kiser et al. 2012; Aravantinou et al. 2012; Major et al. 2012). For these types of microbicides the preclinical safety assessment program must address both the pharmaceutical and the medical device aspects of the product.

The pharmaceutical considerations for these combination products are similar to those outlined above for non-device products. However, a key difference is that, like products intended for oral or parenteral use, the preclinical studies will focus on assessing the toxicity of the active ingredient and will be performed using an alternative formulation to that intended for clinical use. In this case, the intravaginal studies can be conducted using a simple gel formulation of the API.

The regulatory expectations for the biological evaluation of medical devices are described in the International Organization for Standardisation (ISO) 10993 guidelines. The number and type of tests recommended in these guidelines are dependent on the intended use of the device. For example, a vaginal ring intended to be inserted into the vagina for a period of one month and replaced with a fresh ring each month for an indefinite period would be categorized as a surface-contacting device for mucosal membranes for permanent contact (i.e., a device whose single, multiple, or long-term use or contact exceeds 30 days). Based on this categorization, a number of assays are required using extracts of the device intended for clinical use collected under various conditions in polar and non-polar solvents. These include in vitro cytotoxicity and genotoxicity tests, and in vivo guinea pig sensitization and rabbit vaginal irritation studies. Study designs for most of these assays are described in the guidelines, however, based on IPM's experience with the dapivirine vaginal ring, a longer duration of dosing is recommended for the rabbit vaginal irritation study (35 days) with the inclusion of general toxicology endpoints (clinical pathology, and macroscopic and microscopic pathology on a full tissue list) so that an assessment of the systemic effects of any absorbed by-products formed during the manufacture of the device can be evaluated. It is important that the studies are conducted using product identical to that intended for clinical use, bearing in mind that even slight alterations in starting materials, manufacturing procedures or storage conditions could result in changes to the final product which could alter the safety profile. All of the assays should be run in compliance with GLP.

A further consideration that should be addressed is whether the physical presence of the device may accentuate absorption or enhance the local toxicity of the active ingredient. While there is no specific regulation or guidance requiring such studies, there is the possibility that the presence of a medical device in the vagina could result in slight localized changes that increase both the sensitivity of the tissues to irritant effects and the degree of absorption of the active ingredient.

One of the key challenges to evaluating these effects is the size of devices intended for human use, which often precludes their evaluation in species routinely used for toxicology assessments. In such cases, one of two approaches can be used. The first is modification of the device to enable evaluation in standard toxicology species. For example, smaller versions of vaginal rings can be manufactured that can be inserted into the vagina of macaques (Promadej-Lanier et al. 2009; Malcolm et al. 2012; Johnson et al. 2012). Alternatively, segments of the ring can be evaluated in rabbits, however, this generally requires suturing to hold the segment in place in the vagina and avoid expulsion (Clark et al. 2011; Moss et al. 2012). The main limitation with this approach is that alterations to the device could affect both its performance and its safety profile, and in the case of the rabbit there is the risk of artifactual effects resulting from the necessary surgical procedures. The second approach is to use species that allow the unmodified product intended for human use to be evaluated. The sheep has been used successfully for evaluating vaginal rings (Moss et al. 2012; Kiser et al. 2012), and although it is not a standard toxicology species with the body of background data available that is typically associated with more conventional species, good toxicological assessments have been shown to be feasible. The sheep can also be used for pharmacokinetic assessments of vaginal rings that may be useful in determining product performance and selection of drug loads. These assessments can include accurate measurements of drug concentrations in vaginal or cervical tissue, which are valuable in assessing compounds with intracellular sites of action (Holt et al. 2012).

7 Multi-Drug Combination Products

The use of combinations of drugs has been shown to be more effective than single drugs in both the treatment of HIV/AIDS and the prevention of mother to child transmission of HIV (Portsmouth 2007; Vermund 2004). Combinations can both increase the potency of the treatment and broaden the range of virus subtypes that it is active against (Pirrone et al. 2011). For the same reasons it could be expected that a microbicide containing combinations of antiretroviral drugs may provide greater protection than a product containing only one. In addition, efforts are in progress to develop multipurpose prevention technologies that target more than one indication, such as HIV prevention plus contraception, or prevention of HIV along with other STIs (Thurman et al. 2011; Friend 2012).

For any product containing two or more active ingredients, preclinical assessments should be performed to evaluate whether there are any interactions between the agents. These should investigate pharmacodynamic, pharmacokinetic, and toxicological interactions. Pharmacodynamic interactions may be beneficial, such as synergistic or additive effects of combinations of antiretroviral agents, or they may be deleterious, such as antagonistic effects where the therapeutic activity of one drug is reduced in the presence of another. This type of interaction can occur if both drugs exhibit affinity for the same biological target (e.g., a specific receptor or

enzyme). Pharmacokinetic interactions are primarily related to either induction or inhibition of hepatic drug-metabolizing enzymes (Ogu and Maxa 2000; Kumar et al. 2012). If the enzymes involved in clearance of one drug (Drug A) are inhibited by a co-administered drug (Drug B), concentrations of Drug A may increase to toxic levels that would not occur in the absence of Drug B. Conversely, if the same enzymes are induced by Drug B, Drug A will be cleared much more quickly than it would in the absence of Drug B, and consequently concentrations of Drug A may fail to reach therapeutic levels, or the period that they are therapeutically effective for may be truncated. Alternatively, one drug may alter the absorption, distribution, or excretion of another drug, or may compete for serum protein binding, which may result in higher concentrations of free drug that may result in toxicity (Kroner 2002; Boffito et al. 2003). Toxicological interactions can also occur when co-administered drugs have the same target organs (Zimmermann et al. 2006).

The European Medicines Agency and the US FDA have each issued guidelines on the preclinical development of products containing multiple active ingredients (CHMP 2008; US FDA 2006), and more recently the ICH M3(R2) (2009) guideline has been updated to include combinations. It is generally recommended that the separate active ingredients are first evaluated in independent preclinical programs, and the potential for interactions are then evaluated in limited studies that bridge to the individual drug data packages. The types of bridging studies that are required and their timing is dependent upon the stage of development of the individual compounds, with late stage/marketed compounds with significant clinical experience requiring less evaluation, and at a point later in the development program, than NCEs in the early preclinical stages.

Where a combination bridging evaluation is warranted, it should typically comprise a single 90-day repeat dose toxicity study in one species. The design may vary, but the study should be able to distinguish effects and changes in severity of any findings, that are associated with the drug combination from findings that can be attributed to one of the individual drugs. The multiple permutations of drug doses mean that combination toxicity studies can be very large and expensive. Reducing the number of doses tested will reduce the study size, cost, and animal usage, but may also reduce the sensitivity of the assay. Combination studies to evaluate other toxicological end points (e.g., safety pharmacology, genotoxicity, reproductive toxicity, or carcinogenicity studies) are generally not required unless there is a particular concern based on findings from studies with the individual entities.

8 Conclusions

The preclinical development of microbicides is an evolving field, with very few products having previously made it to the later stages of development, and none having achieved registration to date. Therefore, no clear precedent has been

established for what a full program should consist of. Although the development of microbicides bears much similarity to that of other pharmaceutical products, there are a number of aspects in which it differs because of considerations specific to the indication and route of administration. In particular, there is a need to carefully evaluate local effects at the site of application and the potential for changes that could increase susceptibility to infection. Since these effects could be due to active or inactive ingredients in the formulation, or an interaction between the two, the formulation intended for clinical use should be used where possible in the preclinical studies. The program of studies necessary will vary between microbicides, and will depend on the extent of system absorption and the status of the active ingredient(s). Systemically absorbed compounds will require a more extensive evaluation of systemic safety than compounds with little absorption, and products based on active ingredients present in already marketed products can be supported by much smaller programs than those containing new chemical entities. Products containing more than one active ingredient need to be evaluated for drug–drug interactions, and sustained delivery products that comprise a medical device as well as a pharmaceutical need to meet the regulatory requirements for both types of product.

In conclusion, the preclinical development of microbicides requires a rational and scientific approach, based on the regulatory requirements for pharmaceuticals and on an iterative approach taking all existing and newly generated data into consideration. Scientific advice consultations with regulatory authorities are also crucial to ensure all expectations met in this evolving field.

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Clinical Safety and Pharmacology Trial

Christine Mauck, Andrea Thurman and Jill Schwartz

Abstract Guidelines for establishing clinical safety of microbicides in early clinical studies have evolved significantly since the first trials. In addition, because of the difficulty of establishing efficacy of a microbicide prior to Phase III testing, there has been an increasing emphasis on establishing pharmacokinetic (PK)/pharmacodynamic (PD) relationships using genital samples collected in vivo in Phase I studies. A healthy pipeline is critical to success; however, it is unlikely that the majority of microbicide candidates will progress to clinical testing. Those that do enter clinical testing may have different mechanisms of action than early candidates. Given this, drug-specific modifications for early clinical assessment will need to be considered. These emerging issues associated with early clinical trials of microbicides will be reviewed, along with recommendations for future clinical safety and PK/PD evaluation.

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1 Introduction

Microbicides are products that can be applied locally to the genital tract or rectum to reduce sexually transmitted infections (STIs), most urgently HIV. The earliest microbicide candidates were surface active, nonspecific surfactants, such as nonoxynol-9 (N-9) and C31G. These were followed by products that included polyanions such as cellulose sulfate, Carraguard, and PRO 2000, and acid-buffering agents such as BufferGel. Despite entering Phase III trials, none of these candidates were found to be effective (Van Damme et al. 2002; Peterson et al. 2007; Feldblum et al. 2008; Skoler-Karpoff et al. 2008; Van Damme et al. 2008; Halpern et al. 2008; Abdool Karim et al. 2011).

The leading antiretroviral (ARV)-based vaginal microbicidal candidates in clinical development include several reverse transcriptase inhibitors: the nucleotide analog (NtRTI) tenofovir (TFV), alone and in combination with the nucleoside emtricitabine; and a non-nucleoside reverse transcriptase inhibitor (NNRTI), dapivirine (DPV). These targets reverse transcription of the HIV RNA genome to make a DNA copy, preventing multiplication and dissemination. Other classes of therapeutics also in development include maraviroc, which prevents infection by blocking the co-receptor CCR5 on the surface of susceptible cells which is used by HIV to gain entry into the cell.

The first proof of concept of an ARV was demonstrated with TFV gel. A randomized, double-blind, placebo-controlled trial (CAPRISA 004) showed that vaginal TFV 1 % gel reduced male-to-female HIV transmission by 39 % in all users and 54 % in high adherence (>80 % of sexual acts associated with gel) users (Abdool Karim et al. 2010). In this study, women followed a regimen in which dosing could take place up to 12 h before and up to 12 h after intercourse, with no more than two doses administered in a 24-h period (BAT24). The HIV incidence rates in the TFV gel and placebo arms were 5.6 (confidence interval [CI]: 4.0, 7.7) and 9.1 (CI: 6.9, 11.7) per 100 women-years, respectively (incidence rate ratio = 0.61; $P = 0.017$) (Abdool Karim et al. 2010).

An important aspect of drug development is establishing the safety of a product in healthy volunteers before progressing to larger efficacy trials. First-in-human studies are typically required to be small studies, to minimize potential safety risk of new chemical entities, and are followed by larger studies in relevant populations. A review of published safety studies proposed conducting larger and longer

safety studies in order to detect clinically important safety signals and to confirm readiness for large effectiveness trials (Poynten et al. 2009). Given the limited resources available for clinical testing, it remains important to conduct well--designed studies that efficiently collect relevant data. It is also important to understand the pharmacology and PKs of a drug early on. For microbicides, these trials have particular significance because the target population consists of HIV-negative and otherwise healthy individuals, and it is therefore essential to understand whether a product has safety issues that would result in an unfavorable risk: benefit profile before large numbers of people are exposed.

As newer microbicide candidates enter early clinical testing, it is important to re-examine the evaluation pathway based on lessons learned thus far from first and second generation products. In the absence of specific biomarkers of safety, early safety studies relied predominantly on local and systemic adverse events to identify safety signals. The field has evolved significantly since these early studies. Methods of assessing immunity as a measure of safety have been actively developed. Knowledge of local and systemic drug concentrations in ARVs currently in development has helped to characterize the drugs. In addition, emerging PD models can help provide insight into preliminary efficacy of microbicides prior to larger pivotal trials. The mechanisms of action of new classes of drugs will help guide whether drug-specific trial modifications need to be considered for the clinical evaluation of newer microbicide candidates.

2 Guidelines for the Clinical Evaluation of Microbicides

Guidelines for the clinical evaluation of microbicides were first published in 1996 (International Working Group on Microbicides 1996) and updated in 2001 (Mauck et al. 2001). The United States Food and Drug Administration (FDA) recently published a draft Guidance for Industry entitled *Vaginal Microbicides: Development for the Prevention of HIV Infection* (http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm328834.htm. Accessed 1/21/13).

Many of the considerations that were identified in the very first guidelines remain important, although the endpoints and technology to assess them have become more sophisticated. Assessing local and systemic adverse effects as well as systemic absorption when possible was, and is still, recommended in early phases of microbicide development. New methods for the preliminary assessment of PDs are being developed.

Considerations that must be taken into account when planning early trials include study design, dosing regimen, study population, and study endpoints. Early microbicide trials generally involve the use of a placebo as a negative control in either a parallel or crossover design. The Universal HEC placebo was designed as a placebo for use in microbicide trials because it has few, if any, effects of its own on symptoms and signs of irritation or on effectiveness (Schwartz et al. 2007). Studies with a parallel design require a shorter duration of participation by each

subject and thus tend to retain a larger proportion of participants than crossover studies. Crossover studies, however, allow women to serve as their own controls, reducing the effect of intersubject variability on study endpoints.

Trial design must be individualized given the characteristics of the investigational product and the available preclinical and clinical data. In general, first-in-human studies include a small group of 10–12 volunteers for up to a week of dosing. Subsequent Phase I studies have typically included approximately 12–48 volunteers exposed for a duration of up to 2 weeks. Phase IIa expanded safety studies have evolved to include more endpoints, given the cost of trials and the need to accomplish more rigorous safety testing, and can include as many as 100 participants exposed to investigational product. This can range from 2 weeks to up to 6 months of exposure, as longer exposures to evaluate safety may be required prior to embarking on HIV prevention trials. It is important to evaluate safety in at-risk populations; however, less-developed countries with high HIV incidence may not have the infrastructure to perform complex PK sampling. Also, first exposures in humans require healthy low-risk participants and performing biopsies in women at high risk for HIV acquisition may further increase their risk through the creation of portholes for transmission in the disrupted genital mucosa.

While final labeling for the product may call for coitus-associated use (precoital or combined pre- and postcoital as in the CAPRISA 004 study), dosing in the first safety studies should be in abstinent women to avoid the possible confounding effect of sex and semen on study endpoints (Herold et al. 2011). Each woman should use the product(s) assigned to her for at least 7 days, with 14 days being the longest feasible duration of use that avoids menses. For a product being used in humans for the first time, clinical assessment after the first dose is advisable. When the safety of once-daily use has been demonstrated, dosing can be escalated to twice, three times, or even four times per day, depending on how the product will eventually be used.

Use of the product by sexually active women should then be assessed. The women should be in mutually monogamous relationships to reduce the likelihood of STI acquisition. Condom use should be advised for the same reason, and also for contraceptive purposes, and to avoid male exposure to the product until penile safety has been established in a male tolerance study. The use of effective contraception is also required to prevent pregnancy while exposed to an investigational product. Multiple cohorts of sexually abstinent and sexually active participants using escalating doses have been successfully enrolled in a single study, thereby saving both time and resources (Mayer et al. 2006). It should be noted that the challenges involved in demonstrating adherence to instructions to use the product and to avoid exposure to semen in a clinical study are significant but outside the scope of this chapter.

Women in early trials should be healthy and should include the entire age range of potential users, including postmenopausal women, since both PK and PD may differ between pre- and postmenopausal women (Patterson et al. 2011; Rollenhagen and Asin 2011). Participants should not be using any concomitant medication that may affect study endpoints. The effect of hormonal contraception

on the vaginal environment has been the subject of much debate (Wira et al. 2010; Hel et al. 2010; Fichorova et al. 2011), and until it is clarified it may be beneficial to exclude hormonal contraception during early trials, although this can be challenging given that effective contraception is required. The use of hormonal methods may cause changes in the local genital tract that could affect PK and other endpoints, so if these methods are used this should be considered in the analysis of data. Safety in adolescents and pregnant women should be assessed per regulatory guidelines. Although microbicides are not intended for use in HIV positive individuals, some safety trials have enrolled HIV-infected women since it is possible that women who are unaware of their status could use them and safety in that population may differ (Mayer et al. 2006). When studying HIV-infected volunteers, other relevant endpoints may include resistance mutations and genital and systemic viral load. For example, after TFV gel exposure in HIV-infected volunteers, no new reverse transcriptase resistance mutations were detected (Mayer et al. 2006). Routine early testing in HIV-infected women has not been required for all microbicide products.

Consideration should be given to assessing rectal safety in trials of products intended for vaginal use, given the possibility that such products may also be used rectally by women and/or by men who have sex with men (MSM). Although the precise study design that will be required to support registration of vaginal microbicide is not known, the types of investigation that are likely to be necessary have been incorporated in Phase I trials of microbicides for rectal use. One example is a trial of a reduced glycerin TFV 1 % gel specifically formulated for rectal use, which was found to be safe and acceptable when administered rectally for 7 days in 16 sexually abstinent volunteers (McGowan et al. 2013). Endpoints should include subjective symptoms such as genital pain, pruritus, bleeding, and discharge, as well as systemic symptoms and changes in systemic laboratory examinations such as complete blood counts and serum chemistries. Participants should be examined for objective signs of irritation, bleeding, and other findings. Severity of findings should be recorded using the National Institutes of Health Division of AIDS Toxicity tables, including Addendum 3, the Female Genital Grading Table for Use in Microbicide Studies http://rsc.tech-res.com/Document/safetyandpharmacovigilance/Addendum_1_Female_Genital_Grading_Table_v1_Nov_2007.pdf *(Accessed 1/21/13).*

The role of colposcopy in microbicide evaluation has been controversial for years. It is clear that more findings are detected using colposcopy, but the clinical significance of these findings has not been established. A recent paper suggested that colposcopy was not helpful in identifying an unsafe product (N-9) (Mauck et al. 2012). It was recommended that colposcopy can be considered for early studies, such as first-in-human studies, but not in large studies, and that colposcopy should be replaced with a more objective validated biomarker of HIV risk. For example, general increases in leukocytes (CD45+), specific HIV target cells (CD4+), or activated cells (HLA-DR+, CCR5+) in vaginal tissues signal immune cell activation and may serve as biomarkers for mucosal safety. In addition, decreases in epithelial thickness and number of cell layers based on histology and

in epithelial tight junction proteins (e.g., E-Cadherin, Zona Occludin 1, or Langerin) may be a marker of diminished epithelial integrity and reduced barrier to infection.

The normal vaginal flora plays an important role in protecting against infection (see Holt and Nuttall 2013, this volume) and therefore changes in vaginal microflora should also be assessed, including lactobacilli, bacterial vaginosis (BV), and other reproductive tract infections. It should be established beforehand that the product does not interfere with these (or any other) assays since this could potentially interfere with diagnostic testing.

A major change in assessing microbicides since the 2001 update to the guidelines is a greater appreciation of the role of immunity of the lower genital tract. In addition, assessing PKs has advanced well beyond measuring levels of drug in the blood and cervicovaginal lavage (CVL) to include sampling cells and tissues for both the administered drug and, when relevant, its active metabolite. In addition, PD assays have been established involving ex vivo assessment of the anti-HIV activity of cervicovaginal fluids obtained by lavage from study participants (Keller et al. 2006, 2007), and assays are also in development in which biopsies of cervical or colorectal tissue exposed to drug during its use by study participants are tested in the laboratory to determine whether they can be infected by HIV (Anton et al. 2011). These three changes are discussed in more detail below.

3 Assessing Immunity of the Lower Genital Tract as a Measure of Safety

A safe and effective microbicide is one that does not interfere with natural defenses against HIV and other pathogens or otherwise increase the likelihood of infection. Assessment of candidate microbicide effects now includes their effects on innate defenses, including epithelial integrity, antimicrobial peptides, commensal flora, inflammatory cytokines (interleukins, chemokines, and interferons), leukocyte phenotype, and activation status.

3.1 Epithelial Integrity

An intact epithelium is a first line of defense against infection. Epithelial thickness and number of cell layers have been evaluated histologically for many years. However, immunohistochemistry can now be used to evaluate the density of epithelial junctional proteins (ZO-1 and E-cadherin) in vaginal tissue biopsies (Blaskewicz et al. 2011), as well as the number of cells with the Ki-67 protein, a cellular marker for proliferation (Scholzen and Gerdes 2000). While collecting

biopsies is invasive, specimens obtained for this purpose or during the same procedure may be used for other endpoints as described below.

3.2 Antimicrobial Factors and Commensal Flora

Cervicovaginal (CV) secretions normally contain antimicrobial factors which exert antifungal, antibacterial, and antiviral properties (Cole and Cole 2008). The ability of vaginal fluid to selectively support normal flora but inhibit pathogens depends on the presence of abundant antimicrobial substances, including lactic acid, hydrogen peroxide (H_2O_2), and cationic antimicrobial polypeptides (cAMPs) such as lysozyme, lactoferrin, and secretory leukocyte protease inhibitor (SLPI) (Valore et al. 2006; Cole and Cole 2008).

The importance of these factors and a normal vaginal microbiome is supported by data that show the CV secretions from healthy women exhibit ex vivo activity against HIV and herpes simplex virus (HSV) (John et al. 2005; Ghosh et al. 2010; Shust et al. 2010). Assays of specific mediators, such SLPI, trappin-2/elafin, MIP3α, human ß defensins (HBDs), human neutrophil peptides (HNPs), and interleukins sometimes correlate with overall antimicrobial activity. For example, one group showed that when cAMPs were depleted from the CVL, all antimicrobial activity was lost (Venkataraman et al. 2005). However, the precise mechanisms that contribute to this inherent antimicrobial activity are not fully understood. It is likely that endogenous antimicrobial activity represents cumulative interactions between antimicrobial factors, soluble mucosal immune mediators, the local microbiome, and still uncharacterized factors.

Functional assays attempt to model the global antimicrobial effect of CV secretions, in the presence or absence of variables such as topical microbicides. It is important, when performing functional antimicrobial assays, to correlate the results with cell cytotoxicity data (Hillier et al. 2005). For example, early clinical studies supporting the antimicrobial activity of N-9 in vitro also showed that the antimicrobial concentrations needed were cytotoxic to genital tract cells (Bourinbaiar and Fruhstorfer 1996). As such, although N-9 displayed anti-HIV activity in vitro, it caused cell death which was verified by the cell cytotoxicity data. The toxicity of N-9 is believed to be the cause of the increase in HIV infections observed in women using this product as a microbicide (Van Damme et al. 2002). Also, in cell- or tissue-based models, a product may appear to have inhibitory activity against HIV when, in fact, the cytotoxicity of the product may simply be causing the death of the cells in which the virus replicates, so it is important to establish that reductions in viral replication are actually due to the antiviral activity of the product.

Several groups have shown that BV and Trichomonas vaginalis (TV) compromise the normal antimicrobial properties of the genital mucosa (Fichorova et al. 2006; Valore et al. 2006; Cauci and Culhane 2007; Novak et al. 2007; Simhan et al. 2007) One group showed that women with BV had reduced anti-*E. coli*

activity in vitro, an effect that was reversed with effective treatment of the BV (Valore et al. 2006). BV and mycoplasma colonization have been associated with activation of the long terminal repeat region of HIV (Spear et al. 1997; Al-Harthi et al. 1998).

In addition to the effect of lower genital tract infections, substantial inter- and intra-individual variability exists in antimicrobial activity likely due to covariates such as age (Madan et al. 2012), endogenous hormonal milieu (Keller et al. 2007; Shust et al. 2010), and the presence of semen and seminal plasma (Patel et al. 2007) or other temporary alterations of mucosal immunity which may occur after vaginal intercourse (Keller et al. 2010). There is also a gap in the data as to how ethnicity, race, and environmental exposures such as diet or tobacco affect the normal antimicrobial properties of the lower genital tract.

It is hoped that these functional ex vivo assays will correlate with in vivo results from large clinical studies, because this will provide a means of selecting the most promising candidate microbicides and suitable dosages for evaluation in Phase III trials based on data from early small trials. In support of this, women who used TFV gel daily for 14 days, and had a CVL collected at baseline and days 3, 7, 14, and 21 were shown to have significantly more anti-HIV activity of the CVL than women randomized to receive placebo gel (Keller et al. 2011). Conversely, N-9, a former HIV microbicide candidate, was shown to adversely alter the antibacterial properties of the CVL (Fichorova et al. 2001). However, documentation of efficacy in ex vivo studies (Keller et al. 2006, 2007) does not always translate into effective, large-scale clinical intervention trials (McCormack et al. 2010; Abdool Karim et al. 2011), and thus these important functional assays continue to evolve.

3.3 Inflammatory Cytokines (Interleukins, Chemokines, and Interferons)

There is evidence that local inflammation in the lower reproductive tract may increase the susceptibility to HIV infection (van de Wijgert et al. 2008, 2009; Van Der Pol et al. 2008; McClelland et al. 2007). Therefore, biomarkers of inflammation may have utility in identifying candidate microbicides that could make individuals more, rather than less, prone to infection.

Cytokines are small cell-signaling proteins produced by many kinds of cells. Interleukins serve to provide communication between cells, while chemokines mediate attraction and chemotaxis between cells. Interferons have antiviral functions such as shutting down protein synthesis. Binding of cytokines to their cell-surface receptor results in intracellular signaling that may result in up- or down-regulation of genes, which may in turn result in feedback inhibition, production of other cytokines, or production of surface receptors for other molecules. Cytokines can be assessed in clinical trials by testing CVLs or vaginal fluid aspirate.

Significantly higher levels of inflammatory cytokines IL-1β, IL-6, IL-7, TNF-α, MIP-1α, MIP-1β, and GM-CSF in CVL were seen in women in the CAPRISA 004 study who later became HIV-infected (Roberts et al. 2011). However, some of these same cytokines may have been thought to have a suppressive role in HIV infection, such as RANTES (regulated and normal T cell expressed and secreted), MIP-1α, and MIP-1β which are secreted by activated CD8+ T cells (Cocchi et al. 1995). RANTES is also chemotactic for T cells and is secreted by activated CD4+ cells, so an increase may indicate an increased risk for HIV. In the hormonal contraception (HC)/HIV study, cervical RANTES levels among women who later became HIV-infected were higher than in women who remained uninfected (Morrison et al. 2012).

3.4 Leukocyte Phenotype and Activation Status

Immunohistochemistry and flow cytometry can be used to determine leukocyte phenotype using cell-surface molecules to determine both the type of cell and its activation status. Cells from the CVL cell pellet, endocervical brushings, or biopsies can be used to evaluate whether a microbicide causes recruitment and activation of HIV target cells. In one clinical trial, three doses of N-9 were associated with an influx of polymorphonuclear neutrophils into the cervicovaginal secretions, as well as an increase in CD68+ cells (Fichorova et al. 2001). VivaGel® was associated with a trend toward an increased number of CD8+/CD69+ activated T cells after 7 days of twice-daily use, and a higher number of CD11+/DC-SIGN+ cells after 21 days of use (Moscicki et al. 2012). Although the exploratory nature of these assays means that their biological significance is unclear, they do provide evidence of changes that may warrant further investigation in subsequent trials.

4 Pharmacokinetics in Early Microbicide Trials

The goal of intravaginal or intrarectal application is to deliver drug at the site of viral entry, and achieve effective genital concentrations while minimizing systemic uptake with potential toxicity and the development of resistance. Given that early microbicides were surfactants and polyanions and were not readily absorbed, blood and tissue concentrations were not evaluated. However, ARV compounds are generally small molecules that often have modes of action that occur within host cells or tissues, so uptake into the genital tract is of critical importance and the ability to measure concentrations is valuable to early assessment. Two ARV microbicide candidates are in Phase III testing: TFV gel and the DPV ring.

Tenofovir gel is the only vaginal microbicide with proof of concept data for HIV-1 prevention (Abdool Karim et al. 2010) and has extensive PK data

(Mayer et al. 2006; Schwartz et al. 2011; Hendrix et al. 2013; Karim et al. 2011). TFV is a nucleotide reverse transcriptase inhibitor (NtRTI) that prevents transcription of HIV RNA to DNA when converted to its active form, TFV-diphosphate (TFV-DP) (Robbins et al. 1998). TFV 1 % gel has been found to be safe and well tolerated in women and men (Mayer et al. 2006; Schwartz et al. 2009; Hillier 2008; Sokal et al. 2012).

Pharmacokinetic parameters of TFV that have been evaluated have included assessment of concentrations in blood, peripheral blood mononuclear cells (PBMCs), genital tissue, cells, and fluids. The first study to measure female genital tissue concentrations of TFV-DP, the active intracellular metabolite of TFV, showed that tissue concentrations after both single and multiple doses were above the range of in vitro anti-HIV-1 EC_{50}s (0.4–8.5 μM), remained so for at least 24 h postdose, and were also above those required to inhibit K65R-bearing viruses, a mutation that confers resistance to the drug ($EC_{50} = 25.3$ μM) (Schwartz et al. 2011). An open-label crossover study comparing TFV gel versus oral TFV in 144 women in Africa and the U.S. showed that TFV drug concentrations were twofold higher in genital tissue with gel compared to oral administration (Hendrix et al. 2011, establishing that intravaginal application has the potential to achieve high concentrations at the site of viral entry.

TFV concentrations in the cervicovaginal aspirate and the level of protection against HIV infection have been found to be strongly correlated. In the CAPRISA 004 HIV prevention study with TFV gel, women with TFV concentrations >1000 ng/mL in their genital tract aspirates had substantially lower risk for HIV infection (Abdool Karim et al. 2011). These exposure–response relationships identified with gel dosing demonstrate the feasibility of using TFV concentrations in genital fluid and tissue for bioequivalence measures. A pivotal Phase III HIV prevention study with BAT24 dosing, the Follow-on African Consortium for Tenofovir Studies (FACTS 001), is underway to confirm the findings from CAPRISA 004.

Dapivirine is a substituted di-aminopyrimidine (DAPY) derivative that is a NNRTI that binds to, and disables, HIV's reverse transcriptase enzyme. DPV, also known as TMC120, is a promising candidate for a topical microbicide because of its highly potent activity against HIV-1 and its favorable safety profile. A DPV vaginal ring for monthly use has been developed by the International Partnership for Microbicides (IPM), and various DPV vaginal ring formulations have been tested in numerous clinical trials of up to 3 months duration (Gupta et al. 2008; Nel et al. 2009; Romano et al. 2009). The DPV rings were generally well tolerated. Systemic exposure was low for DPV rings (for Ring-004, mean plasma $C_{max} = 0.392$ ng/mL and $AUC_{0-24h} = 8.379$ ng.h/mL) (Nel et al. 2009). Local concentrations in cervicovaginal fluid for the DPV ring were much higher, with a mean C_{max} of 79.9 μg/g and AUC_{0-24h} of 973.2 μg.h/g (Nel et al. 2009). Two Phase III trials are underway to evaluate the DPV ring: ASPIRE (A Study to Prevent Infection with a Ring for Extended Use) and 'The Ring Study.' These mark the first effectiveness trials of a vaginal ring for HIV prevention.

As other microbicide candidates enter early clinical testing, it is important to recognize that different mechanisms of action will present unique challenges for PK testing. The reverse transcriptase inhibitors (RTIs) studied to-date specifically target HIV's earliest intracellular step. With candidates that target HIV later in viral replication (e.g., integrase inhibitors that target insertion of HIV proviral DNA into the host's cellular genome), the timing and type of sampling, and the resulting design of future studies will need to be individualized based on the duration and window of activity. However, determining concentrations of RTIs have proved to be a powerful tool and will continue to be valuable for the evaluation of future candidates.

5 Pharmacodynamics in Early Microbicide Trials: Use of Cervicovaginal and Rectal Explants

Preclinical testing of microbicide candidates (see Holt and Nuttall 2013, this volume) utilizes various laboratory cell lines and PBMCs (Dezzutti et al. 2004; Beer et al. 2006), but these models are not representative of the epithelial tissue and immune cells present in the genital or rectal tract. To address this limitation, models have also been developed using CV and rectal explant tissues obtained either from discarded hysterectomy or colectomy specimens (Abner et al. 2005; Fletcher et al. 2006a; Cummins et al. 2007; Herrera et al. 2009; Rohan et al. 2010; Rollenhagen and Asin 2011) or biopsies from healthy volunteers (Abner et al. 2005; Fletcher et al. 2006a; Schwartz et al. 2010). The tissues are grown in tissue culture and are usually treated in vitro with various microbicide or other test compounds (Abner et al. 2005; Fletcher et al. 2006a; Cummins et al. 2007; Herrera et al. 2009; Rohan et al. 2010). Various endpoints have been assayed to determine the susceptibility of the explant tissue to a productive ex vivo HIV-1 infection. These endpoints include assay of p24 antigen from the supernatant by enzyme linked immunoassay (ELISA) or p24 incorporation into immune cells by co-localization with immunohistochemistry (IHC) after various durations of tissue culture (7–21 days) (Abner et al. 2005; Fletcher et al. 2006a; Cummins et al. 2007; Herrera et al. 2009; Rohan et al. 2010). Others have monitored the efficiency of ex vivo HIV-1_{BaL} uptake by explant tissue by examining the quantity of proviral DNA (long-term repeat) in tissues by real-time polymerase chain reaction (rtPCR) (Fletcher et al. 2006a). Finally, the quantity of HIV-1 RNA can also be assessed in explant tissue after ex vivo infection by PCR (Fletcher et al. 2006a).

Efforts are in progress to adapt these models for evaluating samples collected during clinical trials. Samples of cervical, vaginal, or colorectal tissue can be collected by biopsy and cultured in much the same way as in the established laboratory models; however, to-date the results have been variable. Some success has been achieved in assays involving colorectal biopsies collected following exposure to rectal microbicides (Anton et al. 2011), which may be due, at least in

part, to the number of samples that can be collected per individual relative to the small number of samples of cervical or vaginal tissue that can be collected. In addition, colorectal tissue has high concentrations of CCR5 and CXCR4 co-receptor positive CD4+ cells, making these tissues ideal for ex vivo models (Fletcher et al. 2006a).

Limitations of the explant model include substantial interdonor variability when tissues are obtained from discarded hysterectomy specimens, lack of endogenous hormonal stimulation, loss of epithelial tissue with prolonged tissue culture times, inability to regenerate or repair after application of test products, and inability to model immune cell trafficking in response to product application (Rohan et al. 2010; Schwartz et al. 2010). In addition, there is likely substantial variability in the density of HIV-1 target cells in CV explants, which are usually approximately 15–60 mg of tissue. Many of these limitations also apply to the use of biopsies from trial participants. In addition, for assays using samples collected during trials there are logistical challenges in transferring the samples from the clinical trial site to the testing laboratory. If the two locations are relatively close, it may be possible to process within a short period after collection, but for longer distances it may be necessary to freeze the samples, and the impact of freezing on biopsy samples is not fully understood (Schwartz et al. 2010, McGowan 2012). It is also not clear if ectocervical versus vaginal tissue is the best tissue for efficient infection in an ex vivo model. Some investigators use Phytohemaglutinin A (PHA), a phorbol ester that activates multiple proliferative signaling pathways in cells, along with HIV-1_{BaL} (Abner et al. 2005; Cummins, et al. 2007) to prime the tissue for more efficient ex vivo infection. No standard protocol as yet exists for ex vivo infection assays, and attempts to correlate infection and toxicity experiments among major labs performing preclinical testing of microbicide candidates have demonstrated variability in infectivity and toxicity studies on cell lines and CV tissues (Beer et al. 2006).

Another limitation of the tissue assays is the variability in correlation with the outcome of clinical trials. For example, TFV vaginal gel, which was successful in the CAPRISA 004 trial, was also shown to prevent ex vivo infection in polarized cervical and rectal explant models, as assessed by p24 antigen production at 21 days (Rohan et al. 2010). TFV vaginal gel also inhibited ex vivo HIV-1_{BaL} infection of rectal explants (Fletcher et al. 2006a; Herrera et al. 2009). However, as with the antimicrobial assays, not all microbicide candidates that are effective at preventing ex vivo HIV-1 infection of CV or rectal tissue have been shown to be effective in large efficacy trials. Both PRO 2000 and Carraguard were effective in the cervical explant model (Fletcher et al. 2006b; Cummins et al. 2007), but failed to show efficacy in clinical trials (McCormack et al. 2010; Skoler-Karpoff et al. 2008). Work is ongoing to develop improved infectivity models utilizing human biopsy tissue.

6 Conclusions

The methods to establish clinical safety of microbicides have significantly evolved to become more extensive and more sophisticated since the seminal trials. A robust clinical screening algorithm is essential to select those microbicide candidates that should progress to large-scale clinical testing for efficacy. Assessing immunity as a measure of safety, measuring drug concentrations (PK), and developing models to predict efficacy (PD) have the potential to strengthen early clinical assessments, select the best candidate microbicides and determine the optimum dosage.

As newer microbicide candidates enter early clinical testing, it is important to critically assess how their unique mechanisms of action might influence the testing algorithm. This continues to be an emerging science. Knowledge of genital concentrations can provide rich safety data and help predict efficacy. The susceptibility of tissue biopsies from study participants after exposure to a microbicide candidate to an ex vivo HIV-1 infection can provide an early correlate of efficacy.

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Microbicides for Prevention of HIV Infection: Clinical Efficacy Trials

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Abstract Microbicides are an important HIV prevention technology under development, but the clinical testing of candidate products for efficacy faces many design and ethical challenges. Nevertheless, several microbicide candidates have been tested or are under development. Eight candidate products have entered late stage microbicide effectiveness trials. Following 11 disappointing effectiveness trial results of six candidate products over the past 20 years, substantial progress is now being made in microbicide development following the release of the CAPRISA 004 tenofovir gel trial results in 2010, which provided proof of concept that topical antiretroviral microbicides can prevent sexual transmission of HIV and herpes simplex type-2 infection. A trial is currently underway to confirm the effectiveness of tenofovir gel and two others have recently been initiated to assess ring formulations of the antiretroviral drug, dapivirine.

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1 Introduction

Since the concept of a women-controlled HIV prevention technology was first proposed more than two decades ago (Stein 1990), eight candidate microbicides (some tested as multiple doses and formulations) have entered late stage trials to assess their effectiveness in preventing HIV infection. In this chapter, we describe the limitations in the ability of standard Phase III double-blinded placebo control trials to accurately estimate the efficacy of microbicides, we recount the history of microbicide effectiveness trials and we explore approaches to improve future microbicide efficacy trial design and conduct.

2 Limitations of Microbicide Trials

2.1 Criteria for Making the Decision on Which Products Should Enter Efficacy Trials

Microbicide development has the distinct challenge of not having a precedent to emulate (e.g., HIV vaccine development can follow previous successful strategies used to develop vaccines against other viruses). Dose level selection has, to date, been largely empiric. Since the drug level required for protection against HIV is not known, the aim has been to achieve therapeutic levels, where this has been established, in local genital tissues. Currently, no markers exist for the biological activity of microbicides and no markers have been established as correlates of protection for microbicides. Therefore, in the microbicide field it is very difficult to

predict which products are likely to succeed and which are not. This obstacle presents a major impediment to rapid progress in the field, as reduced HIV infection in humans is the key marker of biological activity and efficacy, and increased infection can be an important indicator of a safety concern. This means that meaningful studies of effectiveness of a microbicide can only be designed with HIV infection as the primary end point. Due to the lack of surrogate outcomes, the path toward licensure typically moves directly from expanded safety studies to expensive, time-consuming Phase IIb/III trials with incident HIV infection as the outcome. The decision to proceed with large-scale Phase III effectiveness studies, therefore, has to be based on a combination of pre-clinical data, animal challenge data, and safety.

2.2 Validated Animal Model

Another significant challenge in the microbicide field is the applicability of the animal model used in pre-clinical efficacy testing. Several animal models have been used in pre-clinical microbicide testing [e.g., the mouse HSV-2 model and the nonhuman primate (NHP) model; see Herrera and Shattock 2013, this volume]. The predominant model being used is the simian immunodeficiency virus and the simian HIV challenge in NHPs, but the biological relevance of this model remains contentious (Stone and Harrison 2010). Owing to the substantial differences between the human and primate vagina (e.g., the primate vagina is neutral pH, whereas the human vagina has a low pH), it is unclear whether the NHP model accurately predicts what would occur in humans. One example of this paradox is PRO2000; despite 0.5 % PRO 2000® (Indevus Pharmaceuticals, Lexington, MA, USA) showing potent activity against HIV in vitro (Dezzutti et al. 2004; Greenhead et al. 2000) and in animal models for vaginal HIV transmission (Lewis et al. 2002; Scordi-Bello et al. 2005; Weber et al. 2001), human studies show that, although safe, 0.5 % PRO 2000® gel may have little or no effect on reducing a woman's risk of HIV infection (Abdool Karim et al. 2010b; McCormack et al. 2010). Furthermore, due to the high costs involved, only a limited number of animals can be used, which makes the generalizability of the results difficult. Usually, multiple exposures or very high doses of SIV are required to infect sufficient numbers of animals intravaginally with SIV. To overcome this, progestins, e.g. Depo-Provera, are used to synchronize the menstrual cycles and thin the vaginal epithelium, which dramatically increases vaginal transmission rates in NHP (Veazey 2008). Other disadvantages of NHP models are that the assays do not involve intercourse or semen and the models are not standardized. The absence of a validated animal model is a major obstacle to microbicide development, as it means that the first point at which the protective effect of a microbicide candidate can be accurately assessed is in costly and time-consuming large-scale human studies.

2.3 Strategies to Demonstrate Microbicide Efficacy with Clinical Trials

Once a decision is made to proceed to effectiveness trials, there have been two schools of thought on how best to proceed; one approach is to proceed immediately for licensure by undertaking two trials concurrently or one single very large clinical trial and the second approach is to undertake a modestly sized screening trial to obtain an efficacy signal as the basis for proceeding with additional trials for licensure. One advantage of the first strategy is that adequate evidence for licensure is obtained in a single step while the second strategy may involve multiple steps. However, the completion of one very large single trial could take as long as completing two separate trials and is usually very expensive without any guarantee of success. The first strategy using two simultaneous trials was pursued with cellulose sulfate (Ushercell^{TM}, Polydex Pharmaceuticals, Nassau, Bahamas) and is currently being used to test the Dapivarine Vaginal Ring. A single large pivotal study was used for testing Carraguard® (product number PDR98-15, FMC, Philadelphia, PA, USA). The advantages of the screening trial strategy (second approach) are that a highly effective product can be found much faster at a much lower cost, but only if no further trials are indicated. The Phase IIb screening trial strategy was pursued for 0.5 % PRO2000® and tenofovir gel (Gilead Sciences, CA, USA). There is no clear evidence on which of the strategies is most efficient or cost-effective as neither has to date resulted in a licensed product.

2.4 Effectiveness Versus Efficacy

Efficacy is defined as the maximum ability of a drug or treatment to produce a result and represents the protection achieved if the drug is delivered and used correctly every time. Effectiveness is defined as the real life ability of a drug or treatment to produce a result under conditions of "real use". It is measured as reduction in infections averaged across all users. Because not all trial participants in the active arm will use the microbicide correctly or as frequently as required, Phase III microbicide trials actually measure the effectiveness of a candidate microbicide rather than its efficacy.

A participants' willingness and ability to use the product as instructed can directly impact on the ability of the trial to demonstrate effectiveness. For example, a 100 % efficacious product, which is used only 50 % of the time will be only 50 % effective. Underutilization or inappropriate use of product, especially in relation to exposure to HIV, is a serious threat to a microbicide trial being able to demonstrate an accurate level of product efficacy. Evidence from the CAPRISA 004 trial (Abdool Karim et al. 2010a) clearly demonstrates how effectiveness can be eroded with inconsistent use. In CAPRISA 004, although overall effectiveness was 39 %, women who used the gel most consistently (gel adherence greater than

80 %) had a 54 % lower HIV incidence compared with women using the placebo (Abdool Karim et al. 2010a). Indeed, low adherence was identified as being responsible for the lack of effectiveness observed in the FEM-PrEP trial, a study of daily oral antiretroviral prophylaxis in women (Van Damme et al. 2012). To overcome some of the adherence challenges with microbicides, new formulations, such as long-acting vaginal rings which reduce the burden of compliance on the user, are being evaluated.

2.5 Ethical Issues

Several ethical issues are involved in the conduct of microbicide trials. To show safety and effectiveness, the product must be tested on large numbers of sexually active people. Trials also need to be conducted among representative populations that are at a sufficiently high risk of acquiring HIV infection that it will be possible to demonstrate whether the investigational product is protective. In addition, it is important that the efficacy trials are performed in populations that are representative of the target populations for marketing, so that investigators and regulatory authorities can be more confident that effectiveness seen in the trials will be representative of real life effectiveness in the same populations. Clinical trials are thus often carried out in developing countries that have high levels of infection (Abdool Karim and Baxter 2010). This has raised concerns about the potential for exploitation of vulnerable populations; consequently, it is essential that high ethical and care standards are maintained in all microbicide trials.

Counseling on use and provision of condoms as a proven HIV prevention method, in addition to the experimental product, is an ethical and moral prerequisite in all HIV prevention trials, including microbicide trials. Under these conditions, the trial can only measure whether microbicides improve upon the protection afforded by condom use. Microbicides will also only work if they are widely accepted and used consistently by women.

Trial sponsors and implementers are also ethically obliged to provide care and treatment for study participants who become HIV positive during a trial (UNAIDS 2011). This means that planning for the care of HIV-positive people needs to take place at the protocol development Phase and long-term logistical planning is needed to ensure that people living with HIV can access lifelong care and treatment.

Care needs to also be taken to ensure that financial compensation in trials with vulnerable populations is not perceived as an undue inducement. Financial compensation to research participants is usually guided by community stakeholder consultation and needs to be approved by local ethical and regulatory authorities.

Other practical, ethical, and scientific challenges that complicate microbicide trial data include behaviors such as anal sex and the use of other intravaginal substances. The estimated per-act probability of acquiring HIV from an infected

source through unprotected receptive anal intercourse is five times higher than through penile-vaginal intercourse (Leynaert et al. 1998; Varghese et al. 2002). The use of vaginal drying agents may also increase a women's HIV risk when these practices result in a disruption to the integrity of the vaginal epithelium. Participants of HIV prevention trial need to be advised of the increased risks of both these practices.

2.6 Factors Affecting Trial Design

Other concerns surrounding the development of microbicides include the potential hazards related to reproductive toxicity. As a result, women who become pregnant during clinical trials of experimental drugs are usually taken off the product for safety reasons. Frequent and prolonged product withdrawal can compromise statistical integrity in microbicide trials (Lagakos and Gable 2008). Some of the earlier microbicide effectiveness trials had very high pregnancy rates, ranging from 21 to 76 per 100 person-years (Feldblum et al. 2008; Lagakos and Gable 2008; Peterson et al. 2007; Raymond et al. 2007; Smart 2006; Van Damme et al. 2008). To overcome the challenge of high pregnancy rates, more recent studies such as the CAPRISA 004 tenofovir gel trial, the MTN 003 VOICE trial, and the FACTS 001 tenofovir gel trial have included contraceptive-related eligibility criteria in their protocols. Results from the CAPRISA 004 trial have shown that this strategy can be successful in managing pregnancies during microbicide trials and an overall pregnancy rate of 3.95 per 100 woman-years was achieved (Sibeko et al. 2011). Additional studies are necessary to establish the safety of microbicide use during lactation and pregnancy. One trial is has already been initiated to investigate the safety of tenofovir use during pregnancy and lactation (National Institute of Allergy and Infectious Diseases 2012).

2.7 Logistical Issues

Participating in a microbicide trial can be burdensome on the participants and trial staff. This is because most microbicide effectiveness trials require monthly visits for up to 30 months. Monthly visits are necessary to check the HIV status of participants, and thus determine the timing of HIV seroconversions. In addition to HIV tests, each monthly visit will typically include medical examinations (including genital examinations; often quarterly), pregnancy testing, and interviews about sexual behaviors. Retaining participants in follow-up over extended periods can be challenging and substantial effort is usually expended on tracking participants who miss visits.

3 History of Microbicide Efficacy Trials

3.1 Early Microbicides: Surfactants, Blockers, and Buffers

The first microbicide gels to enter Phase III trials were surfactants, which act by inactivating pathogens, including HIV, while they are in the lumen of the vagina. The best known product in this category is nonoxynol-9, which had been widely available as a spermicide for many years before it was tested as a microbicide. Various doses (Richardson et al. 2001) and formulations of nonoxynol-9 were tested, including the sponge (Today Sponge, VLI Inc. Irvine, CA subsequently Whitehall Laboratories American home products, New York, NY) (Kreiss et al. 1992), film (Roddy et al. 1998), and gel (Advantage 24; Columbia Research Laboratories, Rockville Center, NY) (van Damme et al. 2002), but none were shown to prevent acquisition of HIV (Fig. 1). The definitive trial of nonoxynol-9 among sex workers in Benin, Côte d'Ivoire, South Africa, and Thailand, the COL-1492 trial, showed that nonoxynol-9 increased the risk of HIV infection among women who used the product more frequently, possibly owing to an increased frequency of epithelial disruption (van Damme et al. 2002). Several years later, another surfactant, SAVVY® (C31G, Cellegy Pharmaceuticals Inc, Huntingdon Valley, PA, USA), was tested in two separate studies in Ghana and Nigeria, but these studies also did not find any significant effect on HIV prevention, primarily as a result of lower than expected HIV incidence rates in the targeted population (Feldblum et al. 2008; Peterson et al. 2007). It would therefore seem that surfactants do not represent a viable option for microbicides.

Studies of the polyanionic sulphated or sulphonated polymers, which had a more limited spectrum of activity, included cellulose sulfate (Ushercell™, Polydex Pharmaceuticals, Nassau, Bahamas), Carraguard® (product number PDR98-15, FMC, Philadelphia, PA, USA), and PRO 2000®. Despite the evidence of activity against HIV in vitro and in animal studies (Lewis et al. 2002; Nunez and Soriano 2005; Pearce-Pratt and Phillips 1996; Rusconi et al. 1996; Saifuddin et al. 2008; Scordi-Bello et al. 2005), none of these products were shown to prevent HIV in large-scale human trials (Abdool Karim et al. 2010b; Skoler-Karpoff et al. 2008; Van Damme et al. 2008).

The cellulose sulfate trial conducted in several African countries and a site in India was stopped prematurely because of safety concerns. Interim analysis suggested that the product may have increased the risk of acquiring HIV. Final analysis, however, suggested no effect on HIV acquisition (Van Damme et al. 2008). Carraguard®, which was tested among 6202 South African women, was also shown to have no effect on HIV (Skoler-Karpoff et al. 2008). HIV incidence was 3.3 per 100 woman-years (95 % CI 2.8–3.9) in the Carraguard® group (134 events) compared to 3.8 per 100 woman-years (95 % CI 3.2–4.4) in the placebo group (151 events) ($p = 0.30$). In 2009, there was a small glimmer of hope in the HPTN 035 study, which showed a 33 % lower HIV incidence in women using 0.5 % PRO 2000® when compared to placebo, although the results were not

statistically significant (Abdool Karim et al. 2010b). However, subsequent findings from the almost threefold larger MDP 301 trial (McCormack et al. 2010), which had 0.5 % PRO 2000® and placebo groups comprising 6,268 women with 253 HIV infections, showed that 0.5 % PRO 2000 had no protective effect against HIV infection (risk ratio: 1.05).

BufferGel® (ReProtect LLC, Balitimore, MD, USA), designed to maintain a healthy vaginal milieu, was also tested alongside 0.5 % PRO 2000® in the HPTN 035 trial, but no effect on HIV acquisition was detected (Abdool Karim et al. 2010b). Given the disappointing clinical trial results with buffering agents, these candidates have essentially disappeared from the product development pipeline.

3.2 Current Microbicides: Antiretroviral Agents

Several antiretroviral drugs, which were originally developed as HIV therapeutics, are now being tested in clinical trials as potential microbicides, because their mechanisms of action suggest they may be able to prevent as well as treat HIV infection. These antiretroviral agents can act either locally in the reproductive tract mucosa or systemically at specific steps in the HIV replication cycle, and it may be that one or both of these compartments are important for microbicide effectiveness. In addition, these compounds generally have specific activity against HIV only, and therefore the potential for unwanted side effects is limited, particularly where systemic absorption is low.

Tenofovir gel, developed by Gilead Sciences, was the first antiretroviral drug that was shown to prevent sexually acquired HIV infection in women (Abdool Karim et al. 2010a). In 2010, the CAPRISA 004 trial showed that tenofovir gel, applied before and after sex, reduced HIV incidence by 39 % (95 % confidence interval 6–60) overall and by 54 % in women who used the gel consistently (Abdool Karim et al. 2010a). This trial provided proof of concept that an antiretroviral agent can prevent sexual transmission of HIV in women and has provided the first evidence that tenofovir gel is a safe and effective microbicide.

Following the results of the CAPRISA 004 study, there was high hope that the first microbicide would soon be available once the study results were confirmed. The VOICE (Vaginal and Oral Interventions to Control the Epidemic) (National Institute of Allergy and Infectious Diseases (NIAID) 2011) trial had already been initiated and included a tenofovir gel arm, along with additional arms with oral tenofovir disoproxil fumarate (TDF) and oral co-formulated emtricitabine and TDF (Truvada®). A notable difference between the CAPRISA 004 trial and the gel arm of the VOICE trial was that the gel was applied once daily in VOICE, whereas in CAPRISA 004 it was applied before and after sex, but it was anticipated that a positive result in VOICE could be sufficient to support registration of tenofovir gel. Disappointingly, none of the three products tested in the VOICE trial were shown to be effective in preventing HIV infection (Marrazzo et al. 2013). An analysis of detectable drug levels in blood has shown that few of the VOICE participants

adhered to the daily dosing regimen; adherence was estimated to be 23, 28, and 29 % in the tenofovir gel, oral tenofovir, and oral Truvada® arms, respectively (Marrazzo et al. 2013). The lack of protection against HIV observed in the VOICE trial is essentially explained by the low levels of adherence to the daily dosing prescribed in this trial.

In the meantime, another placebo-controlled study, the Follow-on African Consortium for Tenofovir Studies 001 (FACTS 001) (CONRAD 2011b), is attempting to confirm and extend the findings of the CAPRISA 004 trial (CONRAD 2011a) using the same before and after sex dose regimen. If proven effective, tenofovir gel has the potential to alter the course of the HIV epidemic. In South Africa alone, it is estimated that, over the next two decades, this gel could avert 1.3 million new HIV infections and over 800,000 deaths (Williams et al. 2011). Implemented on a broader scale, tenofovir gel could save millions of lives over time.

Two nonnucleoside reverse transcriptase inhibitors, dapivirine (TMC120) and UC781, have also been evaluated as candidate microbicides. Although UC781 was found to be well tolerated and safe in women and men in early clinical studies, further research on this candidate has been put on hold because of difficulties encountered with formulating UC781 in alternative dosage forms (e.g. rings and films) and in combination with tenofovir. Dapivirine is being developed by the International Partnership for Microbicides as a monthly vaginal ring and Phase III trials of the ring were initiated in 2012.

3.3 Current State of Clinical Effectiveness Trials of Microbicides

Although there are over 70 microbicides candidates in the preclinical development pipeline (Stone and Harrison 2010), the number of candidates currently in clinical trial testing is limited to three: tenofovir, dapivirine, and maraviroc. Thus, clinical development activities are currently dominated by antiretroviral agents (Fig. 1) and involve testing different formulations and combinations.

Phase III trials currently underway include the FACTS 001 trial, which is testing tenofovir gel using the same before and after sex dose regimen as the CAPRISA 004 trial (Abdool Karim et al. 2010a) and could provide the data needed for regulatory approval. In addition, two trials of dapivirine (TMC120), formulated as a vaginal ring, were initiated in 2012. These trials will enroll over 5,000 women from six African countries.

In preparation for the implementation of tenofovir gel into the public health service, an open label implementation effectiveness study (CAPRISA 008) is underway in the communities where the CAPRISA 004 trial took place. Previous CAPRISA 004 trial participants will be invited to enroll in this study, which aims to address critical implementation questions about how best tenofovir gel could be

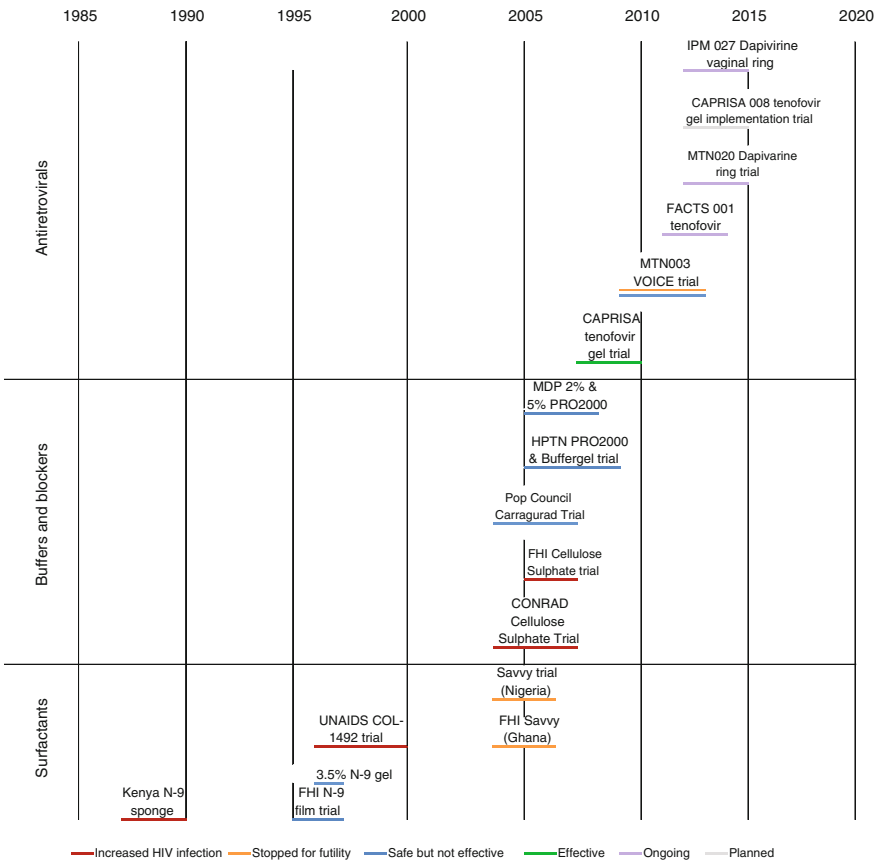

Fig. 1 Past and current microbicide effectiveness trials. Adapted from Stone and Harrison (2010)

incorporated into current health systems and made accessible to women who would benefit most from this product while also providing a mechanism for ongoing posttrial access to the tenofovir gel in these communities.

3.4 Planned Microbicides Trials

Expanded safety studies of a reformulated tenofovir gel for rectal use and the use of 1 % tenofovir gel among adolescents are planned.

A summary of the current clinical effectiveness trials of topical microbicide candidates are summarized in Table 1.

Table 1 Ongoing and planned clinical trials of topical microbicide candidates (March 2013)

Phase	Trial name	Candidate(s)	Mechanism of action	Location	Population
Open label	CAPRISA 008	Tenofovir gel	Replication inhibitor	South Africa	700 women
III	MTN020 (International Partnership for Microbicides 2012b)	Dapivirine vaginal ring	Replication inhibitor	Malawi, South Africa, Uganda, Zambia, Zimbabwe	3476 women
	IPM 027 (International Partnership for Microbicides 2012a)	Dapivirine vaginal ring	Replication inhibitor	Malawi, South Africa,	1650 women
	FACTS 001 (CONRAD 2011a)	Tenofovir gel	Replication inhibitor	South Africa	2900 women

Table compiled from the Global Advocacy for HIV prevention tables on ongoing clinical trials—http://data.avac.org/OngoingMicrobicideTrials.aspx (last accessed 14 August 2012) and from records in ClinicalTrials.gov

4 Future Microbicide Clinical Trial Challenges

4.1 Measuring Adherence

Objectively measuring adherence in microbicide trials has proved challenging. Many of the early microbicide trials relied exclusively on self-reported use, which has several limitations (Mauck and Van de Straten 2008). Dye staining of applicators has been shown to be a reliable and objective method to test vaginal insertion in clinical microbicide trials (Hogarty et al. 2007), but different plastics, dyes, and product formulations may impact the accuracy of this method (Austin et al. 2009). Other novel technologies such as UV light assessment of vaginal applicators (Moench et al. 2012) and wireless technologies, e.g., Wisebag (Gengiah et al. 2010), are also being considered for microbicide trials to monitor adherence. Trials of microbicides containing antiretroviral drugs have made it possible to more objectively assess whether the product has been used or not. Results of recent trials that have measured levels of drug in the vaginal tract or in the plasma have provided investigators with a better understanding of the level of drug needed for protection (Abdool Karim et al. 2011b) or why some products have not worked (Van Damme et al. 2012). The limitation of this method, however, is that we are still unable to measure adherence to the placebo.

With the approval of the first oral antiretroviral drug, Truvada®, for HIV prevention (U.S. Food and Drug Administration 2012), the use of oral Pre-Exposure Prophylaxis (PrEP) may soon become a reality. However, since Truvada® also

contains a form of tenofovir, if it is used by participants in trials of tenofovir gel, the measurement of drug levels as an indicator of compliance may become complicated. The inclusion of some kind of marker in the product as well as the placebo to obtain objective measures of adherence is likely to be required. An exploratory study among 8 women has shown that the alcohol and ketone metabolites from vaginal products that were tagged with esters could be detected using a breath test, suggesting that a breath test for microbicide gel use is physiologically and technically possible (Morey et al. 2012). The limitation of this approach, however, is that the product being tested will not be the same as the one intended to be marketed, which will result in complications for product registration.

Better assessments of exposure risk to HIV and the ability to measure this will be needed. It is not sufficient to assess exposure to semen as current assays such as Prostate Specific Antigen or Y-chromosome set out to do, it is also essential to develop markers of HIV exposure. New HIV PCR assays are able to measure low levels of virus in the vaginal fluids—potentially providing a marker of HIV exposure in the vagina.

4.2 Microbicides for Specific Target Populations

Given the high rates of HIV in adolescents in some regions (Abdool Karim et al. 2011a), they are likely to be an early target population for an effective microbicide, and evaluation of microbicides in this group should be a priority. Unfortunately, none of the studies to date have been conducted in this important group. The first trial of daily tenofovir gel use among 16 and 17 years old (FACTS 002) is, however, planned and will provide important safety data for the use of microbicides in this group.

The development of rectal microbicides has lagged behind vaginal microbicides development but is no less important. The mucosal surfaces in the rectum are vulnerable to physical damage during sex and potentially increase the risk of HIV infection (see McGowan and Dezzutti 2013, this volume). Given that there are distinct structural differences between the vagina and rectum, some candidate microbicides are being designed specifically for rectal use, for example, safety and effectiveness trials of a low osmolality tenofovir gel is already underway.

4.3 Development of Combination Products

Consistent with advances in AIDS treatment regimens that combine antiretrovirals from different classes, microbicides based on a combination of drugs are seen as offering a potential for synergy, reduced drug resistance, and broader activity by targeting of several stages of the life cycle. Although the microbicide candidates

with multiple mechanisms of action or multipurpose products (e.g., microbicide combined with a contraceptive) are already being tested in early clinical trials, no products have advanced to clinical effectiveness trials. The development of these products is considerably more complex than for compounds containing single agents. In the case of microbicides comprising more than one drug, the effectiveness of the combination needs to be shown to be greater than that of the individual drugs alone. For multipurpose products, they may need to demonstrate effectiveness against both indications, which could require multiple trials. Combining physicochemically diverse compounds can be difficult, and if the products are owned by separate companies there are often complicated intellectual property issues. Furthermore, the regulatory pathway for microbicide combinations is unclear.

4.4 Alternative Study Designs

Given the recent successes in the HIV prevention field, including the proof of concept for the use of antiretroviral-based microbicides (Abdool Karim et al. 2010a) and oral PrEP (Baeten et al. 2012; Grant et al. 2010; Abdool Karim and Abdool Karim 2011), some have questioned the ethics of continuing with placebo-controlled trials (Kuhn et al. 2011). However, because we do not yet have an objective marker of adherence for both the active and placebo groups, equivalence studies are challenging. This is because an equivalence trial may produce a biased result where poor adherence in a trial may lead to similar outcomes in each of the arms in a trial, falsely creating equivalence. Further, suboptimal adherence to a highly efficacious product may be as good as high adherence to a low efficacy product. Therefore, superiority designs are likely to be necessary (Grobler and Abdool Karim 2012), which will be even larger, more costly and more logistically challenging than placebo-controlled trials. Alternate adaptive designs are also being considered for future microbicide trials. This approach could increase the efficiency of the trial thereby reducing its duration.

4.5 Funding

Although funding of microbicide research has significantly increased over the years, it still lags far behind research and development funding for other HIV prevention technologies such as HIV vaccines. In 2011, the total global investment for microbicide research and development was US$186 million. This is compared to funding of US$845 million for HIV vaccine-related research and development in the same year: 4.5 times more than microbicides (HIV Vaccines and Microbicides Resource Tracking Working Group 2012). A successful microbicide product will require extensive and sustained investment in research and development.

The product pipeline, in general, needs a large number of products in Phase I because of the high attrition rate before a product warrants assessment for efficacy against HIV infection. It is important to note that a particular microbicide may have only a limited life span because of increases in circulating drug-resistant strains. Therefore, a pipeline of new products will be necessary to address the declining utility of previous microbicides because of drug resistance. At present, the dearth of products from new classes in the Phase I pipeline is a source of major concern.

Funding for microbicide research and development may become even scarcer in the future if limited financial resources are redirected to implementation of PrEP, other HIV prevention strategies, HIV treatment, or other diseases. The widespread availability and accessibility of PrEP may take several years to realize as the implementation of this strategy faces many challenges. Even when PrEP is widely available, women will need access to a range of methods to protect themselves from HIV. The development of other HIV prevention technologies like microbicides therefore remains important, particularly for women.

4.6 Resistance

Some challenges unique to antiretroviral products being developed as candidate microbicides are concerns about the potential for development of drug resistance. Although resistance cannot develop in people who do not have HIV, it could possibly develop if the person taking the prophylactic regimen becomes infected with HIV and continues to take the prophylactic product. The contribution of acquired resistance from prophylactic use of antiretrovirals is anticipated to be a much smaller contributor to drug resistance than the use of antiretrovirals in treatment (Abbas et al. 2011). Encouragingly, to date, the studies investigating tenofovir gel as prevention have not detected resistance (Abdool Karim et al. 2010a). The main issue regarding resistance, however, is whether this will compromise the ARV treatment options for such individuals in several years' time when they may require ART. At present, there are no data to answer this question. It is, therefore, important that effectiveness trials of microbicides include assessments of the development of resistance, either as part of the same trial or through continued monitoring of seroconverters in separate trials. As data are gathered through such studies, they will provide a better understanding of resistance developing due to the use of microbicides.

A separate concern about resistance is the use of the same drugs (e.g., tenofovir) in therapy and prevention. Therapy failure is associated with the development of resistance and thereby the spread of resistant viruses which in turn may compromise the efficacy of the same drugs (or occasionally, the same class of drugs) used for prophylaxis. Some consideration about setting aside a class (or classes) of ARVs for use in prevention only is warranted; however, the options available for doing this are currently very limited due to the small number of ARVs available to microbicide developers.

5 Conclusion

Microbicide effectiveness trials have several inherent challenges, principal among these is adherence to study product. This shortcoming in current effectiveness trial designs is compounded by the inability to accurately measure adherence. Despite this, at least one candidate microbicide has been shown to be effective. Future trials will need to improve measurement of HIV exposure and HIV infection endpoints within the context of new approaches that fundamentally change the traditional double-blinded placebo-controlled randomised trial. Substantial progress has been made in the microbicide development field and, for the first time, the field is optimistic. There is now proof that a safe and effective microbicide, in the form of tenofovir gel, is possible. Despite numerous scientific, ethical, methodological, and implementation challenges, microbicides provide real potential to influence the course of the HIV epidemic, as they fill an important gap for women-initiated prevention methods and could potentially offer an alternative HIV prevention option for men who have sex with men.

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Rectal Microbicide Development

Ian McGowan and Charlene Dezzutti

Abstract The last few years have seen important progress in demonstrating the efficacy of oral pre-exposure prophylaxis, vaginal microbicides, and treatment as prevention as effective strategies for reducing the risk of acquiring or transmitting HIV infection. There has also been significant progress in the development of rectal microbicides. Preclinical non-human primate studies have demonstrated that antiretroviral microbicides can provide significant protection from rectal challenge with SIV or SHIV. Recent Phase 1 rectal microbicide studies have characterized the safety, acceptability, compartmental pharmacokinetics (PK), and pharmacodynamics (PD) of both UC781 and tenofovir gels. The tenofovir gel formulation used in vaginal studies was not well tolerated in the rectum and newer rectal-specific formulations have been developed and evaluated in Phase 1 studies. The PK/PD data generated in these Phase 1 studies may reduce the risk of advancing ineffective candidate rectal microbicides into late stage development. Tenofovir gel is currently poised to move into Phase 2 evaluation and it is possible that a Phase 2B/3 effectiveness study with this product could be initiated in the next 2–3 years.

Contents

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1 Introduction

The field of vaginal microbicide (VM) development began as researchers in women's health and contraception contemplated whether a virucidal gel could be developed that would have activity against sexually transmitted diseases including HIV-1 (Stein 1990). Nonoxynol-9 (N9) had demonstrated these properties in laboratory experiments (Jennings and Clegg 1993) and was rapidly advanced into effectiveness studies. In addition it was also evaluated as a potential rectal microbicide (RM) (Gross et al. 1999a; Tabet et al. 1999). Unfortunately, N9 was not found to be satisfactory as a VM (Van Damme et al. 2002) and was not further developed as an RM. However, these early studies set the framework for how future microbicides were evaluated and emphasized the importance of characterizing safety and acceptability in Phase 1 studies. Following the early N9 studies, the field focused, without much success, on surfactant and polyanion VM candidates (McGowan 2006). During this period there was no research on specific RM. However, it was clear that men who have sex with men (MSM) were interested in the concept of RM and would be willing to enroll in clinical trials evaluating the safety and effectiveness of these products (Carballo-Dieguez et al. 2007b; Gross et al. 1998). It was also apparent that sexual lubricant use, a possible model for RM use, was common among MSM (Carballo-Dieguez et al. 2000). Other important early studies included exploring product formulation preferences in MSM (Carballo-Dieguez et al. 2008a, b) and assessment of the acceptability of different volumes of rectal gels (Carballo-Dieguez et al. 2007a). The HPTN-056 study provided data on the variability of rectal mucosal safety parameters that might be measured in future RM studies (McGowan et al. 2007) and set the stage for the first RM Phase 1 studies evaluating antiretroviral products.

2 The Biology of Rectal HIV-1 Transmission

Ideally, a rectal microbicide should block transmission of HIV-1 regardless if it is the receptive or insertive partner for anal intercourse (AI). The specific processes underlying HIV-1 transmission are still not fully understood, but are dependent on several factors that include the stage of infection (Pilcher et al. 2004), the presence of other sexually transmitted diseases (Cohen et al. 1997; Vernazza et al. 1997), and successful suppression of HIV-1 replication in the infected partner (Baeten et al. 2012; Cohen et al. 2011; Donnell et al. 2010). The risks for HIV-1 acquisition for the receptive partner are about 10-fold higher than for the insertive

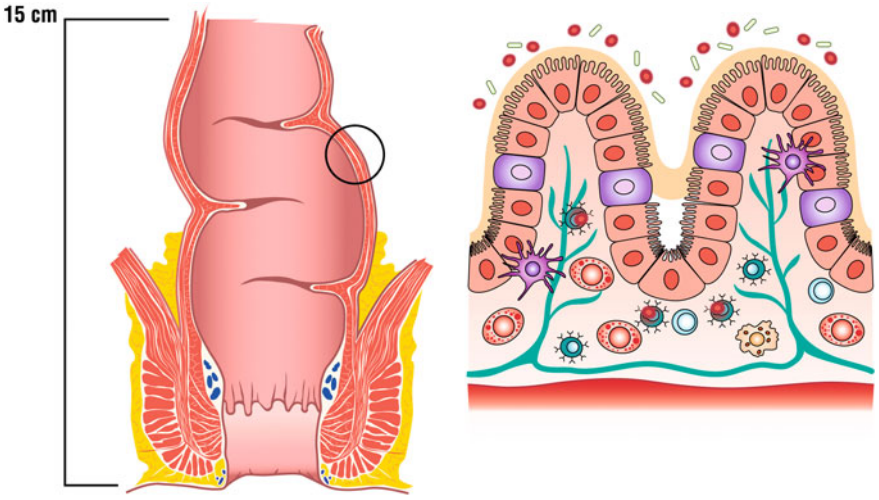

Fig. 1 A detailed view of the colon. The colon is lined with a single layer of epithelium, covered by mucus that separates the luminal contents from the underlying lamina propria. The lamina propria is a rich source of immune cells that includes dendritic cells (*purple*), T cells (*blue*), plasma cells (*pink*), and tissue macrophage (*tan*). Virus (*red*) can reach the lamina propria through micro tears, transcytosis through the epithelium, or by binding to epithelial cells or dendritic cells. Recent data suggest cell-free virus reaches immune targets to infect the resident immune cells (T cells in *blue/red*)

partner (Boily et al. 2009; Varghese et al. 2002). However, these estimates are quite variable due to the factors discussed above (Baggaley et al. 2010).

The GI tract is a rich source of HIV-1 target cells (Fig. 1). Isolated lymphoid follicles, which serve as inductive sites for immune responses, are located throughout the colon (Koboziev et al. 2010). The number of follicles generally increases toward the anus with the greatest numbers found in the rectum (Langman and Rowland 1986, 1992). Antigen presenting cells (macrophage and dendritic cells) along with effector and regulatory T cells are found within the follicles. These cells are generally activated and express HIV-1 co-receptors, CCR5, and CXCR4, as well as soluble immune mediators (Anton et al. 2000; McGowan et al. 2004; Poles et al. 2001; Zhang et al. 1998) thus creating the perfect environment primed for HIV-1 infection.

HIV-1 can reach these activated immune cells in several ways. While micro-tears in the epithelium can occur during coitus, the envelope of HIV-1, gp120, has been shown to increase the permeability of the epithelium allowing HIV-1 to easily traverse to the lamina propria (Nazli et al. 2010). Even with an intact mucosa, epithelial cells can bind and transfer HIV-1 either through active transport, transcytosis (Bomsel 1997), or non-specifically (Dezzutti et al. 2001; Meng et al. 2002; Wu et al. 2003). Finally, dendritic cells extend dendrites through the tight-junctions of the epithelium to sample the luminal environment (Rescigno et al. 2001). HIV-1 can take advantage of this by binding to DC-SIGN and

subsequently infect activated lymphocytes (Gurney et al. 2005). Once past the epithelium, HIV-1 preferentially infects local lymphocytes expressing CCR5 (Anton et al. 2000; Meng et al. 2000). Cell-free and cell-associated virus are both present in the ejaculate and so it is still not clear which virus is the primary driver of mucosal infection (Anderson et al. 2010). However, recent sequence analysis of viral RNA and integrated proviral DNA suggests that cell-free virus contributes the most to mucosal transmission (Butler et al. 2010). These early infection events are known to initiate from a single founder virus in heterosexual transmission (Keele et al. 2008; Sagar et al. 2009), but RAI is associated with a more diverse founder virus population (Li et al. 2010) likely due to direct access to underlying immune cells. This information suggests that an ideal rectal microbicide should protect the epithelium and be active against a swarm of viruses.

2.1 *Epidemiology of HIV Infection Associated with Receptive Anal Intercourse*

Epidemiological studies have confirmed that receptive anal intercourse (RAI) is a common practice among MSM in the Global North (Hart and Williamson 2005; Wolitski and Fenton 2011). Unfortunately, a significant proportion of RAI among MSM is unprotected (Begley et al. 2008; Chen et al. 2003) and contributes to the growing number of new infections among MSM (Beyrer et al. 2010). More recent data have clearly identified at risk MSM in the Global South (Baral et al. 2012; Beyrer et al. 2012). There are also increasing data demonstrating that women in both the US and Sub-Saharan Africa practice RAI (Gorbach et al. 2009; Kalichman et al. 2009). In the VOICE study (Microbicide Trials Network (MTN-003)) of oral and vaginal tenofovir or Truvada® that was conducted in South Africa, Uganda, and Zimbabwe, approximately 7–20 % of the 5,029 women enrolled in the study reported a history of RAI in the prior 3 months (Marrazzo and VOICE MTN 003 Study Team 2012). This high frequency of RAI in women is important from a public health perspective but it also has the potential to interfere with the ability to conduct effectiveness trials of vaginal microbicides as they may not provide protection against rectal infection (Masse et al. 2009; McGowan and Taylor 2010). These observations emphasize the need to develop new prevention strategies, including RM, in populations at risk of HIV infection through unprotected receptive anal intercourse (URAI) who are unable or unwilling to use a condom.

2.2 *The Rectal Microbicide Pipeline*

In theory, the RM pipeline could be derived from the VM pipeline. However, the majority of RM candidates have only been evaluated in preclinical studies and the only products to have been evaluated in clinical studies are the non-nucleoside

reverse transcriptase inhibitor (NNRTI) UC781 (Anton et al. 2011) and the nucleotide inhibitor, tenofovir (Anton et al. 2012). The first generation of antiretroviral rectal microbicides only contained one active pharmaceutical ingredient (API) but there is increasing interest in evaluating combination products with two, three, or even four API (Herrera et al. 2009, 2011). The majority of research has been conducted on nucleotide reverse transcriptase inhibitors (tenofovir) or NNRTIs (UC781 and dapivirine (TMC120)). Recent studies have also evaluated L'644, a fusion inhibitor that demonstrated post-exposure activity in both colorectal and cervical explants as well as activity against reverse transcriptase resistant isolates (Harman et al. 2012). Protease inhibitors are also being considered as candidate RM (Herrera and Shattock 2012; Stefanidou et al. 2012). Potential RM candidates are listed in Table 1 together with their current stage of development.

2.3 Formulation Development

Qualitative and clinical studies have suggested that acceptable RM formulations could include gels, suppositories, or douches (Carballo-Dieguez et al. 2008a, b). Gels were considered more acceptable than suppositories (Carballo-Dieguez et al. 2008b) and a volume escalation trial of a placebo gel demonstrated that up to 35 mL of gel with the physical properties of Femglide® (transparent and odorless) was acceptable to the majority of participants (Carballo-Dieguez et al. 2007a). The first RM clinical trials evaluated the rectal safety of gels that were being developed as VM. These products had a number of characteristics that were suboptimal for an RM. They were extremely hyperosmolar and had an acid pH. Hyperosmolar products are known to induce mucosal damage (Begay et al. 2011; Fuchs et al. 2007) and may be associated with increased risk of acquiring STIs (Gorbach et al. 2012). Rectal use of the vaginal formulation of tenofovir 1 % gel was associated with gastrointestinal adverse events including diarrhea, bloating, urgency, and abdominal pain (Anton et al. 2012). Consequently, efforts are under way to develop rectal-specific microbicide formulations that are iso-osmolar with a neutral pH (Agashe et al. 2012; Wang et al. 2011). Initial preclinical evaluation of a reduced glycerin formulation of tenofovir (RG-TFV) 1 % gel suggested that the new gel induced less mucosal damage than the original formulation but was equally effective in explant models of HIV infection (Dezzutti et al. 2012a). The RG-TFV 1 % also appeared to have improved in vivo safety and acceptability in Phase 1 studies (McGowan et al. 2012a).

2.4 Preclinical Evaluation of Rectal Microbicides

Preclinical testing of API, has been standardized to define the API, but there are subtle variations in the specific assays used based on the preferences of the

Table 1 Products that have been evaluated as candidate rectal microbicides[a]

Product	Development stage	Study	Status	ClinTrials.gov	Reference
Cellulose acetate PRO2000 SPL7013 Vena Gel UC781	Preclinical	Explant model	Completed		Abner et al. (2005)
PRO2000 Dextrin sulfate	Preclinical	Explant model	Completed		Fletcher et al. (2006)
Tenofovir	Preclinical	Explant model	Completed		Dezzutti et al. (2012)
C34 T20 T1249 L'644	Preclinical	Explant model	Completed		Harman et al. (2012)
Tenofovir Emtricitabine UC781 Dapivirine (TMC120)	Preclinical	Explant model	Completed		Herrera et al. (2011)
Saquinavir	Preclinical	Explant model	Completed		Stefanidou et al. (2012)
Maraviroc Griffithsin MIV-150 Carageenan Zinc acetate	Preclinical	Explant model	Ongoing		
BufferGel® Nonoxynol-9 C31G Octylglycerol Polystyrene sulfate Cellulose sulfate SPL7013 Carraguard UC781	Preclinical	NHP/safety	Completed		Summarized in Patton et al. (2009)
Cyanovirin gel	Preclinical	NHP/Efficacy	Completed		Tsai et al. (2003)
Tenofovir gel	Preclinical	NHP/Efficacy	Completed		Cranage et al. (2008)
MIV-150 gel	Preclinical	NHP/Efficacy	Completed		Singer et al. (2011)
Nonoxynol-9	Phase 1	HIVNET-008	Completed	NCT00000929	Tabet et al. (1999)
UC781 gel	Phase 1	RMP-01	Completed	NCT00408538	Anton et al. (2011); Ventuneac et al. (2010)
Tenofovir 1 % gel (VF)	Phase 1	RMP-02/ MTN-006	Completed	NCT00984971	Anton et al. (2012)
Tenofovir 1 % gel (RGF)	Phase 1	MTN-007	Completed	NCT01232803	McGowan et al. (2012)
Tenofovir 1 % gel (RGF)	Phase 1	Project Gel	Ongoing	NCT01283360	N/A
Tenofovir 1 % gel (VF, RGF, RF)	Phase 1	CHARM-01	Q3 2012	NCT01575405	N/A
Tenofovir 1 % gel (VF, RGF, RF)	Phase 1	CHARM-02	Q3 2012	NCT01575418	N/A
Tenofovir 1 % gel (RGF)	Phase 2	MTN-017	Q3 2012	NCT01232803	N/A

[a] Products that have been studied in colorectal explant systems, animal models, or humans

VF vaginal formulation, *RGF* reduced glycerin formulation, *RF* rectal-specific formulation, *NHP* non-human primate

laboratory doing the testing (Buckheit, and Buckheit 2012; Lackman-Smith et al. 2008; Lard-Whiteford 2004). Initial tests using primary immune cells, such as peripheral blood mononuclear cells, and indicator cell lines are performed to determine mechanisms of action and potency of the API against standard laboratory and primary clinical isolates of HIV-1. Some testing is now being conducted with the newly identified primary isolates known as transmitter/founder viruses from persons who acquired HIV-1 through penile-vaginal or penile-rectal coitus (Dezzutti et al. 2012b; Keele et al. 2008). The incorporation of biological fluids such as semen, cervicovaginal fluid, or their simulants is also used early in the testing to ensure the API remains potent during coitus (Neurath et al. 2006; Patel et al. 2007). More recently, mucosal tissues have been used to evaluate API activity. To screen APIs, non-polarized mucosal tissue (ectocervical, vaginal, and colorectal) has been used (Fletcher et al. 2006; Greenhead et al. 2000; Herrera et al. 2009, 2011; McElrath et al. 2010). Typically, small pieces of tissue are exposed to the drug for a short period and then exposed to HIV-1. After an incubation period, the tissue is washed and placed back in culture to monitor for HIV-1 infection. These assays have characterized many potential rectal microbicide candidates from non-specific entry inhibitors (Fletcher et al. 2006) to more specific non-nucleoside and nucleotide/nucleoside reverse transcriptase inhibitors, fusion inhibitors, and protease inhibitors (Fletcher et al. 2006; Harman et al. 2012; Herrera et al. 2009, 2011). These candidates have been typically evaluated as single entity agents. However, combinations of APIs are now being tested and much like therapy, they show that combinations of up to three APIs (tenofovir or emtricitabine with UC781 and dapivirine) are much more potent against HIV-1 infection in colorectal tissue even against drug resistant HIV-1 (Herrera et al. 2011). These data will help inform microbicide developers as to which candidates/combinations are optimal to pursue for further development.

2.4.1 Formulation Considerations in Preclinical Assessment

Formulation of these APIs for rectal use will likely be in a liquid or semi-solid dosage form to cover areas that are at most risk for HIV-1 exposure (Wang et al. 2011). Preclinical testing of formulated APIs adds additional complexity because pH, osmolality, and viscosity of the product will impact the results. For instance, the polymers used in the formulation may enhance toxicity or efficacy due to smothering of individual cells or non-specifically binding HIV-1. These results may be over interpreted by inexperienced researchers. Therefore, it is critical to include the vehicle control—the same formulation but without the API—in all assays to accommodate the impact the formulation may have on the testing results. As with unformulated APIs, testing algorithms have been developed (Rohan et al. 2010). Typically, the testing done with the formulations is to ensure the APIs activity has not been impaired and the formulation is safe. Mucosal tissues are used for testing the formulations, but are polarized, keeping the apical surface at the liquid/air interface (Abner et al. 2005; Cummins et al. 2007; Rohan et al.

2010). The formulation with or without HIV-1 can be applied to the apical surface recapitulating the use by a person. Using this testing algorithm, it was recently reported that the hyperosmolar 1 % tenofovir vaginal gel formulation demonstrated epithelial facture and sloughing in polarized mucosal tissue (Rohan et al. 2010). The gel was reformulated to reduce the glycerin content and thus reduce the osmolality (Dezzutti et al. 2012a). The reduced glycerin 1 % tenofovir gel showed improved epithelial retention in polarized rectal and ectocervical tissue explants. These data support the clinical trial results (discussed below) that showed that gastrointestinal adverse events where significantly more common when the original 1 % tenofovir gel was used rectally compared to the reduced glycerin gel formulation (Anton et al. 2012; McGowan et al. 2012b). The preclinical testing of formulations is thus important to provide assurance that those products that move into clinical trials are safe as well as likely to be effective.

2.4.2 Animal Models

In addition to in vitro and ex vivo testing, animal models are used to test the safety and effectiveness of an API/microbicide (see Holt and Nuttall 2013, this volume). Several microbicide products have been evaluated for rectal safety and include BufferGel (Patton et al. 2004), Savvy (Patton et al. 2006b), VivaGel (Patton et al. 2006a), and UC781 (Patton et al. 2007). In general, there has been a good correlation between vaginal and rectal safety findings suggesting no more safety concerns for these vaginal formulations when used rectally.

Animal models also are used to evaluate the efficacy of microbicide candidates against HIV-1, SHIV, and other sexually transmitted diseases and include immunodeficient mouse strains (severe-combined immunodeficient (SCID) and non-obese diabetic (NOD)-SCID) and macaques (rhesus, pig-tailed, and cynomolgous) (see Herrera and Shattock 2013, this volume). The SCID mouse strains have been reconstituted with human fetal tissue, typically liver, thymus, and bone marrow, which has allowed them to be repopulated with human immune cells throughout their bodies including mucosal tissues (Berges and Rowan 2011; Denton and Garcia 2011). These animals are susceptible to infection with HIV-1 through systemic, vaginal, and rectal routes. Recently, Denton et al. demonstrated that engraftment of intestinal immune cells in the NOD/SCID model was most efficient in mice that had an intact interleukin-2 common γ chain which suggests that these mice would be superior for rectal microbicide efficacy testing (Denton et al. 2012). All of the published microbicide work to date in the "humanized" mice has focused on vaginal evaluation of microbicide candidates, but work is currently ongoing to adapt this model to evaluate rectal microbicides.

NHP infection models use two different challenge schemes that involve either a multiple low-dose or single high-dose challenge with simian immunodeficiency virus (SIV) or a chimeric SIV/HIV construct (SHIV) (Veazey et al. 2012). Typically the virus used to evaluate microbicides is an SIV modified to express the HIV-1 envelope (SHIV) or the HIV-1 reverse transcriptase (RT-SHIV) (Pal et al.

2012). Using the NHP model, gel formulations of an entry inhibitor, cyanovirin-N (Tsai et al. 2003), and the reverse transcriptase inhibitors, tenofovir, and MIV-150 (Cranage et al. 2008; Singer et al. 2011) prevented rectal challenge of SHIV or RT-SHIV. These data are informative regarding the potential of a low volume of gel to block atraumatic exposure to infectious virus. Of note, there were correlations between plasma drug levels of tenofovir and protection from macaque infection (Cranage et al. 2008). To begin to address where drug distributes after dosing in a more formal way, a multi-compartment pharmacokinetic study in macaques after vaginal or rectal dosing with tenofovir gel was done (Nuttall et al. 2012). Macaques dosed with tenofovir gel vaginally showed rectal drug levels only 1 $\log_{10}$ lower than the vaginal drug levels. And both mucosal compartments were 4–5 $\log_{10}$ higher than plasma drug levels. Similar results were found when the macaques were dosed with tenofovir gel rectally. These data suggest that vaginal or rectal dosing of a soluble microbicide could protect against HIV-1 regardless of the route of exposure.

2.5 Clinical Development of Rectal Microbicides

As with VM, the purpose of Phase 1 RM studies is to generate preliminary data on the safety, acceptability, PK, and PD activity of the candidate microbicide. However, in contrast to VM development where there have been multiple Phase 1 studies of surfactant, polyanion, and antiretroviral candidates, there have only been four Phase 1 rectal microbicide studies conducted to date; HIVNET-008 (Nonoxynol-9 (N9) gel) (Tabet et al. 1999), RMP-01 (UC781 0.1 and 0.25 % gel) (Anton et al. 2011; Ventuneac et al. 2010), RMP-02/MTN-006 (oral tenofovir and tenofovir 1 % gel (original formulation)(Anton et al. 2012), and MTN-007 (N9 gel, HEC placebo gel (Schwartz et al. 2007), and tenofovir 1 % gel (reduced glycerin formulation)) (McGowan et al. 2012a). These studies are discussed in more detail below.

HIVNET-008. The HIVNET-008 study was designed to assess the safety of N9 when applied one to four times daily to the rectum and penis. Twenty-five HIV-negative and ten HIV-positive, monogamous gay male couples were enrolled in Seattle, WA. Each partner was exclusively insertive or receptive while using N9 gel and served as his own control during placebo gel use compared to during N9 gel use. The study was conducted over 7 weeks. During the first week participants used the placebo gel. Thereafter, couples used the N9 gel and the frequency of use was escalated from once daily to two applicators twice daily in the final week of the study. Despite the frequency of administration, adverse events (AEs) were generally mild and transient. No rectal ulcers were detected; superficial rectal erosions were noted in two HIV-negative participants. Abnormal or slightly abnormal histologic abnormalities of rectal biopsies were detected in 31 (89 %) of receptive participants after N9 gel use compared to 24 (69 %) of participants after 1 week of placebo gel use. Excluding participants who felt no need for an HIV

prevention method, 58 % said they would use N9 if approved for rectal use; 69 % of receptive users reported rectal fullness and related side effects after insertion of the gel, and 68 % reported applicator-related discomfort; 59 % of insertive participants found the gel too sticky (Gross et al. 1999b).

RMP-01. Thirty-six HIV-1 seronegative, sexually-abstinent men and women were enrolled in Los Angeles, CA, and randomized into a double-blind, placebo-controlled trial comparing UC781 gel at two concentrations (0.10, 0.25 %) with a placebo gel (1:1:1). Safety and acceptability were primary study endpoints. Changes in colorectal mucosal safety biomarkers and UC781 plasma drug levels were secondary endpoints. Ex vivo explant infectibility with HIV-1 was an ancillary study endpoint. Samples were collected at enrollment, after a single rectal dose of study product, and after seven daily doses. The majority of AEs were mild. Product acceptability was high, including likelihood of future use. No changes in mucosal safety biomarkers were identified. Plasma levels of UC781 were not detected. Ex vivo infection of biopsies using two titers of HIV-1_{BaL} showed marked suppression of HIV-1 p24 in tissues exposed in vivo to 0.25 % UC781 (Fig. 2).

RMP-02/MTN-006. Eighteen participants were enrolled from Pittsburgh, PA, and Los Angeles, CA. All participants received a single 300 mg dose of oral tenofovir and were then randomized 2:1 to receive a single then seven daily doses of tenofovir (TFV) 1 % gel or the HEC placebo gel. Safety endpoints included clinical AEs and mucosal safety biomarkers. Participants were assessed at enrollment, after single doses of oral tenofovir and study gel, and after seven daily doses of study gel. Blood and colonic biopsies were collected for PK analysis and ex vivo challenge with HIV-1. No serious AEs were reported. However, AEs, especially gastrointestinal AEs, were significantly increased with seven-day use of the tenofovir 1 % gel. Only 25 % of participants liked the tenofovir gel; however, likelihood of use, if the product was somewhat protective, was high (75 %). No significant mucosal injury was detected. Tissue TFV diphosphate (TFV-DP) C_{max} 30 min after single rectal exposure was 112 times greater than single oral-exposure with tissue seven-day exposure 5 times greater than single rectal-exposure. Seven-day exposure to rectal TFV was associated with significant suppression of ex vivo infection of colorectal explants collected by biopsy. Increased AEs suggested that the vaginal formulation of tenofovir 1 % gel used rectally was not entirely safe or fully acceptable, suggesting a need for improved formulations.

MTN-007. The study was designed to assess the safety, adherence, and acceptability of the reduced glycerin formulation of tenofovir 1 % gel (RG-TFV 1 % gel). An N9 arm was included as a positive control for the mucosal safety biomarker assays. Sixty-five participants (45 men and 20 women) aged 18–61 were recruited from Pittsburgh, PA; Boston, MA; and Birmingham, AL. Participants were randomized 1:1:1:1 to receive the RG-TFV 1 % gel, a HEC placebo gel, an N9 gel, or to a no treatment arm. Participants were evaluated at baseline, after a single dose, and after seven daily doses of study product. Systemic and mucosal safety, acceptability, and adherence were evaluated at all three visits. Comprehensive mucosal safety biomarker evaluation included histology, fecal calprotectin, epithelial sloughing, cytokine expression (mRNA and protein),

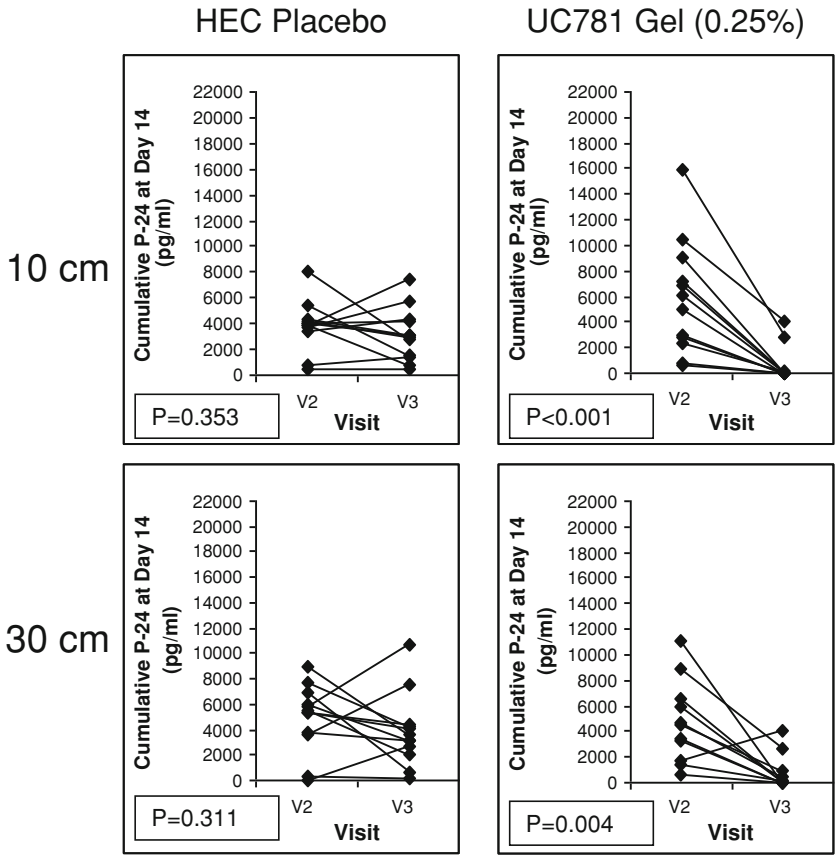

Fig. 2 Colorectal biopsies were collected before (V2) and 30 min after (V3) a single rectal dose of UC781 gel. The biopsies were challenged ex vivo with 10^4 $TCID_{50}$ of HIV-1_{BaL} (Fletcher et al. 2006) and explant supernatant was collected for HIV-1 p24 quantification every 3–4 days following infection (Anton et al. 2011). The pharmacodynamic response was calculated as the difference between V2 and V3 Day 14 cumulative HIV-1 p24 levels

microarray analysis, flow cytometry of mucosal T cell phenotype, and rectal microflora. Acceptability and adherence were determined by computer-administered questionnaires and interactive telephone response, respectively. Product adherence was ≥94 %. AEs were generally mild or moderate. There was no significant difference in the prevalence of AEs across the four arms of the study. Likelihood of future product use (acceptability) was 86.7 % (RG-TFV 1 % gel), 93.3 % (HEC gel), and 62.5 % (N9 gel). Fecal calprotectin and epithelial sloughing did not alter during the study. In contrast, significant changes were seen in mucosal cytokine/chemokine expression, T cell phenotype, and rectal microflora, which were mostly confined to the N9 gel arm. Overall, the study suggested that the RG formulation of 1 % tenofovir was safe and well tolerated and should be advanced to Phase 2 RM development.

Table 2 Composition and characteristics of the currently available formulations of tenofovir 1 % gel

Formulation study	Original VF, % w/w (CAPRISA 004)	RGF, % w/w (MTN-007)	RF, % w/w (CHARM-01)
Glycerin	20.00	5.00	2.50
Hydroxyethyl cellulose	2.50	2.75	0
Carbopol 974	0	0	0.50
Sodium carboxymethylcellulose	0	0	1.00
Methylparaben	0.18	0.22	0.18
Propylparaben	0.02	0.024	0.02
Purified water	75.23	89.936	94.78
Disodium edetate (EDTA)	0.05	0.05	0.01
Citric acid	1.00	1.00	0
PMPA	1.00	1.00	1.00
Sodium hydroxide	As needed	As needed	As needed
Diluted hydrochloric Acid	As needed	As needed	As needed
pH	4.5	4.6	7
Osmolality (mOsmol/kg)	3111	836	479

VF vaginal formulation, *RGF* reduced glycerin formulation, *RF* rectal-specific formulation

A number of additional RM studies are ongoing or will start enrollment in 2012. The National Institutes of Health (NIH) has funded a project entitled "Microbicide safety and acceptability in young men" that attempts to evaluate RM safety, adherence, and acceptability in young ethnic minority MSM in Boston, MA; Pittsburgh, PA; and San Juan, PR. The study design has two stages: A clinical and behavioral evaluation (Stage 1A) with an acceptability and adherence trial (Stage 1B), followed by a Phase 1 randomized, double-blind, multi-site, placebo-controlled safety trial (Stage 2). The first 120 eligible participants who complete Stage 1A and report unprotected RAI in the previous 3 months will continue on to Stage 1B. During Stage 1B, participants will be given condoms and a placebo gel to use during receptive anal intercourse. Over a 3- month period they will report the frequency of product use and be interviewed about the acceptability of the product. The first 24 participants who complete Stage 1B will be eligible to participate in Stage 2 where they will be randomized to receive an actual microbicide (RG-TFV 1 % gel) or matched placebo. It is hoped that data from this study will provide unique insights into the acceptability, safety, and adherence of rectal microbicides in young MSM.

The Combination HIV Antiretroviral Rectal Microbicide or CHARM Program will develop and evaluate a combination antiretroviral rectal-specific product. Tenofovir and maraviroc are the two lead compounds and the ultimate goal is to develop a tenofovir/maraviroc combination product. Two Phase 1 studies, CHARM-01, and CHARM-02 will start in 2012. CHARM-01 will assess the safety, acceptability, and PK/PD profile of three tenofovir gel formulations; the original tenofovir 1 % gel used in vaginal microbicide studies, the RG-TFV 1 % gel, and a rectal-specific TFV gel (Table 2). CHARM-02 will evaluate the safety, PK, and distribution of the same three gels. Similar techniques have been used to

characterize the distribution of semen surrogates and microbicide products in the presence and absence of simulated receptive anal intercourse (Cao et al. 2012; Louissaint et al. 2012). Collectively, these studies will provide unique data on the influence of formulation characteristics, including osmolality, and product safety, PK/PD, and distribution.

The final RM study to start in 2012 will be MTN-017, a Phase 2 expanded safety study of the RG-TFV 1 % gel used in MTN-007. The study will enroll 186 MSM and transgendered women in the US, Peru, Thailand, and South Africa. Each participant will receive 8 weeks' exposure to oral Truvada, daily RG-TFV 1 % gel, and use of RG-TFV 1 % gel before and after sex (analogous to the regimen used in the CAPRISA 004 vaginal microbicide study (Abdool et al. 2010)). Apart from general safety and acceptability, a subset of approximately 30 participants in the US and Thailand will undergo more intensive mucosal sampling for evaluation of mucosal safety biomarkers and PK/PD. If successful, it is hoped that MTN-017 will set the stage for a Phase 2B/3 RM trial in 2015.

3 Rectal Microbicide Advocacy

Drug development does not occur in a vacuum and from the outset advocacy groups have played a critical role in RM development. The International Rectal Microbicide Advocates group (IRMA; http://www.rectalmicrobicides.org/) has helped focus attention on RM development including conducting community/internet-based studies on lubricant usage (Javanbakht et al. 2010). IRMA has also lead efforts to define the need for RM for men and women at risk of HIV infection associated with URAI in Africa. IRMA convened a meeting in Addis Ababa, Ethiopia in November 2011 that has helped catalyze community interest in RM within Sub-Saharan Africa (http://www.rectalmicrobicides.org/ProjectARMreport2012.pdf). Unfortunately, MSM activity is stigmatized, illegal, and even punished by death in many countries across the world (Altman et al. 2012) and conducting RM trials or indeed rolling out RM as prevention in these communities would currently be difficult if not impossible (Kyomya et al. 2012; Semugoma et al. 2012). From a human rights perspective, as well as a drug development perspective, there is much to be done.

4 Conclusions

Given the ongoing epidemic of HIV infection associated with URAI it is clear that there is an urgent need to develop safe and effective methods of HIV prevention that the target population will be willing to use. Although the iPrEx study demonstrated the efficacy of PrEP in MSM (Grant et al. 2010), overall adherence in the participants receiving Truvada was estimated to only be about 50 %. This suggests

that an RM, possibly used in addition to oral PrEP, might increase overall protection from HIV infection. The design of Phase 1 RM studies has evolved such that key data on safety, acceptability, and PK/PD can be generated at an early stage of product development. This will allow for refinements of rectal-specific formulations and the development of combination antiretroviral products. Collectively, this will increase the likelihood of developing a safe and effective RM. However, RAI is a stigmatized human behavior and research in this area remains an important but difficult endeavor.

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Developing Regulatory Strategy for Microbicides

Ronald Nardi, Linda Arterburn and Lisa Carlton

Abstract Ever since the discovery that a virus was responsible for AIDS, prevention of HIV infection has been a drug/vaccine development target in therapeutic research. Microbicide products are a viable, globally applicable option; however, to date, no product has been approved anywhere in the world. Development of such a product will need to account for the changing disease landscape and will need to leverage available regulatory pathways to gain approvals in the developed world and emerging markets. In countries where the regulatory pathway is not clear which is the case in several emerging markets, sponsors will need to employ a flexible approach to gather and meet local regulatory requirements and ultimately gain product approvals.

Contents

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1 Introduction

AIDS was recognized as a sexually transmitted disease even before HIV was confirmed as the infectious agent, and since the first confirmation that a virus was responsible for the disease, scientists have been seeking a means to prevent HIV infection of individuals. As a consequence, methods that could prevent infections associated with sexual activity have received significant attention. The use of condoms is well recognized as a physical barrier method to prevent sexual transmission of the virus (Pinkerton and Abramson 1997), and circumcision has also been shown to reduce female to male HIV transmission (Auvert et al. 2005; Bailey et al. 2007; Gray et al. 2007). Chemical and pharmacotherapy approaches to interfere with sexual transmission of the virus have been a drug development target in the pharmaceutical industry, and there are some promising findings on this front, including a recent United States Food and Drug Adminstration (FDA) approval of oral Truvada® (emtricitabine and tenofovir disoproxil fumarate) for prevention (Food and Drug Administration 2012b), and the first large-scale microbicide trial to demonstrate effectiveness of a microbicide gel containing 1 % tenofovir (Karim et al. 2010). Despite this progress, this area requires more research to better define the optimal prevention approaches that can be exploited globally.

Vaccines and non-specific microbicides were among the earliest prevention therapies evaluated in the biomedical intervention setting. Vaccine efforts continue with some promise but also with recognition that vaccination against the virus is not a near-term solution to prevention of HIV infections. Recent microbicide efforts have shifted to the development of products containing specific anti-retroviral (ARV) drugs that directly interfere with viral transmission or replication.

Development and approval in Africa of ARV-based microbicides as a means to prevent sexual transmission of HIV is an important approach to curbing the HIV epidemic. These ARV development efforts have been continuing for more than a decade and are encouraged by the prevention successes in post-exposure prophylaxis and in reductions in mother to child transmission using ARVs. The recent results with Truvada and tenofovir gel further encourage efforts to develop and gain approval for a prevention armamentarium that is globally applicable (Abdool Karim et al. 2010; Grant et al. 2010).

To date, topical pre-exposure prophylaxis (PrEP) prevention therapies have not been approved anywhere in the world. The data required to enable those regulatory decisions are not yet fully understood, but are being pursued in several drug development projects. This chapter will outline the current understanding of the

regulatory approval requirements and mechanisms, and the environment for those approval decisions.

2 Disease Landscape

The characteristics of the HIV epidemic have been changing with the development and wider use of new, effective treatments for HIV. As described below, regulatory authorities will need to review new microbicides in light of this changing face of the epidemic and are expected to place a stronger emphasis on prevention efforts.

2.1 Status and History

According to UNAIDS reports (UNAIDS 2012b), the 2011 estimates of HIV-infected individuals were approximately 34 million total with over 23 million of those living in Sub-Saharan Africa (SSA). SSA is home to almost 70 % of individuals infected with HIV in the world (UNAIDS 2012b). In this region, 60 % of the infected individuals are women, usually young (15–24 years) and often the primary caregivers for children (UNAIDS 2011). There is some year-to-year variability in these estimates, but from 2001 to 2011, the total number of individuals living with HIV infections has risen about 16 % (approximately 2 % per year) from 29.4 million (Fig. 1) (UNAIDS 2012b). In SSA, the growth in the HIV-infected population is about 12 % (approximately 1.2 % per year) over the same timeframe. The continued growth of the infected population worldwide confirms that new infections outpace HIV-related deaths each year both globally and in SSA (see Fig. 2).

As suggested previously, the profile of the HIV epidemic is quite different today compared to several decades ago. Much of this change is the result of drug discovery and development efforts that have exploited the molecular-level basic research on HIV. In contrast to the early association with AIDS and its associated sequelae, appropriate antiretroviral treatment (ART) has transformed HIV infection from a usually fatal condition to a chronic condition. With early diagnosis, HIV-infected patients can expect to live a near normal lifespan if they remain diligently compliant with effective ART (May and Ingle 2011; Mills et al. 2011; Nakagawa et al. 2013). Therefore, HIV infection is essentially a disease and epidemic unto itself and is no longer equivalent to AIDS. In this context, although ART is not a means to cure the viral infection, it has the potential to prevent AIDS (or at least delay the onset), and importantly, suppression of viral replication reduces the risks of transmission.

Nonetheless, individual risks in light of the HIV epidemic should not be underestimated since HIV positive individuals can infect other individuals, and if they do not receive ART they will eventually develop full-blown AIDS. Rather,

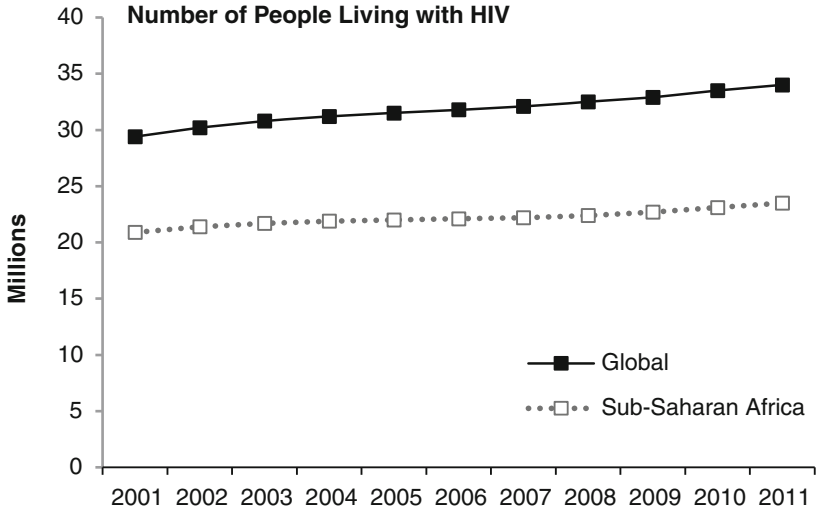

Fig. 1 HIV epidemiology. Data from UNAIDS (2012a, c)

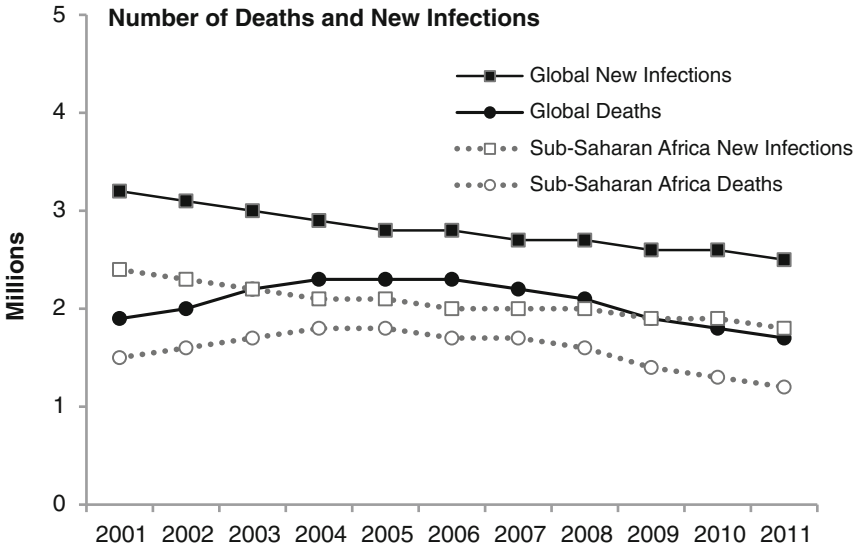

Fig. 2 HIV deaths and new infections. Data from UNAIDS (2012a, c)

understanding these risks will help place into context the environment in which regulatory authorities, especially those in SSA and other developing nations, will make drug approval decisions for HIV prevention. Furthermore, because the full potential of ART is not yet realized throughout these regions, the medical and regulatory communities of those nations must balance the needs of the HIV/AIDS

epidemic with the need to prevent an HIV infection epidemic within their populations in order to manage the necessary transition from treating, or prolonging the progression to AIDS to preventing HIV infection.

2.2 Prevention

In the context of the HIV epidemic, preventing new infections is necessary to shrink the proportion of the population that becomes virus "carriers."

ART effectively prolongs life of infected patients but as noted above it does not eradicate the HIV infection. For infected individuals, ART not only has the potential to prevent the onset of AIDS, it also reduces the potential for transmission to an uninfected partner (Cohen et al. 2011). In addition, it is also clear now that treating the uninfected partner in a serodiscordant couple with an ARV greatly reduces the rate of new infections among the uninfected partners (Polis 2012; Thigpen et al. 2012). Consequently, treatment has the potential to effectively have a neutral effect with respect to the infected population, i.e., the treated HIV positive individual can maintain an almost normal life-span and if they remain adherent to ARV treatment, the probability for the transmission of new infections is greatly reduced.

Unfortunately, the HIV-infected population continues to grow, in part, because not all infected patients receive treatment:

- ART is not routinely initiated upon a positive diagnosis of an HIV infection since some HIV positive patients are not eligible for treatment according to the national treatment guidelines/local healthcare policies.

- The window between the occurrence of new infections and the diagnosis of those newly infected individuals is a time when transmission of the virus to an uninfected sexual partner may occur.

Both situations can produce multiple newly infected individuals as a result of unprotected sexual activity, and in SSA, there are about 500,000 more new infections than deaths each year, which contributes significantly to the growing population of infected individuals (UNAIDS 2010).

It may be possible to reduce the number of new HIV infections by about 3 million over the next 10 years by adding new effective PrEP to the existing options within the prevention armamentarium (see items 1 and 2 below) and by expanding the ability to reach individuals at the greatest risk for infection (Abbas et al. 2007).

1. Barrier methods, such as male and female condoms, are available, and they reduce the risk of transmission and can be used widely regardless of HIV status or exposure risk.
2. ART for HIV positive patients reduces the risk of transmission by suppressing viral loads to near zero.

3. PrEP (systemic and topical) treats uninfected individuals regardless of HIV exposure risk.
    a. Systemic PrEP for the uninfected partner in a discordant couple reduces transmission events.
    b. Coitus-associated vaginal administration of a microbicide as topical PrEP reduces transmission events in a general population of HIV negative women.
    c. Extended-release ARV-based products for topical and systemic PrEP may enable broader utility of prevention-directed ARVs among uninfected individuals.

Recruiting the uninfected and otherwise healthy population into one or more of the prevention modalities is necessary to reduce the size of the HIV-infected population. However, such a decline must be sustained for several decades to achieve the desired control of the epidemic.

2.3 Resistance

In the context of using a range of prevention modalities over an extended period, the potential for development of viral resistance must be considered. The current efforts in PrEP exploit mechanisms known to suppress viral replication. Further, some of the compounds in development for PrEP are an important part of the ART armamentarium. Some are being reformulated to enable the PrEP indications while others are being administered as they are in ART. The primary concerns with this approach are:

Broader use of approved ARVs may increase the risks of developing resistance if the individual becomes infected during use of the product, and thereby, future treatment options with that product become limited and consequently shorten the product's lifespan.
Emergence of widespread resistance to existing ARVs could potentially reignite the AIDS epidemic since treatment options may become limited.

These issues are not new nor are they limited to prevention efforts per se. In the treatment arena, highly active ART (HAART)—use of a combination of ARVs having multiple mechanisms of action—is a means to combat resistance and ensure therapeutic success. In addition, a combination therapeutic approach avoids the increased risk of safety events that may be attendant with increasing the dosage of any single therapeutic component. This approach is also likely to be effective in the prevention arena, and it may become attractive to combine multiple mechanisms of action to lower the potential for development of resistance.

2.4 Summary of HIV Landscape

The HIV epidemic is currently characterized by an increase in prevalence of HIV infections, and new infections are outpacing AIDS-related deaths, primarily as a result of the availability of effective ART and HAART therapies. HIV infection no longer necessarily leads to full-blown AIDS and death; therefore, HIV infection can be considered a chronic, non-life threatening condition that creates an expanding pool of viral carriers. In the absence of widespread use of effective prophylaxis, a larger carrier pool can lead to an increased incidence of new infections. If new infections are not prevented, even though the clinical manifestations of the infection can currently be managed, viral resistance may eventually emerge since the potential for development of resistance increases along with expanded use of ART (for both treatment and prophylaxis). Importantly, the implication of a widespread emergence of resistance is that HIV infection could once again become inextricably linked to the development of AIDS.

As a consequence of the changing face of the HIV epidemic, a greater emphasis is being placed on developing drug products for the prevention of HIV infection. Regulatory authorities will consider these factors and conduct benefit risk assessments for new microbicides in light of the current characteristics of the epidemic. Given the otherwise healthy target population and the fact that HIV infection no longer absolutely results in death, the burden of demonstrating efficacy will be high while the tolerance for side effects will be low. The tendency for the drug to cause resistance or cross-resistance will also be a critical component of the assessment.

3 Regulatory Environment

3.1 ICH Regions

In order to plan for approvals for ARV-based microbicide products used in PrEP, it is helpful to review the global regulatory environment.

A traditional "pharma" industry perspective for most products in development usually has an initial development and commercialization focus in the developed world, primarily in the International Conference for Harmonization (ICH) regions of North America, Europe, and Japan. As a result of the harmonization efforts among the regulatory authorities and experts in these regions, it is possible to define a single development program that meets the scientific data requirements to enable decisions in these regions. The core requirements for the drug product, the nonclinical evaluation of pharmacology and safety, and the clinical evaluation of dosage regimen, efficacy, and safety are established and accepted across these regions. While some regional differences do remain and require some attention, essentially a similar dossier can be filed to the three ICH regions to cover

approximately 30 countries that represent the dominant portion of the anticipated market for a given drug product in the developed world.

3.2 Rest of World

In the HIV arena, the ICH regions are still important, but the rest of world (ROW), comprised of more than 100 countries, is home to the dominant patient population, and all major companies developing drugs to treat HIV infections pursue efforts to make products available to the countries where the HIV epidemic is largest. For these treatments, the challenge is to prosecute the core dossier in this much broader array of national regulatory authorities (NRAs). These NRAs are staffed by scientists and physicians with a commitment to their own public health mission. Consequently, it is expected that each NRA will identify concerns to which the sponsor must respond in order to gain regulatory approval of a new drug product.

For ARVs to treat individuals with HIV infections, products are usually approved and commercialized in one or more of the ICH countries before filings in Africa are initiated. Consequently, the World Health Organization (WHO) and the more than 40 NRAs in Africa will likely have not only the drug development data from the core dossier, but also some of the Phase 3B/Phase 4 data and post-marketing follow-up information available for review. As a result, any new chemical entities (NCE) gaining approval in the African countries often receive these African approvals more than 5 years after their initial commercialization.

As an example, oral PrEP products are based on formulations approved for treatment in many countries including those in Africa. Consequently, a product like Truvada® (Gilead Sciences, Inc.), which has been approved for prevention in the US, is likely to gain approvals in Europe and other ICH countries before the indication is added to the labeling of the product in Africa. However, for ARV-based prevention products, broad commercialization in ICH countries is not likely because the primary burden of new infections occurs outside of these regions. In the case of an ARV-based microbicide product containing an NCE, it is expected that product registration in an African country will enable the first commercial distribution in the world regardless of whether an ICH authority reviews the dossier. For prevention indications involving product reformulations of ARVs approved for treatment, the situation is likely to be similar. In these cases, the regulatory strategy may need to focus primarily on African country requirements as opposed to the ICH regions to enable the most expedient pathways to approvals in Africa.

Table 1 Key regulatory discussions on microbicides

Date	Meeting	Discussion item(s)	Reference
April 2011	FDA CDER Forum for International Drug Regulatory Authorities	Regulatory Considerations for Microbicide Development	Mullick (2011)
May 2010	WHO Symposium—Regulatory considerations for the review of microbicide clinical trials and product registration	• Statistical Issues in Microbicide Trials • Critical issues in Ph 2b and 3 trials: Special populations (pregnant women and adolescents)	Birnkrant (2010), Soon and Hammerstrom (2010)
December 2009	World Health Organization: Regulatory Issues in Microbicide Development	Chemistry, manufacturing, formulation, nonclinical and clinical considerations for the development of microbicides	Stone (2009)
March 2004	Microbicides 2004	Clinical Development of Topical Microbicides (T. Wu)	Wu (2004)
August 2003	FDA Antivirals Advisory Committee	Clinical trial design issues in the development of topical microbicides	Food and Drug Administration (2003)

4 Dossier Content for a Microbicide Product for Prevention

With the recognition that microbicides are going to be a unique product class, the discussions of the approval requirements have been ongoing for more than a decade. Much of this discussion has occurred without focus on specific products (see Table 1), but the EMA recently initiated a discussion via a "reflections" paper on oral and topical PrEP. The FDA has also recently released a draft guidance document on microbicides for comment. In addition, two microbicide products have reached advanced clinical development stages (see below).

4.1 EMA Reflection Paper

A draft reflection paper was recently released for comments (European Medicines Agency 2012). This paper addresses important regulatory issues related to the preclinical and clinical development of topical and oral PrEP products, and in addition to EMA views, incorporated input from the WHO and African Institutional Body as well as academics and patient groups. The paper highlights expectations for a dossier submission, including nonclinical (mechanism of action, proof of concept, dose finding), pharmacology (condom functionality and

drug–drug interaction studies), clinical development (e.g., trial design, primary and secondary endpoints, trial populations and adherence measures), and post-authorization concerns (e.g., risk and risk compensation).

4.2 FDA Draft Guidance

A draft guidance was released for comment by the FDA in November 2012 (Food and Drug Administration 2012a). This guidance specifically addresses the development of topical microbicides for HIV prevention and covers a range of important topics related to the nonclinical and clinical development of microbicides. The guidance discusses expectations for nonclinical development (e.g., virology, toxicology and other nonclinical studies) and general and specific issues in clinical development, including safety, efficacy, and clinical pharmacology considerations as well as clinical trial design guidance for the various phases of development. It also includes a discussion about combination products containing multiple drugs or a drug and device.

4.3 Microbicides in Advanced Development

Two drug products, tenofovir gel and dapivirine vaginal ring, are sufficiently advanced to be considered late-stage development projects. The regulatory discussions concerning the content of an approvable dossier are summarized below. While much of this discussion occurred before the EMA and FDA were in position to publish a guidance document, the programs for each drug reflect the considerations outlined in the draft guidance documents.

4.3.1 Tenofovir Gel

Tenofovir is a nucleotide reverse transcriptase inhibitor (NRTI). A prodrug of tenofovir, tenofovir disoproxil fumarate (TDF), is marketed as Viread® by Gilead Sciences, Inc. for the treatment of HIV/AIDS, and is also present in the fixed dose combination products Truvada® (TDF and emtricitabine), Atripla® (TDF, emtricitabine and efavirenz), Complera® (TDF, emtricitabine and rilpivirine), and Stribild® (TDF, emtricitabine, elvitegravir and cobicistat). Tenofovir gel is a topical formulation of tenofovir for vaginal administration. The product has been developed by a consortium which carefully evaluated the vaginal gel in a series of Phase 1 and Phase 2 trials conducted in the US and Africa (Mayer et al. 2006; Peterson et al. 2007; Rosen et al. 2008). CONRAD (the developer of the dosage form) and agencies of the US government and the Government of South Africa have collaborated with other donors and Gilead to advance this program.

A proof of concept Phase 2 trial (CAPRISA 004) was organized and executed in South Africa. The results, reported in 2010, showed that compared to a placebo gel, the tenofovir vaginal gel administered in association with coitus (before and after treatment over 24 h, BAT24) significantly reduced the rate of HIV transmission among sexually active, HIV negative, women 18–35 years old (Karim et al. 2010). This trial was the first demonstration that an ARV-based microbicide prevented HIV infections.

While the results of this trial have been well received, discussions with the FDA indicated that they would require a confirmatory study for decision making. The VOICE trial, sponsored by the US National Institutes of Health (NIH), included a tenofovir gel arm and a placebo gel control and used a once daily dosing regimen. This trial was acceptable to the FDA as a confirmatory trial but the gel arms were stopped for insufficient treatment effect. A confirmatory trial (FACTS 001) using the same BAT24 regimen as used in the CAPRISA 004 study is actively enrolling subjects with an expected completion date in 2014. Whether the Medicines Control Council (MCC) of South Africa will make regulatory decisions based on CAPRISA 004 alone is currently unknown.

4.3.2 Dapivirine Vaginal Ring

Dapivirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) being developed by the International Partnership for Micobicides (IPM) with funding from European funding agencies, the US government, and several philanthropic organizations. The drug product is a sustained-release vaginal ring containing 25 mg of dapivirine suspended in the silicon matrix. The ring is inserted into the vagina and used for 28 days. Drug release in vivo continues for at least 35 days with high concentrations of drug observed in vaginal fluid and lower levels observed in plasma throughout its use period. These data suggest that locally high concentrations of dapivirine are in the vagina and surrounding tissues.

This product has also followed a conservative clinical drug development process to insure that local and systemic adverse event profiles were acceptable for full clinical development. Scientific advice was provided via the Parallel Scientific Advice Procedure coordinated by the EMA and involving the US FDA, WHO, and invited African authorities. Based on that advice, the dapivirine development program includes two pivotal trials evaluating the efficacy and safety of the product. This Phase 3 program started in 2012 and anticipates completion and dossier filings in 2016.

4.4 Global Dossier Submission Planning

The ICH regulatory authorities have developed a dossier template to facilitate the presentation of product-related characterization data from the preclinical and

clinical development activities. This Common Technical Document (CTD) provides a standardized organization that enables both sponsors and regulatory reviewers to understand the data supporting new product information. It also has flexibility to organize and present information that is of unique interest to some but not all regulators. For example, studies of populations of particular interest in a given country can be integrated in the dossier. The developed world authorities currently accept CTD dossiers in either electronic or paper formats.

As indicated earlier, many developed world countries have fairly small populations for whom microbicide products would be of interest. In these countries, the dossier requirements can be easily discussed, and submissions should be timed based on availability of decision enabling information requested. The FDA and EMA may have slightly different emphasis but generally the dossier requirement will be similar. In particular, the Article 58 procedure (see below) with its emphasis on the African population may lead to some differences in requirements, but overall sponsors can expect the requirements to be similar. It is notable that pediatric population study requirements may be of particular interest for all regulatory authorities but for both the EMA and FDA such plans, including some data in an adolescent population, will be needed for the dossier filing and for labeling.

Microbicide development efforts to date have focused on Africa, but as the current development programs mature it will become necessary to explore the value of these products in other countries (e.g., China and India) as an understanding emerges of the profile of the HIV epidemic in other regions. The regulatory efforts to bridge the available data to these populations will become an important topic in the near future.

For both products in late development and any subsequent microbicide products, the global submission challenge is gaining approvals in individual African countries or other emerging-market territories in addition to gaining review and approvals in the ICH regions. Each of these nations has an approval process which makes for resource-intensive, multiple approval prosecutions proceeding in parallel, with much of the core submission based on the ICH CTD template. As indicated above, the specific requirements such as expert reports can be integrated into this dossier. This core dossier format will also facilitate the review efforts that may be collaborations among several African authorities or review collaboration with the WHO and EMA in the Article 58 procedure. Additionally, with respect to other ROW regions that are greatly impacted by HIV infection, e.g. from Asia and South America, these populations represent an expansion of the target population that will be evaluated as the efficacy data for microbicide products currently in development become available.

Final submission plans will depend on communications with the individual NRAs. This activity is more challenging than simply requesting a meeting with the FDA or EMA but no less critical to the successful prosecution of the dossier. Consequently, some filings may be delayed until it is clear that the filing contains all the desired/required elements. Despite an element of political influence in making health policy decisions in African nations, meeting the requirements of each regulatory body is still a necessary and important step. As a consequence, the

market preparation discussions, which may require policy decision making, will need to progress in parallel with the regulatory discussions.

Three broad categories are identifiable among the African NRAs with respect to their dossier review process.

Requirement for Prior Approval and Market Experience

Not surprisingly, many countries depend on approval and marketing experience in a "home" or source country (i.e., country of origin/manufacture) in their approval review process. There is a long history with this process. For most products, this approval is usually a starting point as discussed above. For PrEP products and microbicides, in particular, this prior experience requirement may delay submissions in some countries. Among the reasons to make every effort to communicate the sponsor's submission plan is to clarify the level, if any, of marketing experience required as that may be the rate-limiting issue for submission in individual countries.

Article 58 Review and Opinion

The EMA and WHO have collaborated with some African countries to develop an Article 58 procedure (named after the legislation), which enables this EMA review and recommendation procedure. There is explicit recognition that some products do not have target populations in Europe but have broad public health utility in other regions of the world. The regulatory review process follows the EMA review procedures except that the Committee for Medicinal Products for Human Use (CHMP) does not grant registration in Europe but rather recommends that the product meets the standards for an approval. Experience with this procedure is limited but encouraging. Some African countries will focus their review and decision making based on the information provided by the EMA reviewers. Submission in countries where this process is acceptable should be timed based on the EMA review.

Independent Review

As several regulatory harmonization collaborations develop on the African continent and as individual countries (e.g., South Africa) develop the necessary resources to review a marketing authorization application (MAA) for a novel product, they will choose to conduct independent reviews of a dossier. Presubmission communication will be very important since the timing of submissions in these countries will be driven by meeting all country-specific requirements.

5 Conclusions

The changing face of the HIV epidemic (and the potential for future changes if widespread resistance emerges) contributes significantly to the changing regulatory landscape for microbicide products, and drug development programs will need to provide adequate safety data to allow for risk assessments in an otherwise healthy population. This presents a challenge to sponsors developing products for a global market since the tolerance for risk is likely to differ in different regions of the world, and therefore, the regulatory strategy will need to consider each region individually. The submission package containing the core data that will enable regulatory decision making should be built on a CTD platform so as to facilitate multiple country submissions and approvals, but the package should also be flexible enough to accommodate local country-specific requirements. While it is generally accepted that an approval in an ICH region will facilitate approvals in ROW countries, sponsors should be mindful that ICH regions are critical but not sufficient for obtaining approvals worldwide.

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Preparing for Microbicide Introduction, Rollout, and Sustained Access

Cynthia Woodsong, Elizabeth McGrory and Tim Farley

Abstract Two topical vaginal microbicide candidates for HIV prevention are at an advanced stage of clinical testing, with efficacy results from three clinical trials expected within the next 2 years. Therefore, preparations for introducing and ensuring access to these products in the event that they are proven safe and effective now require increased attention. Microbicides are expected to fill an important global public health need for HIV prevention options for women. They have been developed almost exclusively with public and private funding through academic and nongovernmental institutions and minimal involvement of commercial pharmaceutical partners. Efficient and rapid introduction of a new public health technology requires a broad range of expertise and collaborations, some of which are new to the microbicide field as products are at last completing late-stage pivotal licensure studies. Strong leadership, political commitment, and considerable financial investments will be required to ensure successful distribution as well as uptake and continued access to this new product class. This paper highlights work conducted since 2000 by scientists, advocates, and public health officials to prepare for microbicide introduction, and discusses some of the needed actions to ensure that products will become readily accessible to the women who need them.

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1 Background

The path to the development of a safe and effective vaginal microbicide has been long, with numerous setbacks as well as promising developments. Microbicides are a new product class, and only one trial to date has demonstrated efficacy. Thus, preparations for introducing and ensuring access to future microbicide products have received less attention in the scientific literature than preclinical and clinical development. Research findings must be effectively applied to achieve product introduction, rollout, and sustained access, and the need for the translation "from research to reality" is increasingly apparent (AVAC 2013).

Microbicides are positioned as public health goods that are expected initially to be used by women in low and middle income countries in high HIV incidence settings. Product development has been funded almost exclusively through public and philanthropic sources, with limited financial involvement of the pharmaceutical industry. The processes for introduction and marketing of public health goods must follow similar steps to those for commercial products, but additional challenges are expected for microbicides. These include the lack of established regulatory or logistical processes for introduction of novel products, and poor healthcare infrastructure in the countries where microbicides will be introduced. While all partners are aligned around the common goal of increasing HIV prevention options for women and reducing the high incidence of HIV infection in this vulnerable group, the main cohesive factor that drives private sector collaboration—maximizing commercial return on investment—is not present. With three late-stage trials (IPM 2012; FACTS 2013; MTN 2013) of the safety and effectiveness of topical microbicide products nearing completion in five sub-Saharan African countries, the planning and work for microbicide introduction and rollout now requires increased efforts.

Delays in bringing new health technology to low and middle income countries are well documented (Brooks et al. 2012). UNITAID/WHO's recent "HIV Preventive Technologies and Market Landscape" report (WHO 2013a, b) states that "delays in roll-out of new HIV prevention technologies have stemmed from numerous factors, including insufficient financial and human resources, inadequate

political support, technical uncertainty regarding optimal programme implementation, and systemic weaknesses, including problems with commodity procurement and supply management… market factors also often play a role in impeding scale-up. These factors include unfavourable commodity prices and insufficient demand" (WHO 2013a, b, p. 67). Although it is not possible to control all of these factors, nor anticipate how they will play out for microbicides, it is incumbent upon those working in the microbicide research arena to contribute to the work needed for introduction, rollout, and continued access.

Considerations for the process of microbicide introduction have been ongoing since the initial conceptualization of these novel products. In 2000, the Rockefeller Foundation provided support for the formation of working groups charged with accelerating microbicide development, including an Access Working Group. The passage below from this group (McGrory and Gupta 2002) provides a comprehensive highlight of what is necessary to bring microbicides to those who need them.

> In order for a woman or girl to use a microbicide, she must perceive herself at risk, the product must be acceptable to her, and she must know how to use it properly. The products must be available in locations that users can easily access at a price they can afford. A woman's ability to access and use microbicides will be facilitated if there is a political and social environment that supports women's use of those products by actively promoting and incorporating them into policies and programs. For all this to be possible, microbicide products will need to be approved by relevant regulatory authorities and promoted as an essential component of a comprehensive HIV prevention package. (McGrory and Gupta 2002, p. 9)

Though the products under development as well as the landscape of HIV prevention and treatment have changed significantly since publication of that report, the issues remain salient. This paper provides an overview of work conducted since 2000 by scientists, advocates, and public health officials to prepare for microbicide introduction, rollout, and access, and discusses some of the actions that will be needed between the time a product is demonstrated to be safe and effective in clinical research settings to when it becomes readily accessible to the women who need it. Following a description of the trajectory of clinical testing which has led to the ARV-based microbicide products that are currently in advanced trials, we briefly review the efforts made to date to prepare for microbicide introduction and then discuss the broader areas needed for successful microbicide introduction and access.

2 Progress in Microbicide Clinical Testing

The first large Phase 3 clinical study of a candidate microbicide was conducted in four countries between 1996 and 2000 under the aegis of the World Health Organization Global Program on AIDS and tested the safety and efficacy of the spermicide nonoxynol-9 (N-9) for the prevention of HIV infection. N-9 had been

shown in the laboratory to prevent HIV, gonorrhea, and chlamydia infections (Van Damme et al. 2002) and was registered as a nonprescription over-the-counter spermicide for pregnancy prevention in many countries. If the trial had confirmed that the product was safe and effective in preventing HIV infection, a change in product labeling, registration in additional countries, manufacturing and distribution scale-up, and increased access would have likely been straightforward. Distribution, marketing, and promotion mechanisms were in place through family planning services, the private sector and social marketing programs, global manufacturing capabilities were scalable, and the cost of goods and finished product were modest. However, these assets were never put to the test. The results from the clinical study showed that women assigned to the active product acquired HIV infection at a 43 % higher rate than women assigned to the matching placebo, putting an end to further research on N-9 for HIV prevention.

Subsequent candidate products that have been developed and clinically assessed for use as topical microbicides involved either novel uses of already registered products or new chemical entities. The latter required preclinical and clinical safety profiles to be established de novo before definitive Phase 3 clinical safety and efficacy trials could be conducted. The "first generation" of candidate microbicides that progressed to clinical safety and efficacy testing were gels containing surfactants, buffering agents, or large polyanions that either broke down the viral envelope, maintained the low pH of the vagina making it inhospitable to HIV, or prevented the virus from coming into contact with target cells—Savvy (Feldblum et al. 2007), BufferGel™ and PRO2000 (Abdool Karim et al. 2011), Carraguard™ (Skoler-Karpoff et al. 2008), and cellulose sulfate (Van Damme et al. 2008). In 2009 the first study of PRO2000 showed a promising but not statistically significant 30 % reduction in HIV incidence compared with placebo, yet a parallel Phase 3 trial showed no reduction in incidence (McCormack et al. 2010). Trials of the remaining first generation products failed to demonstrate any reduction in HIV incidence, and as the disappointing results of these early trials accumulated, there was an increased focus on microbicides containing antiretrovirals (ARVs) that directly targeted HIV entry and/or replication.

The first microbicide study to demonstrate a statistically significant reduction in HIV incidence was the CAPRISA 004 trial, conducted in South Africa in 2007–2010. This study tested a vaginal gel containing a 1 % concentration of the ARV tenofovir used before and after sexual intercourse. Results showed a 39 % reduction in the incidence of HIV among women allocated to active gel compared with those allocated a matching placebo gel (Abdool Karim et al. 2010). A confirmatory Phase 3 placebo-controlled study (FACTS 001) using the same product in the same dosing regimen is currently underway in nine South African sites (FACTS 2013). A parallel study (the VOICE trial) was conducted in 2009–2013 by the Microbicide Trials Network (MTN) at 15 sites in Uganda, South Africa, and Zimbabwe, and included an arm testing daily use of the same 1 % tenofovir gel product. No efficacy was demonstrated in the trial. However, subsequent analysis indicated that this was likely due to low adherence (Marrazzo et al. 2013). Currently, the International Partnership for Microbicides (IPM) and MTN are each

Table 1 Microbicide products in efficacy trials

Active ingredient	Clinical trial and trial site locations	Product delivery mode	Use requirements
Tenofovir gel	CAPRISA 004 Phase IIb South Africa *(study completed in 2010)*	Prefilled single-use plastic HTI vaginal applicator	Single application applied within 12 h before sex and a second application applied within 12 h after sex
	FACTS 001 Phase 3 South Africa (ongoing)		
Dapvirine ring	IPM 027 Phase 3 Uganda, South Africa (ongoing)	Silicone vaginal ring	Vaginal ring inserted and worn continuously for 28 days, then removed and replaced
	MTN 020 Phase 3 Malawi, South Africa, Uganda, Zimbabwe (ongoing)		

conducting parallel Phase 3 safety and efficacy trials testing a vaginal ring that releases the ARV dapivirine over a 30-day period (IPM 2012; MTN 2013). Research sites for these dapivirine ring studies are in Malawi, South Africa, Uganda, and Zimbabwe.

Table 1 provides some comparisons of the two microbicide products that are furthest advanced—tenofovir gel, a 2-dose coitally associated product in prefilled applicators, and the dapivirine ring, which is replaced every 28 days.

At the time of this writing, it appears likely that results from the efficacy trials of dapivirine ring and tenofovir gel will be available at about the same time. Assuming that plans for introduction, rollout, and access continue to progress, and appropriate supportive activities take place according to plan, it is also possible that these two very different products could enter the market at similar times. Preparations for introduction and access must be made in anticipation of successful results from these trials, even though these plans may never be implemented if the products are not proven safe or effective. Such preparations require significant resources and time, but concerns about the cost of investment in an uncertain product must be weighed against the potential public health and political costs of delays in making an effective product rapidly available.

3 Conceptualizing Microbicide Introduction

Microbicides have the most potential for vulnerable women with inadequate options to reduce their risk of HIV infection. It is recognized that a complex alignment of interests and expertise is required to facilitate introduction, ensure sustained product availability, maximize demand, and ensure that resources are available to meet that demand. For example, royalty-free licenses and subsidized access to the products

have been key components of development plans from the beginning (Mandelbaum-Schmid 2004; Ratzan 2007). This has been reflected in mathematical forecasting models that consider cost, supply, and demand, offset by infections averted at different levels of efficacy and use-adherence, in multiple international settings (Watts 2001; Vickerman et al. 2006; Wilson and Coplan 2008; Verguet and Walsh 2010; Hankins and Dybul 2013; Terris-Prestholt et al. 2014). The costs and regulatory requirements for microbicides are major concerns for sponsors and advocates, and numerous consultations have occurred to discuss these issues and chart the way forward (Stone 2009; Stone and Harrision 2010). Social science and behavioral research has steadily built upon experiences with the introduction of new sexual and reproductive health technologies such as contraceptives and the female condom to provide a body of information to contribute to development of service delivery programs, counseling messages, and social marketing strategies to strengthen introduction, initial uptake, and continued use (Darroch and Frost 1999; Elias and Coggins 2001; Severy 2005; Woodsong et al. 2013).

A large number of not-for-profit research groups and universities have conducted microbicide preclinical and clinical research and engaged in studies, projects, and programs with the aim of supporting microbicide introduction, rollout, and access (Alliance for Microbicide Development 2007). Three organizations were formed expressly to support microbicide development, and their remit included aspects of introduction, rollout, and access – the Global Campaign for Microbicides, the Alliance for Microbicide Development, and the International Partnership for Microbicides.

The Global Campaign for Microbicides, founded in 1998, was "a civil society organization that worked to ensure the ethical and accelerated development and widespread access to new and existing HIV-prevention options—especially for women" (www.global-campaign.org). The Alliance for Microbicide Development was also formed in 1998 "to advocate for and educate about microbicide development, track and communicate product development and regulatory status, contribute to enhanced efficiency in preclinical and clinical processes and, later, help establish a base for matching funds to support combination and comparative studies" (Harrison 1999, p. S39) It also served as the Secretariat for the Quick Working GroupQuick Working Group, convened in 2004 to provide a platform for information exchange amongst those working on advanced microbicide trials, including scenarios for the work that will be needed for introduction once efficacy results are achieved. (Harrison 2007). In 2002, the IPM was established as a product development partnership (PDP) charged with accelerating the development and availability of microbicides for use by women in low and middle income countries. IPM's mission to support the pipeline includes efforts to secure royalty-free licenses, and work with organizations at the global, national, and local levels to provide support for microbicide introduction and access (www.ipmglobal.org).

One of the first efforts to outline the elements necessary for successful microbicide introduction and access was spearheaded by the Access Working Group of the Rockefeller Foundation Microbicide Initiative (McGrory and Gupta 2002). Observing the history of lengthy delays between drug approval and availability in

low and middle income countries, the Working Group encouraged the scientific agenda for microbicides to include priorities to ensure access. The group built on work of other partnerships designed to develop and bring public goods to market, such as the Medicines for Malaria Venture and the International AIDS Vaccine Initiative (Widdus 2005) and laid out a framework of goals, objectives, and activities to facilitate women's access to microbicides. Fundamental elements included (1) considering the acceptability, preferences, and needs of users as important for product development, (2) creating a supportive policy and public health environment for microbicide delivery, (3) ensuring availability of products through appropriate and well-functioning distribution channels, (4) establishing an affordable price for individuals and/or governments, and (5) achieving regulatory approval and licensing.

These concepts are also reflected in conceptual frameworks that have helped focus thinking about microbicide introduction and access. One of the most widely cited adaptations is Frost and Reich, which conceptualizes the "Architecture," that supports the "Access" needed to accomplish successful microbicide introduction and rollout (Frost and Reich 2009). Three pillars of this architecture are:

- "Availability"—ensures that there is sufficient high-quality production and supply of the microbicide product, and reliable channels for distribution, to meet user demand.
- "Affordability"—directs attention to the costs of microbicide products and programs to deliver them, and seeks to ensure products are affordable to purchasers, funders, and end users.
- "Adoption"—considered at multiple levels, from individual to global. Adoption also includes the notion of "Acceptability"—that microbicides are satisfactory to end users (women and their sexual partners) as well as the gatekeepers who control access to them.

In 2006, the Alliance for Microbicide Development produced the Microbicide Development Strategy (Alliance for Microbicide Development 2006), which included considerations for manufacturing, commercialization, and access, noting that "the process will have to be actively and jointly managed by sponsors, product developers, industry partners, and donors" (p. 12). It was recognized that pharmaceutical industry experiences with introduction must be effectively applied to a public health setting, with attendant social marketing, cost and demand forecasting models, manufacturing scale-up in a government-subsidized environment, and financing for product procurement and distribution provided by a range of public and private partners.

Although the Microbicide Development Strategy stated that these costs and the related demand issues would continue to be informed by the process of preclinical and clinical research, it encouraged efforts to plan and execute pilot studies for introduction strategies, drawing on experiences from other health technologies such as female condoms, diaphragms, vaccines, and over-the-counter products. The strategy document also called for concerted efforts to ensure communication with the US Food and Drug Administration (FDA), the European Medicines

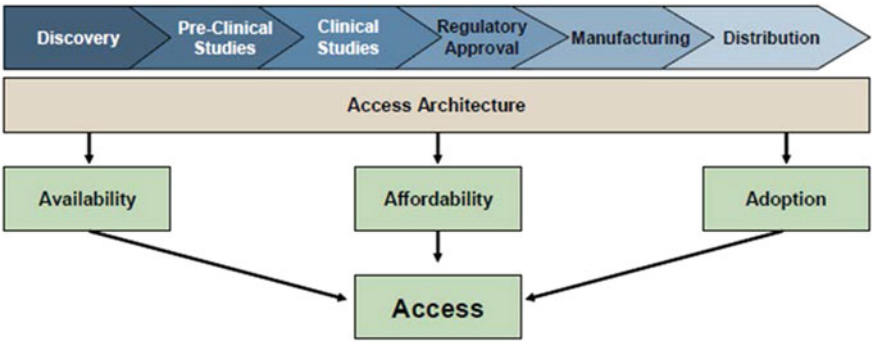

Fig. 1 Access framework and the product development process Herman and Oudin 2010. Reprinted with permission from the Product Development Partnership Access Group (www.pdpaccess.org)

Agency (EMA), and regulatory authorities in the countries where microbicides trials were being conducted.

In 2008, a Product Development Partnership (PDP) Access Group was formed to serve as a forum for access information exchange among 12 PDPs working on new technologies for diseases affecting people in low and middle income countries (notably, malaria, tuberculosis, and HIV/AIDS). Microbicides were one of the technologies included, through IPM's membership in the group. The PDP Access Group maintains a website to improve performance and facilitate efficiencies in access activities (www.pdpaccess.org). Building on the Frost and Reich framework, the PDP Access Group, added a conceptualization of the process pathway from "Discover" to "Distribution," shown in Fig. 1 (Herman and Odin 2010). This framework was used in an assessment of the roles that PDPs will play in post-licensure access to the health technologies they develop. The assessment noted that product developers' specific activities in support of Access varied according to where their technology sits in the pathway, but it was stressed that developers should keep the complete pathway in mind, and be prepared to contribute throughout the process.

4 Progress in Planning for Microbicide Introduction

Dialogue and linkages with regulatory authorities from developing countries with high HIV incidence were brokered by the World Health Organization (WHO). WHO sponsored a series of workshops beginning in 2002 to discuss minimum preclinical requirements to be met before initiating clinical research, and the sequence of clinical studies necessary before launching pivotal Phase 3 studies to establish safety and efficacy for HIV prevention. These workshops served to underline the importance that regulatory authorities in low and middle income countries place on the thorough review conducted by better-resourced national regulatory authorities

with sufficient resources to carefully examine all elements of the dossier, while nevertheless conducting a final assessment of risks and benefits in the context of their own country setting. It was noted that health care systems in countries with high incidence and generalized HIV epidemics are severely stretched to provide care to individuals living with HIV. As a result, the balance of risks and benefits for investment in microbicides for HIV prevention in countries with generalized epidemics differs markedly from the situation in the USA or in European countries.

A series of "Access Forums" were convened in 2007, 2008, and 2010 by the World Health Organization Department of Reproductive Health and Research (WHO/RHR), IPM and the Population Council to flesh out understandings and agreements about what is needed for microbicides to be accessible (WHO 2007, 2008). Informed by experiences with introducing and improving access to sexual and reproductive products, these forums analyzed the challenges and successes of HPV vaccines, the female condom, subdermal implants, and intrauterine contraceptive devices. The candidate microbicide products in efficacy testing at that time were gels designed to be used before sex. The other lead candidate was IPM's vaginal ring, which was similar to those used for contraceptive delivery.

Introduction of the female condom, as a family planning as well as HIV prevention product, was considered particularly relevant. The experiences with introducing the female condom in South Africa and Zimbabwe highlighted the importance of public and private sectors and social marketing structures working synergistically using consistent technical information and promotional materials, as well as supporting programs long enough to allow users to become comfortable with the product. Lessons from the female condom are useful, although current ARV-based products like tenofovir gel and dapivirine ring will likely need to be delivered through a more formal health system. The female condom also illustrated the complexity of product pricing; the price the user was willing to pay was well below the cost of producing the product, let alone distributing it. Subsidies by national governments and/or bilateral donors were able to bring down the cost to the user, but had to compete with other demands on HIV prevention resources, such as male condoms with a unit cost 10–20 times lower. Lessons from contraceptive introduction provided numerous similar and additional insights (Brown 2007).

Over the past decade, the roadmap for specific streams of work needed for microbicide introduction has become clearer. The Bill and Melinda Gates Foundation sponsored a conference in 2009 to outline processes for implementation of new HIV prevention technologies, including topical microbicides, noting that clinical trial results are "only the beginning" (Kim et al. 2010). WHO convened meetings to establish a consultative process for introduction of Pro2000 (WHO 2010), which proceeded until the Phase 3 trial results failed to show efficacy (McCormack et al. 2010). Nevertheless, this work provided instructive, if sobering, insights into the process and timelines to be expected for other microbicide products advancing through the pipeline (Stone 2010). Conceptual frameworks were revisited, and vulnerabilities identified. The positive results of the CAPRISA 004 tenofovir gel trial spurred efforts to address these gaps and timeline needs, and again, WHO convened consultative meetings. A steering committee was formed in 2011 to coordinate

preparation for the introduction of tenofovir gel, and facilitate communication between the research teams, license holders, manufacturers, and donors (WHO/UNAIDS 2010), while the FACTS001 efficacy trial is underway. Some of the key knowledge gaps identified in the Pro2000 and tenofovir consultative process are reflected in USAID's "Shared Vision and Strategic Plan for Microbicide Introduction" (USAID 2013) which overviews a portfolio of projects to be funded by USAID. To-date, a number of these projects have been completed, are ongoing, or planned.

5 Components of Microbicide Introduction

This section briefly summarizes critical components of the architecture that will be needed for microbicide introduction, rollout, and access. These include:

- clinical and regulatory issues,
- social and behavioral science,
- community participation,
- policy and advocacy
- operations and implementation research,
- marketing, and
- manufacturing/supplychain/distribution.

Figure 2 provides a schematic for these components, adapted from Walker (Walker 2006) and benchmarked for demonstration of efficacy.

These components are not discrete, and work in these areas is best done concurrently. AVAC (Global Advocacy for HIV Prevention) has observed that although the prerequisite steps for microbicide introduction follow a linear process (such as that outlined in Fig. 1), feedback between these steps is fluid, and often iterative (AVAC 2013). For example, as ARV-based microbicides have moved forward in the development pipeline, an understanding of what will be needed for their introduction is becoming clearer. The architecture for their delivery will require a health care delivery system capable of providing periodic HIV testing. This will be necessary to monitor and manage the potential for drug resistance among women who become infected while using the ARV prophylactically. If non-ARV microbicides, which are at earlier stages of development, are introduced, service delivery requirements and distribution options will differ.

The sub-Saharan countries where microbicide trials have been conducted will be priority countries for introduction. This sets a geographic stage for where introduction policies and programs will be needed, even though it is not yet clear how or if introduction efforts will focus on women at highest risk. Since the geographic setting, dosing strategy, and active ingredients can now be taken into consideration for tenofovir and dapivirine products, the manufacturing, supply, and distribution costs can now be more accurately forecast (Wilson and Coplan 2008). Furthermore, it is now possible to work more concretely on expected regulatory and policy requirements.

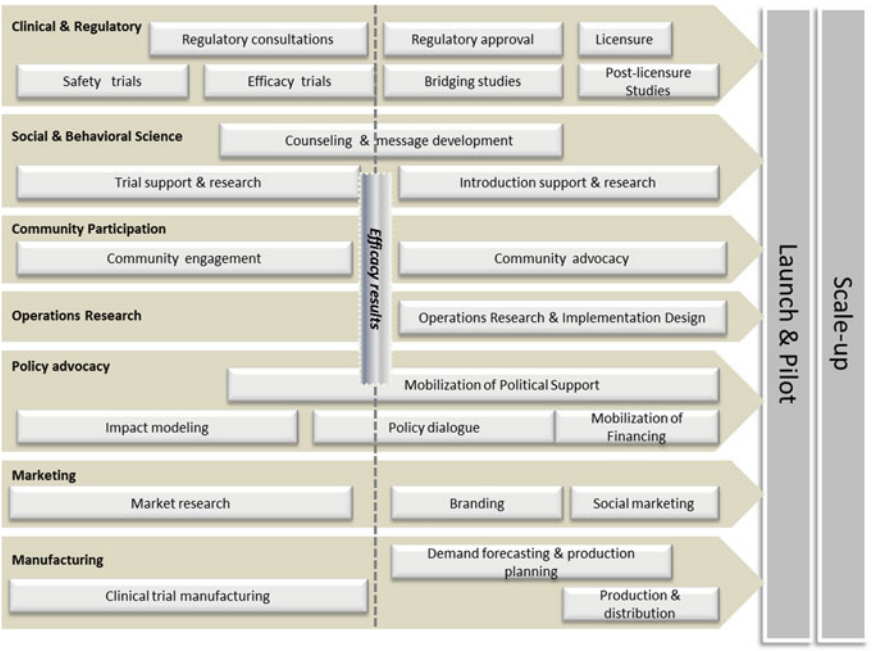

Fig. 2 Process flow for components of microbicide introduction

5.1 Clinical and Regulatory Issues

As a new product class, to be introduced to women in low and middle income countries, the regulatory requirements for approval and licensure for microbicides have yet to be clearly specified (see Nardi et al. 2014, this volume). Initial guidance from the US FDA and the EMA detailed aspects of trial design needed to provide sufficient evidence for licensure. There are multiple regulatory pathways that could be used for microbicide licensure, based primarily on FDA or EMA requirements, and these should in turn be considered by national regulatory authorities in the countries where microbicides will be introduced. EMA's article 58 provides for the EMA, in cooperation with WHO, to provide a scientific assessment of a new drug, yet leave authorization to the national regulatory authorities. Some countries have decided to recognize FDA's assessments, and adopt products that have FDA approval. Guidance for microbicide development continues to be developed and is available on the EMA and FDA websites (FDA 2012; EMA 2012).

It has been observed that although the regulatory bodies in some of the countries where microbicides will be introduced continue to develop their expertise and strengthen the resources needed to approve first-in-class products, many countries will need considerable support (Coplan et al. 2004; Stone 2009). In recognition of these challenges, organizations developing microbicides have

engaged in consultations with regulatory authorities, to keep themselves apprised of the evidence that will be expected of them as well as to provide information to aid in agency deliberations. For example, in 2008, IPM began convening annual meetings that brought together representatives of the ethics and regulatory bodies in the African countries where microbicide research is being conducted. The meetings update these bodies on progress in microbicide development, and serve as a forum for considering the requirements for microbicide registration and licensure in the countries represented.

As microbicide products advanced through clinical trials, communication between research teams was facilitated by the Quick Working Group, and meetings convened by WHO, as mentioned above. Consultations with regulatory bodies, particularly the FDA, the EMA and the South African Medicines Control Council have also occurred, and are the furthest advanced for tenofovir gel and dapivirine ring. These bodies have indicated additional data that will be needed to support licensure, and as a result, a range of supportive clinical studies have been fielded or planned. These include studies of drug–drug interactions, use by women with specific health conditions (e.g., Hepatitis B, impaired liver function), female and male condom compatibility, and use by adolescents and postmenopausal women. Pregnancy registers have been developed for those who became pregnant while using microbicides, as well as studies enrolling women who became infected with HIV while participating in a microbicide trial. These clinical studies have been carefully developed with feedback from the regulatory bodies that requested them, to ensure that the data that will be required are collected. Finally, bridging or follow-on studies are being planned for the post-efficacy/prelicensure period, if the trials show the product(s) are effective, including those that will provide trial participants with continued access to the products they helped to prove effective.

Normative guidance provided by WHO is widely accepted and has a strong influence on policy, accelerates implementation in resource-limited settings, and is necessary for accessing funding for implementation from some donors such as the Global Fund. WHO guidelines contain clinical, public health, or policy recommendations about health interventions, and provide information about what policy makers, health care providers, or patients should do (WHO 2012b). While national regulatory authorities have the mandate and responsibility to allow a product into the market in their jurisdiction, WHO Guidelines provide advice on issues to consider when national program managers and policy makers decide whether and how a product should be used, whether and how it should be prioritized to certain segments of the population or risk groups, and how to deliver the new product in an efficient and cost-effective manner with due consideration to other priority health interventions. The process to develop normative guidance on microbicides is underway and is aimed to be published soon after the products are first licensed. The guidance will be informed by pilot introduction and acceptability research conducted in high priority settings and communities if the new products have been shown to be safe and effective in the late-stage clinical studies.

A parallel procedure that will accelerate introduction and availability of the new microbicide products is the WHO prequalification process which is applied to

medicines and medical devices in priority areas (WHO 2013a, b). This procedure is usually focussed on medicinal products with established markets and several generic manufacturers, and prequalifies specific manufacturing sites and facilities to supply product for international procurement agencies for distribution in resource-limited settings. In this respect the prequalification program concentrates on the quality of the manufacturing process and the finished product. However, when applied to novel pharmaceutical products, the WHO prequalification process also assesses the safety and efficacy of the product in a similar manner to national regulatory authorities. This assessment will be strongly influenced by the result of any reviews by and decisions from stringent regulatory authorities. At present, prequalification requirements have not been developed for microbicides but the procedures should be initiated once data on the safety and efficacy of the new microbicide products are available.

5.2 Social Science and Behavioral Science Research

A decade ago, a landmark social and behavioral science study on preparing for microbicide introduction provided an overview of issues relevant to community, service delivery, policy development, and advocacy (Becker 2004). This work highlighted the range of potential contributions from social science and behavioral research. Earlier social and behavioral science research focused on acceptability of hypothetical vaginal products, and conceptual models framed acceptability as influencing adherence to use of microbicides proven effective (Woodsong and Koo 2002).

As experience with clinical trials increasingly pointed to adherence problems, significant effort has been devoted to measuring and motivating adherence to product use (van der Straten et al. 2012; Woodsong 2013; Tolley in press), and further understanding how (or if) acceptability and adherence are related (Montgomery, Gafos et al. 2010). Microbicide clinical trials include collection of behavioral data on acceptability and adherence, and provide behavioral counseling for risk reduction and adherence to the study protocol. Additional ancillary studies have further investigated factors influencing use within clinical trials, collecting data from trial participants as well as their male partners, health professionals, and community stakeholders (Pool et al. 2010; Whitehead et al. 2011; van der Straten et al. 2012; Woodsong et al. 2012). This body of research provides data that can inform much of the workstreams portrayed in Figure 3, particularly once a product with demonstrated clinical effectiveness becomes available outside of a research setting.

For example, the adherence counseling approaches used in microbicide trials are being further scrutinized and studied to ensure quality counseling in trials as well as provide an evidence base for operations research to develop future counseling on use of an effective product (Hoffman et al. 2008; Evangeli et al. 2009; Amico 2012). Social and behavioral science has identified gender issues (Mantell et al. 2009; FHI360 2014) and traditional sexual practices (Hilber et al. 2007; Braunstein et al. 2011) that could influence uptake and use of microbicides

proven effective. Marketing research will benefit from what has been learned about user perceptions of microbicide products and their use within sexual relationships (Bentley et al. 2004; Tolley et al. 2006; Morrow et al. 2007; Woodsong and Alleman 2008). Community engagement, education, and advocacy all require an understanding of social, cultural, and behavioral factors that will influence initial uptake and use, and it is expected that social scientists and behavioral scientists will be engaged in this work.

5.3 Community Engagement and Education

Community engagement is an essential component in the process of developing new health technologies, and facilitating introduction, uptake, and sustained use. It will be critically important for microbicides, as they will be introduced in settings where non-Western health belief systems are common, and the products are to be used for prevention (not treatment) of a highly stigmatized disease which is frequently associated with rumors, misinformation, and suspicion. Community stakeholders serve as an interface between government policy makers, program developers, civil society groups, and potential microbicide users. The HIV care and treatment field has demonstrated that community stakeholders and other influential individuals must be involved from the initial stages of human trials, and preferably even earlier (Tindana et al. 2007; Tedrow et al. 2012; AVAC 2013). Guidance and training in "Good Participatory Practice" has been developed by UNAIDS/AVAC to provide basic tools for effectively educating and engaging with communities in research (UNAIDS/AVAC 2011).

Microbicide trial researchers have routinely solicited community input, by working with existing community advisory groups or forming new ones. Initially, microbicide activities with communities were focused on increasing awareness of the need for a woman-centered HIV prevention product. As clinical research has progressed, community engagement has expanded to strengthen local knowledge about scientific research, as well as provide education on HIV prevention that can support the emergence of demand for microbicides. There is considerable variability in the form and function of community advisory groups (Morin et al. 2003), but the community groups currently engaged with microbicide trials are poised to provide support for a wide range of introduction activities.

5.4 Policy and Advocacy

The timing for policy and advocacy work is critical, and must be synchronized with the product development process. Microbicide advocates focused initially on the basic concept of a vaginal method that women can use for protection, followed by details about the products that advanced to large-scale efficacy testing. Next

will be concerted efforts to champion the development of policies and programs to deliver the microbicides that prove efficacious. The process of policy development, and attendant national guidelines and budgets, requires lengthy and multitiered discussions. Although policy makers may be reluctant to engage in serious consideration of a new class of product that is not yet proven effective, past experiences with developing HIV and population policies demonstrate that the policy process is lengthy and requires consultation and buy-in from many sectors (Stover 1998). Thus, it is appropriate to engage with policy makers and other influential parties during the clinical research process, to set the stage for action once efficacy is demonstrated. The possibility exists that a microbicide trial could be discontinued early because of high levels of efficacy, and in such a situation, advocates and policymakers will be keen to take advantage of the momentum of such a positive situation and move for speedy introduction.

The policy development process varies between countries, but the steps in this process are generally known, and approaches for engagement in these steps have been articulated. A number of assessment tools, techniques, and templates useful to organize information for policy makers to use in decision-making can be used for development of microbicide policies (Schwartlander et al. 2001; Stover 2003). Furthermore, policy development must be done in parallel with regulatory development, as the policies will reflect the regulations for how, who, when, and for what the products can be made available. For innovations in the international public health sector, the interface between product developers and policy makers has historically been supported by international aid agencies, and it is likely that such agencies will provide support for microbicide introduction in sub-Saharan Africa.

5.5 Operations Research and Implementation Science

International aid agencies, particularly USAID, provide funding support for operations and implementation research, and will likely be instrumental in microbicide introduction. This work will draw from all the streams of activity shown in Fig. 2 to plan programs in accordance with the capacity and requirements of local health service delivery systems. The Population Council, with funding support from USAID, has convened consultative meetings with nongovernmental, governmental, advocacy, and donor organizations to consider how experience with introducing other healthcare technologies, particularly contraceptives, can inform development of pilot and demonstration projects, and operations research for microbicide introduction (Brady and McGrory 2007, 2012). FHI 360, with funding support from the Bill and Melinda Gates Foundation, has completed a "proof of deliverability" study, to highlight issues that could facilitate or hinder introduction of new HIV prevention methods, including microbicides (Evens et al. 2012).

Since the current lead microbicide candidates are ARV-based, the products will initially be available as prescription-only medicines. Thus, they must be delivered through formal health care delivery systems, and the service provider must be able

to provide HIV testing to ensure that users are and remain uninfected. The service delivery avenues that have been discussed most frequently are family planning services and/or HIV services. Both types of service delivery points have advantages and disadvantages, which vary considerably in different country settings and with different target populations.

In addition to requiring that users be HIV negative, the first microbicide delivery services will likely require that users be on contraceptives until the safety of the products during pregnancy has been established. Family planning centers are generally "woman friendly" service delivery settings that could provide both contraceptives and microbicides. An open-label follow-on study to CAPRISA 004 is providing tenofovir gel in family planning clinics to assess provision of microbicides in this service delivery setting, compared to the research clinic setting (Karim and Baxter 2013), and study results will inform decisions about co-locating microbicide delivery with family planning.

Not all women who are at risk of HIV infection access family planning services. HIV service delivery facilities reach out to general or targeted at-risk populations, and may provide a range of testing, care, and treatment services. However, such facilities are primarily resourced to provide care, and are less well-resourced to provide the necessary counseling and support for HIV prevention. In addition, the stigma that may be associated with HIV treatment and care facilities could be a deterrent to potential microbicide users. Furthermore, in order to deliver microbicides, an HIV service facility must have capacity to provide more than voluntary counseling and testing. Operations and implementation research will be needed to inform decisions about locating microbicide in these settings, to ensure that both practical and socio-cultural issues are addressed. Making microbicides available to young women will be a particular challenge as they already have limited access to family planning and other reproductive health services. Yet this high HIV incidence group is one of the populations most in need of new HIV prevention approaches and should be prioritized for access.

Regardless of the type of service delivery setting used for microbicide delivery, the basic elements of microbicide delivery programs will include determining potential users' eligibility (e.g., must be HIV negative) and contraindications for use, establishment of testing requirements (e.g., STI, pregnancy and HIV testing), and a resupply schedule. The products will require resources and space for storage, tracking, disposal, etc. Training programs will be needed to encompass service provision and counseling. Although in most settings, processes and systems for meeting needs are generally understood by in-country program developers, adding a completely new technology will require creativity and flexibility.

Microbicides are intended to fit within a range of HIV prevention options with different levels of use-effectiveness (Nuttall 2004; Brown 2007), and preferably be used with other effective methods, such as condoms. Thus health professionals must be able to provide information so that prospective users can combine microbicide use with other prevention methods, if they wish. Lack of provider support is cited as a significant factor contributing to poor uptake of female condoms and intrauterine contraceptive devices (Hubacher 1994; Kerrigan 2000; Hoffman et al. 2008;

Mantell et al. 2011). This underscores the importance of leadership and guidance from national and international health professional associations, as well as the promulgation of clear national policies and service delivery protocols. It is likely that microbicide introduction and delivery will require additional scarce resources in health care delivery systems that are already stretched very thin.

5.6 Marketing

It is likely that microbicides will be introduced in government-subsidized programs in sub-Saharan Africa. Social marketing campaigns will be needed for microbicide introduction, and establish the initial positioning of these products. Some market research has been conducted during the microbicide development process to inform decisions about product formulations and means of delivery to the vagina. For example, market research studies assessed user preferences for the different types of products being considered for further development (e.g., gels, rings, vaginal tablets) (Nel et al. 2011), and studies assessed user preferences for products and their delivery modes (e.g., gel applicators) (Cohen et al. 2007, 2013). These studies provide information relevant to user interests and the products' appeal to potential users, which can inform marketing strategies.

Past experiences with condom social marketing campaigns and HIV prevention messages illustrate the importance of getting the introduction message right (Hanson et al. 2001). Doing so requires tailoring the message to the setting and potential user group. Social and behavioral science research, advocacy experiences, and community engagement activities conducted to date provide important insights into how to position microbicides as women's HIV prevention products (Boyce 2008). In the early days of microbicide development, they were referred to as "woman-controlled" and claims were made that they would empower women (Elias and Coggins 1996). It is now more common to use the term "woman-initiated" and to temper claims for the impact of microbicides on gender roles. Positioning microbicides as products to improve women's sexual pleasure could have negative ramifications if women's sexual pleasure is not a culturally appropriate topic for open discussion, but for some populations it could increase interest in the product.

Although preliminary work on product branding and social marketing research can be conducted earlier in microbicide development and be included in the regulatory dossiers, realistically it is probable that the main thrust will be conducted once efficacy is demonstrated. It is likely that these branding and marketing activities will include commercial firms with experience in marketing health products in low and middle income countries, as well as not-for-profit groups that have previously conducted social marketing campaigns for HIV prevention and contraceptives. Since the initial positioning of microbicides could have a long-term impact on perception and uptake (Brady and McGrory 2012), there is keen interest in making sure this receives thoughtful attention. The basic concept of

asking women to insert a microbicide product in their vagina and keep it there when having sexual intercourse, possibly without the partner's knowledge or approval, could become negatively sensationalized. It will be also important to monitor negative and erroneous publicity about health interventions and new technologies, and take appropriate steps to counter this when necessary (Robinson 2010; Larson et al. 2013).

5.7 Manufacturing, Supply Chain, Distribution

Manufacturing products for testing in clinical trials is done on a much smaller scale than will be required for introduction and rollout. Careful coordination will be needed to manufacture and deliver the increasingly larger amounts of products that will be needed for introduction and scale-up. Processes that are compliant with Good Manufacturing Practice must be achieved and validated, incremental increases in quantities must be estimated, and supply must be ensured through distribution channels that are monitored to ensure quality is maintained. As tenofovir gel and dapivirine ring advance in development, and potential manufacturing firms are identified, these considerations are becoming clearer. For example, there may be advantages to in-country manufacturing, both in terms of cost of production and transport, and because governments may require it. With improved clarity in the manufacturing process, the determination of microbicide cost per unit is also becoming more focused.

Microbicides are unlike other drug products that have been introduced in low and middle income countries long after being made available in the USA, Europe and other developed country markets, and they will require significant subsidies for manufacture and distribution. Demand forecasting and economies of scale are governed by policy and program, in accordance with funding for procurement. Again, the female condom experience is instructive, since low demand hindered economies of scale and potential cost reductions, and even when cost reductions were achieved, low demand persisted in areas where potential users, health professionals and policymakers continued to think of them as too expensive (Bertrand 1995, 2002; Horn 1998; Shane 2006). The importance of sustained supply is highlighted by contraceptive experiences in low and middle income countries, where sustained use of contraceptive products has been impeded by stock-outs, and these interruptions have affected use-effectiveness as well as provider and user-confidence in plans to continue to use the method (Bertrand 1995; Horn 1998).

Finally, women's experiences in clinical trials point to the importance of developing simple user-friendly instructions about how to correctly and consistently use microbicides (Woodsong et al. 2013). Increasingly, trials have used illustrated instruction sheets, with minimal text in the local language, to supplement the instructions given by clinical staff. These materials can be adapted for use with licensed products, as has been done by PATH for development of package inserts and labeling for the SILCS diaphragm and Woman's Condom.(PATH 2005;

PATH 2005; PATH 2006; Major et al. 2013) PATH conducted studies to assess women's ability to use the products by following the package instructions and work is currently underway to develop prototype packaging that meets regulatory requirements for labeling and package inserts in such a way that the preponderance of required information will not confuse users and that key messages are kept clear and comprehensible for users and providers. Similar user tests will be needed for microbicide packaging and use-instructions.

6 Conclusion

There is widespread agreement that women need more options for HIV prevention, and that vaginal microbicides could help meet this need. The challenges of introducing a new product class into overstretched healthcare systems in low and middle income countries are sobering, but work that is needed has become more clear since microbicide research first began.

Much of the work conducted thus far for the explicit purpose of planning for microbicide introduction focuses on the supply side, and as candidate products advance in efficacy testing, this type of planning will become more concrete. Certainly, there is much to be done to ensure that a reliable supply of quality products gets to where they are needed. However, the demand side of microbicide access also requires significant attention and planning. There is currently no space for microbicides on the shelves in public health clinics, people don't ask for them, and providers don't recommend them—this demand will have to be created. Furthermore, their use may challenge existing norms for sexual behavior (Becker 2004; Mantell et al. 2009). This has led to calls for a new paradigm for product introduction (Nuttall et al. 2007).

A compelling reason why microbicides are needed is that the cornerstones of HIV prevention—sexual abstinence, mutual sexual fidelity, and condom use—which have been so successful in reducing HIV incidence in many communities and populations, are inadequate for many women at risk of infection. This is evidenced by the stubbornly high HIV incidence in young women in sub-Saharan African countries. By intention or design, microbicide introduction could become a leverage point for empowerment and other broader improvements in women's positions in those regions targeted for microbicide introduction. In order to accomplish this goal, microbicides must be introduced in a way that provides appropriate access to those who need them most, and ensures that users are adequately empowered to use them correctly and consistently so that they can better protect themselves against infection.

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