Intravaginal rings as delivery systems for microbicides and multipurpose prevention technologies

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Abstract: There is a renewed interest in delivering pharmaceutical products via intravaginal rings (IVRs). IVRs are flexible torus-shaped drug delivery systems that can be easily inserted and removed by the woman and that provide both sustained and controlled drug release, lasting for several weeks to several months. In terms of women's health care products, it has been established that IVRs effectively deliver contraceptive steroids and steroids for the treatment of postmenopausal vaginal atrophy. A novel application for IVRs is the delivery of antiretroviral drugs for the prevention of human immunodeficiency virus (HIV) genital infection. Microbicides are antiviral drugs delivered topically for HIV prevention. Recent reviews of microbicide IVRs have focused on technologies in development and optimizing ring design. IVRs have several advantages, including the ability to deliver sustained drug doses for long periods of time while bypassing first pass metabolism in the gut. IVRs are discreet, woman-controlled, and do not require a trained provider for placement or fitting. Previous data support that women and their male sexual partners find IVRs highly acceptable. Multipurpose prevention technology (MPT) products provide protection against unintended/mistimed pregnancy and reproductive tract infections, including HIV. Several MPT IVRs are currently in development. Early clinical testing of new microbicide and MPT IVRs will require a focus on safety, pharmacokinetics and pharmacodynamics. Specifically, IVRs will have to deliver tissue concentrations of drugs that are pharmacodynamically active, do not cause mucosal alterations or inflammation, and do not change the resident microbiota. The emergence of resistance to antiretrovirals will need to be investigated. IVRs should not disrupt intercourse or have high rates of expulsion. Herein, we reviewed the microbicide and MPT IVRs currently in development, with a focus on the clinical aspects of IVR assessment and the challenges facing microbicide and MPT IVR product development, clinical testing, and implementation. The information in this review was drawn from PubMed searches and a recent microbicide/MPT product development workshop organized by CONRAD.

Keywords: contraception, HIV, microbicides

Introduction

Globally, 34 million people are currently infected with human immunodeficiency virus (HIV), and incident infections are more common in women than men, especially in sub-Saharan Africa.¹ Recent data from the Microbicide Trials Network's Vaginal and Oral Interventions to Control the Epidemic (VOICE) study revealed HIV incidence at the clinical sites as high as 9%, especially in young, single women.² The prevalence of HIV in South Africa (SA) has increased from 10.6% in 2008 to 12.3% in 2012, according to data presented recently at the 6th SA AIDS Conference in Durban.³

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Condom use “at last sex” has fallen in all age groups, most dramatically, from 85.2% to 67.4% among men aged 15 to 24 and from 66.5% to 51% in women of the same age.³ This highlights that the need to develop and implement preventive strategies continues to be one of the highest public health research priorities.

Microbicides are topical products designed to prevent HIV acquisition when applied to the cervicovaginal or rectal mucosa. The first successful microbicide was tenofovir (TFV) 1% gel. Pericoital use of TFV 1% vaginal gel was demonstrated to be safe and partially effective at reducing HIV-1 and herpes simplex virus-2 (HSV-2) incidence in the Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 Phase IIb study.⁴ In addition to showing effectiveness in this landmark study, TFV has shown efficacy in preventing infection in macaque and humanized mouse models and in several clinical trials, after oral administration.^{5–8} TFV gel was shown to provide 39%–54% protection from HIV acquisition, depending on adherence and the time of use.⁴ Further analysis of efficacy among drug compliers suggests levels of protection between 74% and 90%.^{9,10} Adherence and protocol compliance have plagued microbicide trials, and new, less user-dependent dosage forms and delivery systems are needed if significant effectiveness is to be attained. Intravaginal rings (IVRs) are intended to provide continuous, discreet protection without the need to disrupt the sex act. There is hope that they represent a more acceptable form of microbicide delivery, thereby increasing adherence and anti-HIV effectiveness.

Almost half of all pregnancies worldwide, estimated to be over 100 million annually, are unintended.^{11–13} Despite the existence of a variety of effective contraceptives available, discontinuation or nonuse remains high, primarily due to cost, side effects, inconvenient dosing schedules, poor access to prescription products, and/or poor acceptance of the method by the male partner, resulting in the unacceptably high rate of unintended or mistimed pregnancies.¹⁴ Statistics clearly show an unmet need for highly effective contraception, especially in less developed countries, where 99% of worldwide maternal deaths occur.¹² Not surprisingly, these countries, especially the ones of sub-Saharan Africa and south Asia, are also at the core of the acquired immunodeficiency syndrome (AIDS) epidemic.

Poverty, malnutrition, lack of education, and gender inequality fuel both unplanned pregnancies and HIV transmission. There are a significant number of women, especially in less developed countries, needing protection against sexually transmitted infections (STIs), in particular

HIV/AIDS, and long-term, highly effective contraceptive methods to prevent unplanned or mistimed pregnancies and provide optimal birth spacing and family size. Highly effective contraceptives (eg, sterilization, intrauterine devices, and hormonal contraceptives) typically provide little or no protection against STIs, while the barrier methods (eg, condoms) have unacceptably high contraceptive failure rates with typical use. Therefore, there is an urgent need to develop multipurpose prevention technologies (MPTs) providing both contraception and microbicidal activity, that are safe, highly effective, acceptable, and low-cost, especially ones that are suitable for use in less developed countries.

In the past, the main strategies for developing combined HIV prevention and contraceptive technologies were the use of physical barriers.¹⁵ The physical barriers comprise male and female condoms, diaphragms, and cervical caps. The typical contraceptive use failure rates for these methods range from 15%–30%.¹⁶ Physical barriers are heavily dependent on user adherence and partner cooperation and, in most cases, need to be applied immediately before intercourse. Physical barriers for contraception, while having few side effects, offer none of the health benefits typically found with hormonal contraceptives, such as reduction in menstrual blood loss.¹⁷ In terms of HIV prevention, a randomized controlled trial of the diaphragm showed no further reduction of the rates of HIV or STI male-to-female transmission over the condom.¹⁸

Recently, there has been a renewed interest in developing MPT IVRs. IVRs are flexible torus-shaped drug delivery systems that can be easily inserted and removed by the woman and provide both sustained and controlled drug release, lasting for several weeks to several months. IVRs, which deliver contraceptive steroids and steroids for the treatment of postmenopausal vaginal atrophy, have already been approved and are available worldwide. Antiretroviral (ARV) medications can also be released from IVRs, at concentrations that are expected to prevent the acquisition of HIV-1.¹⁹

Herein, we review the history and advantages of IVRs, microbicides, and the MPT IVRs currently in development, focusing on the clinical aspects of IVR evaluation and the challenges facing microbicide and MPT IVR product development, clinical testing, and implementation.

History

The first report of vaginally administered drugs was published in 1918 by Macht, who demonstrated that several drugs, including morphine, atropine and potassium iodide, could be vaginally absorbed.²⁰ The field of vaginal drug delivery has since flourished to include a diversity of applications, with

numerous marketed products currently available for contraception (eg, IVRs: NuvaRing® [etonogestrel/ethinyl estradiol] [Merck & Co, Inc, Whitehouse Station, NJ, USA] and Progering® [progesterone] [Silesia Laboratorios, Santiago, Chile]; film: Vaginal Contraceptive Film® [nonoxynol-9] [VCF; Apothecus Pharmaceutical Corp, Oyster Bay, NY, USA]; and the sponge: Today® sponge [nonoxynol-9] [Mayer Laboratories Inc, Berkeley, CA, USA]), for hormone replacement therapy (IVRs: Femring® [estradiol acetate] [Warner Chilcott LLC, Rockaway, NJ, USA], Estring® [estradiol] [Pfizer, Inc, New York, NY, USA], and Fertiring® [progesterone] [Silesia Laboratorios]; creams: Premarin® [conjugated estrogens] [Pfizer, Inc, New York, NY, USA], Estrace® [estradiol] [Shire plc, Dublin, Ireland], Estrasorb™ [estradiol] [Medicis Pharmaceuticals Corp, Scottsdale, AZ, USA], and Ogen® [estropipate] [Pfizer, Inc]; and tablets: Premarin® and Vagifem® [estradiol] [Novo Nordisk, Bagsværd, Denmark]), for antimicrobial treatment (gel: Metrogel® [metronidazole] [Galderma Laboratories, Fort Worth, TX, USA]; creams: Cleocin® [clindamycin] [Pfizer, Inc], Clindesse® [Ther-Rx Corp, Chesterfield, MO, USA] or ClindaMax® [clindamycin phosphate] [Nycomed, Zurich, Switzerland], Gyne-Lotrimin® [Schering-Plough HealthCare Products, Inc, Memphis, TN, USA] or Mycelex® [Bayer HealthCare Pharmaceuticals Corp, Montville, NJ, USA] or Femcare® [clotrimazole] [Schering-Plough HealthCare Products, Inc, Memphis, TN, USA], Gynezol® [Parke-Med, SA] or Femstat® [butoconazole] [Bayer HealthCare Pharmaceuticals Corp], Monistat® [miconazole nitrate] [Insight Pharmaceutical LLC, Trevose, PA, USA], Vagistat-1® [tioconazole] [Bristol-Myers Squibb, New York, NY, USA], and Terazol® [terconazole] [Janssen Pharmaceuticals, Inc, Titusville, NJ, USA]; suppositories: Cleocin, Terazol, and Monistat; tablets: Gyne-Lotrimin or Mycelex or Femcare), cervical ripening (vaginal insert: Cervidil® [dinoprostone] [Forest Laboratories, Inc, New York, NY, USA] and gel: Prepidil® [dinoprostone] [Pfizer, Inc]), and pregnancy termination (suppositories: Prostin E2® [dinoprostone] [Pfizer, Inc] and tablets: Cytotec® [misoprostol] [GD Searle and Co, Skokie, IL, USA]). The development of vaginal drug delivery systems for new uses, most notably as topical microbicides, continues to expand and progress at a fervent pace.

Several decades following the introduction of vaginal drug delivery, IVR development efforts began in earnest, in 1966, with the discovery that steroids could be delivered, through silicone elastomers, at constant rates for several days.²¹ The first IVR tested clinically in the 1970s

was for the delivery of medroxyprogesterone acetate for contraception.^{22,23} Also in the 1970s, the World Health Organization (WHO) developed three contraceptive rings containing either progesterone, norethisterone, or levonorgestrel (LNG). The progesterone and norethisterone rings showed higher than desired side effects (eg, menstrual irregularities) and pregnancy rates,²⁴ so the 90-day LNG IVR was selected for further development, ultimately showing a 3.6% pregnancy rate among women who wore the IVR continuously for 1 year (replacing the IVR every 90 days).²⁵ While effective, the IVR was firm and relatively inflexible, leading some women to experience expulsion, vaginal abrasions/ulcers and asymptomatic vaginal irritation^{26,27} and was subsequently redesigned to be smaller and more flexible. The redesigned LNG IVR was shown not to have adverse effects on the vaginal epithelium,²⁸ but a lack of funding precluded further testing.

Today, there are two contraceptive rings commercially available: the NuvaRing (available in more than 40 countries worldwide), and the Progering (available in Chile, Peru, Bolivia, Ecuador, Guatemala, and Honduras). The NuvaRing is made of ethylene vinyl acetate (EVA), a thermoplastic that allows the IVR to be 4 mm thick in cross-sectional diameter, which is significantly thinner than silicone IVRs. It delivers 120 µg of etonogestrel and 15 µg ethinyl estradiol (E₂) per day. The NuvaRing is approved for 3 weeks of use, with removal for 1 week to allow for menses. However, a small study using vaginal ultrasound and serum progesterone as markers of ovulation supports that ovulation is suppressed for 5 weeks with the NuvaRing.²⁹

Progering, which is made of silicone elastomer and approved for use by lactating women, delivers 10 mg of progesterone per day for 3 months. Other hormonal IVRs have been developed over the years but were not brought to market for various reasons, usually related to undesirable side effects (eg, menstrual irregularities and reduction of high-density lipoprotein cholesterol levels). Work is ongoing in contraceptive IVR development, including the Population Council's Nestorone® (NES) and NES/E₂ IVRs, the latter of which is intended to last 1 year using a 21-day-in and 7-day-out dosing cycle.³⁰

Steroid-releasing IVRs have also been developed for hormone supplementation or replacement therapy, with a handful of silicone elastomer-based products commercially available. The Fertiring, similar to the Progering, releases progesterone but for the indication of hormone supplementation and pregnancy maintenance during *in vitro* fertilization. The Estring (7.5 µg/day estradiol) and Femring (0.05 mg/d or 0.1 mg/d estradiol acetate dose options) are both 90-day IVRs

for use in estrogen replacement therapy in postmenopausal women with urogenital atrophy.

The first IVR developed preclinically for microbicide release was a silicone matrix IVR designed to release the non-specific microbicide/spermicide surfactant nonoxynol-9.³¹ Development of this ring was halted when a clinical trial showed that frequent use of nonoxynol-9 could increase the risk of HIV-1 transmission.³² In the decade since, the development of microbicide and more recently, MPT IVRs has rapidly expanded to include the development of numerous classes of microbicide candidates, including ARVs with different mechanisms of action. Because of the physiochemical diversity of these drug candidates, this has also led to a boom in the development of new IVR designs and technologies that may better deliver these drugs or drug combinations.³³

Advantages of IVRs for delivery of microbicides and MPTs

The IVR is a unique and appealing vaginal drug delivery system, as it is female initiated and controlled, and provides sustained drug release, in the order of weeks to months. Some of the major advantages of IVRs for the development of microbicides and MPT products include the local delivery of microbicides, the maintenance of steady-state hormone serum concentrations, extended dosing regimens that do not require daily action, and the discreet woman-controlled use.

Local delivery of microbicides

IVRs can effectively deliver high concentrations of microbicides directly to the vagina and specifically, to the cells and tissues targeted by most of the microbicide drug candidates in development.^{34–37} This local delivery also allows for substantially lower drug doses compared with oral dosing regimens. The clinical data on the delivery of TFV via an oral pill (using 300 mg dose of the prodrug tenofovir disoproxil fumarate) or via vaginally applied TFV 1% gel (~40 mg TFV) in women show that the vaginal tissue concentrations of tenofovir-diphosphate, the active metabolite of TFV, were ≥ 130 -fold higher with vaginal dosing compared with oral dosing.³⁸ Compared with vaginal gels, the rate and duration of microbicide delivery from IVRs to the vagina is more controlled, providing reduced doses that may better maintain prophylactic concentrations for longer duration. Moreover, the local delivery of microbicides from IVRs also helps reduce the systemic exposure to these ARVs, potentially improving their safety profiles (ie, reduced systemic side effects) and minimizing the risk for developing resistance.

Pharmacokinetic (PK) studies will need to confirm that the microbicide doses delivered from IVRs remain high in the genital compartment but low in the systemic circulation, to reduce the risk of developing ARV resistance, as this is a concern with oral dosing.

Maintenance of steady-state hormone serum concentrations

For microbicide/contraceptive MPTs, IVRs are capable of simultaneously providing the local delivery of microbicides and the sustained systemic delivery of contraceptive hormonal steroids. Delivery of hormones via IVRs eliminates the burst effect seen with injectable contraceptives^{39,40} and minimizes the daily peak and trough fluctuations seen with orally administered hormones.⁴¹ For example, NuvaRing use results in maximum serum hormone concentrations that are 30%–40% lower than those seen with orally administered contraceptive hormones.⁴² Further, by avoiding first pass metabolism in the gut, IVR users typically have fewer hormonal side effects than do oral hormonal contraceptive users, including adverse effects on the coagulation system.^{43–45} Many ARVs either induce or inhibit hepatic cytochrome P450 enzymes, which are required for the metabolism of commonly used contraceptive steroid hormones.^{46,47} In addition, vaginal drug absorption, unlike oral dosing, is not altered by gastrointestinal disturbances.

Extended dosing regimens possible with IVRs

The clinical evaluation of effectiveness in the microbicide and oral preexposure prophylaxis (PrEP) trials, to date, has struggled greatly with the issue of poor adherence, which might be attributed in part, to short action and daily or pericoital dosing regimens. Adherence to vaginal gel dosing in the microbicide gel trials has been a major challenge. TFV 1% gel was shown to be 39% effective overall in the CAPRISA 004 trial when dosed pericoitally, with the effectiveness increasing to 54%–90% in the high-adherence users.^{4,9,10} TFV 1% gel, dosed daily in a coitally-dissociated regimen, was determined to be ineffective in the VOICE trial, with the wide consensus that poor adherence was a major contributor to the gel's failure.² The analysis of drug levels in the VOICE participants' blood samples showed detectable levels of TFV in only 23% of the participants in the TFV gel arm, indicating that only one out of four participants actually used the product.² Similarly, the poor adherence to daily oral PrEP dosing regimens has also affected efficacy in clinical efficacy studies.⁴⁸ In the FEM-PrEP study,

for example, the self-report and pill count showed >90% and >80% adherence, respectively, while detection of drug in blood indicated that no more than 30% of women used the product as instructed.⁴⁸

Although daily dosed combined oral contraceptives are the most commonly used contraceptive method worldwide, this dosing regimen remains problematic for many women, with nonadherence and discontinuations being major contributors to contraceptive failures.⁴⁹ Recent initiatives have focused on long-acting reversible contraceptive (LARC) methods because it has been shown that as the uptake of LARC increases, unintended pregnancies decrease.⁵⁰ While not typically considered LARC methods, the currently available contraceptive IVRs have durations of action of 3 weeks–3 months,⁵¹ eliminating the need for daily or coitally-dependent action. Because a woman can insert an IVR and forget about it for a determined time period, there is a great potential to improve adherence to product use with IVRs.⁵²

Though it remains to be seen whether IVRs will in fact improve adherence to microbicide use, a study comparing the adherence to contraceptive IVRs with adherence to daily oral contraceptive pills provides encouraging results.⁵² IVR users were more likely to report perfect use compared with daily pill users.⁵² The IVRs that are currently in contraceptive, microbicide, or MPT development are being designed to have a 30–365 day duration.³³

Discreet woman-controlled use

The most disadvantaged women in society are also at highest risk for HIV. These women include commercial sex workers, women who cannot negotiate safer sex, women who cannot ensure their partners' faithfulness, and those who often cannot leave a relationship, for economic or social reasons. Therefore, the development of microbicide and MPT products that require no partner cooperation and that can be used discreetly is a priority. Many of the highly effective LARC methods, such as the contraceptive implant or the contraceptive intrauterine system, require insertion by a trained provider, which may be a barrier to access in resource-constrained areas.⁵³ IVRs, on the other hand, provide the promising balance of not requiring daily action and being woman initiated and controlled.

Microbicide and MPT IVRs in development

Microbicide IVRs

Currently, the most clinically advanced microbicide IVR is for the 28-day delivery of dapivirine (DPV), a nonnucleoside

reverse transcriptase inhibitor of HIV developed by the International Partnership for Microbicides (IPM). While several types of DPV IVRs (including matrix- and reservoir-type IVRs, all comprised of silicone elastomer) were evaluated in the early clinical studies,^{36,54,55} the matrix DPV ring (a simpler ring design with a 56 mm outer diameter and a 7.6 mm cross-sectional diameter) was selected for further development and is currently in Phase III testing, with results expected in 2015. IPM is also conducting clinical trials of silicone IVRs containing maraviroc, a chemokine receptor type 5 (CCR5) entry inhibitor, alone or in combination with DPV.⁵⁶

Building on the success of the leading topical microbicide product TFV 1% gel, which has demonstrated prophylactic effectiveness in both animal models and women (CAPRISA 004), long-acting TFV-based IVRs are being developed for the 3-month delivery of TFV, with Phase I testing planned later this year. These IVRs, developed by CONRAD in collaboration with Dr Patrick Kiser (University of Utah and Northwestern University), are comprised of polyurethane, which is a more versatile thermoplastic than the EVA used in the NuvaRing, yet have dimensions more similar to the NuvaRing than to the bulky silicone-based IVRs (the TFV IVR has a 5.5 mm cross-sectional diameter and a 55 mm outer diameter). Notably, in sheep, the drug concentrations throughout 90 days of treatment with TFV IVRs were similar to or higher than those seen with TFV 1% gel.^{19,57} A 1-month tenofovir disoproxil fumarate IVR that is similar in design to the TFV IVR is also nearing Phase I testing. This IVR has been tested in a nonhuman primate HIV efficacy model and was recently reported to demonstrate 100% protection against simian–human immunodeficiency virus (SHIV) acquisition in a repeated low-dose challenge model.⁵⁸

Several additional microbicide IVRs are in preclinical development and have been recently reviewed elsewhere.^{1,33} A detailed table of past, ongoing, and future microbicide trials, including IVR trials is available at <http://www.avac.org/ht/a/GetDocumentAction/i/3109>.

MPT IVRs

Worldwide, there is a significant unmet need for both effective contraception and medications to prevent genital acquisition of STIs, namely HIV. The most advanced MPT IVR in development is the 90-day TFV/LNG IVR for the dual purpose of HIV prevention and contraception, which is expected to enter Phase I testing later in 2013. Developed by CONRAD in collaboration with Dr Kiser, this IVR builds on the development of the TFV IVR. The combination IVR has been demonstrated

preclinically to provide steady-state dosing of approximately 10 mg/d TFV (similar to the TFV-only IVR) and 20 µg/d LNG for 90 days, both in vitro and in animal models.^{19,57} This low-LNG dose was selected based on the previous work done by the WHO in the 1970s, described above, in which 20 µg/d LNG was found to be effective and acceptable, working primarily through local effects (eg, cervical mucus thickening) rather than by disrupting normal ovulation.^{25,59} Previous studies of the 20 µg/d LNG IVR found that 40% to 60% of users ovulated normally.^{24,60} The systemic levels of LNG found in the 20 µg/d LNG IVR users were in the same low range as those measured among users of the 20 µg/d LNG intrauterine system (IUS) (Mirena®; Bayer HealthCare Pharmaceuticals) (0.2–0.5 ng/mL)^{61–63} and the 14 µg/d LNG IUS (Skyla™; Bayer HealthCare Pharmaceuticals) (0.1–0.2 ng/mL).⁶⁴ These highly effective and acceptable contraceptive products work primarily by exerting local effects on the cervical mucus,⁶⁵ which we believe will be the primary contraceptive mechanism of action of the proposed TFV/LNG IVR. The additional contraceptive action from the LNG IUS likely results from local effects on the endometrium,⁶³ impairment of the fallopian tube transport of sperm and ova, and some level of ovulation suppression.⁶⁶ In vitro data even support that the LNG levels delivered from the LNG IUS interfere with sperm–zona pellucida interactions.⁶⁷ We plan to assay cervical mucus LNG levels in early phase PK studies and compare these levels in TFV/LNG IVR users with the levels found in LNG IUS users.⁶⁵

IPM is also involved in the early development stages of a microbicide/contraceptive MPT IVR comprising the combination DPV/LNG; the LNG dose targeted for this IVR is 35 or 70 µg/d, which is expected to have both systemic (ie, anovulatory) and local contraceptive effects.

Other MPT IVRs in preclinical development include the Population Council's Medivir-150/zinc acetate IVR (for prevention of HIV and HSV-2) and Medivir-150/zinc acetate/LNG IVR (for the same indications plus contraception). CONRAD also initiated the development of an acyclovir/TFV IVR for the treatment of HSV-2 and prevention of HIV,⁶⁸ but the development was halted due to reallocation of funding.

Objective assessment of product adherence with long-term use of IVRs

As discussed above, the monitoring of product adherence in microbicide/PrEP efficacy trials is a major issue facing the field currently, and while great strides are being made

to develop better measures of adherence for gel products, monitoring the correct and consistent use of long-acting IVRs will present new, additional challenges. Some groups have proposed assessment of the presence of the biofilm on the surface of IVRs to monitor adherence to the dosing regimen. However, it is not known how quickly a biofilm forms on the IVR surface, as the limited number of studies have only tested 28 days of use.^{69,70} In comparison, urinary catheters have been shown to become colonized with bacteria, in the sterile bladder, within 48 hours.⁷¹ Biofilms form within 60 hours on intrauterine devices, in the relatively sterile uterine cavity.⁷² This suggests that monitoring vaginal bacteria could determine whether an IVR was inserted vaginally but would unlikely be able to differentiate hours from weeks of use or determine the duration of use.

An alternative to assessment of the vaginal biofilms on IVRs would be to quantify the level of drug substance left in an IVR after a participant returns the IVR. This method is currently being employed in the IPM Phase III study of the DPV IVR. The current TFV and TFV/LNG IVR prototypes about to enter Phase I testing are transparent, revealing a white drug-loaded core that transitions to clear as TFV is released during use; similar to determining the residual drug content in the returned IVRs, the level of pigment/opacity in these IVRs could potentially be standardized to correlate with duration of usage, potentially providing a new, rapid, and more field-ready measure of IVR adherence.

However, all of these methods described thus far have the limitation that they do not provide time-associated adherence data, and given that IVRs may be removed intermittently during use, time-stamped measures would be most useful.

Clinical assessment of IVRs

The need for microbicide and MPT products is clear. STIs and unintended/mistimed pregnancies are leading causes of morbidity and mortality among women worldwide. Progress has been made in the preclinical setting to develop both IVRs, which can deliver concentrations of contraceptive hormones, and ARVs, which are expected to be effective in preventing pregnancy and HIV. Going forward, Phase I testing in the clinic must include an evaluation of product safety and acceptability, particularly in women in developing, resource-poor countries, who are at high risk of HIV acquisition.

Acceptability

There is high acceptance of IVRs for the delivery of contraceptive hormones.^{73,74} An international study of the NuvaRing found that approximately 97% of women reported they

would recommend the IVR to their friends.⁷⁵ Perhaps the best assessment of user acceptability was reported in a study of women who were currently using oral contraceptive pills and who reported being happy with this method.⁷³ These oral contraceptive pill users were transitioned and randomized to either the contraceptive patch or NuvaRing.⁷³ The women who transitioned to the IVR were significantly more likely to plan to continue this method than were the women who transitioned to the contraceptive patch.⁷³ The acceptability assessments for the IVR were significantly higher than for the oral contraceptive pill ($P < 0.001$), and the women randomized to receive the contraceptive IVR reported shorter and less painful menses than did the women randomized to the contraceptive patch.⁷³ These data are in accordance with another head-to-head study of the contraceptive IVR versus oral contraceptive pills, in which IVR users reported equal or higher acceptability with the IVR compared with the oral contraceptive pills.⁷⁶ Women using a NES/E₂ IVR for 13 cycles, using a 21 days in/7 days out regimen, also found this extended use to be acceptable.⁷⁷

An IVR acceptability study conducted in Africa enrolled 157 HIV-negative women who were asked to use a placebo IVR (56 mm outer diameter, 7.7 mm thick) for 12 weeks. Based on focus group discussions with women and in-depth interviews with a subset of male partners, the IVRs were found to have low expulsion rates (3%), and most women (82%) did not remove the IVR for any time during the 12 weeks of use, with most of the removals occurring for less than a 12-hour period.⁷⁸ There was high acceptability of the placebo IVR in this patient cohort, with 94% of women saying that they were either interested or very interested in trying the IVR.⁷⁸ Very few women (2%) reported that they could feel the IVR during daily activities.⁷⁸ The biggest concern for women trying the placebo IVR was that it would “get lost inside their bodies,”⁷⁸ suggesting that counseling on product use, risks, and benefits would be important prior to delivery.

The most advanced microbicide IVR to date is the DPV IVR (developed by IPM). Early safety and acceptability studies, in African populations, of this IVR have found no product-related serious adverse events,³⁶ and the adverse events were not higher than those reported by placebo IVR users.⁵⁴

Side effects associated with IVRs

Breakthrough bleeding (contraceptive component)

Another major determinant of acceptability of an IVR product includes the side effects experienced by the user. For IVRs, systemic side effects are usually minimized, as

first-pass metabolism through the liver is avoided.⁷⁹ However, a major concern in terms of the acceptability of the product, specifically, when a hormonal contraceptive is selected, is the effect on the menstrual cycle, with breakthrough bleeding being a major reason for product dissatisfaction and discontinuation.^{25,80} Breakthrough bleeding was a leading reason for the Population Council to discontinue development of its NES-only IVR for contraception.³⁰ Of note, ovulation was inhibited in approximately 97%–99% of cycles, using the NES IVR,⁸¹ supporting our hypothesis that disruption of ovulation is one factor associated with breakthrough bleeding in progestin-only methods. However, with use of the previous 20 µg/d LNG IVR, during which approximately 40%–60% of women continued to ovulate normally,^{24,60} bleeding disturbances were less likely – at approximately 17%.⁸⁰ This hypothesis is further supported by prior studies of LNG and norethisterone IVR users,^{82–84} showing that anovulation was associated with disrupted bleeding patterns. It has previously and recently been recommended that to decrease breakthrough bleeding, one should increase the progestin dose,^{30,81} but we believe that the opposite is true. By lowering the progestin dose, the main contraceptive action would be local,⁶⁵ and breakthrough bleeding would be minimized by allowing normal ovulatory cycles and physiologic estrogen and progesterone levels.^{82–84}

Vaginal expulsions, disruption of intercourse, vaginal abrasions/ulcers

Early clinical safety testing of microbicide (and MPT) IVRs includes a focus on the effect of the IVR on the subclinical and physical properties of the vaginal mucosa, as well as any systemic effects. One of the most basic safety precautions is that an IVR does not have any adverse physical effects on the vaginal epithelium. The reason for this is that women who are at risk of unintended pregnancies are similarly at risk of STIs, including HIV-1. It is known that ulcerative infections, such as syphilis and HSV-2, increase the efficiency of sexual transmission of HIV-1, likely through frank breeches in the genital epithelium or chronic inflammatory infiltrates after ulcer healing.^{85–89} Any breeches in the protective mucosa caused by IVRs would be a safety concern.

As discussed above, an early LNG IVR prototype developed by the WHO had a cross-sectional diameter of 9.5 mm and released 20 µg/day of LNG for 90 days.²⁵ This was a silastic ring and was firm and relatively inflexible, requiring 6.4 N to compress the ring²⁸ (thus, approximately fourfold more rigid than the NuvaRing). These physical properties resulted in 29% of women experiencing an unintended

expulsion of the IVR in the first year of use, mainly with defecation, urination, or menstruation.²⁶ Repeated unintentional expulsion of the IVR resulted in 7.1% of women discontinuing the product in the first year of use.²⁶ This bulky IVR was also electively removed by 12% of women, due to vaginal discharge, irritation, vaginal pain, or dyspareunia.²⁶ The 3.6% pregnancy rate found in the older generation 20 µg/day LNG IVR might have been primarily the result of the high rates of expulsions and removals seen with this less flexible IVR.²⁶ A later trial of 139 women using the early generation silastic IVR found that 35% had erythematous lesions, which were usually asymptomatic.²⁷ Although most of the lesions resolved quickly and spontaneously, the findings prompted considerable concern over safety, and manufacturing of that IVR ceased.²⁷

A redesigned, softer 20 µg/d 90-day LNG IVR (IVR-2), which had a smaller cross-sectional diameter (6 mm) and required only 1.3 N to compress, was later developed and tested for cervicovaginal irritation. This IVR showed no clinically significant changes in the vaginal and cervical mucosa.²⁸

Modern day IVRs have thus evolved to incorporate smaller cross-sectional diameters and more flexible polymers. The progesterone IVR (Progering) has a cross-sectional diameter of 8.4 mm and an outer diameter of 5.5 cm.⁹⁰ In one study, 6% of the progesterone IVR users reported frequent expulsions, and 8.1% discontinued use due to this reason.⁹¹ The NuvaRing has a cross-sectional diameter of 4 mm and an outer diameter of 5.4 cm and requires approximately 0.75 N to compress through 20 mm. In studies, women have reported that this IVR was easy to insert and remove^{74,92} and did not interfere with intercourse.⁷⁴ Among the couples using the NuvaRing for contraception, approximately 15% of men reported being infrequently able to feel the IVR during intercourse⁷⁵ but noted that it was not bothersome and did not interfere with intercourse. The Population Council is currently testing a NES/estradiol IVR for contraception. The colposcopic safety data on the NES/estradiol IVR demonstrate no adverse physical effects on the vaginal epithelium. For example, in a large study of women using IVRs with various combinations of ethinyl estradiol and/or NES or norethindrone acetate, there was a low incidence of subclinical abnormal findings, with colposcopy; all of the subclinical abnormal findings resolved within 1 month, and there were no differences found in the incidence of colposcopic abnormal findings, based on the four types of IVRs evaluated.⁹³ The IVRs used in this study ranged in size from 7.6 × 56 mm to 9.0 × 56 mm.⁹⁴ The rigidity of these IVRs was expressed as the force, in grams,

required to push the sides of the IVR together in the center, and ranged from 270–1000 grams.⁹⁴ The results of the IVR users in this study were also compared with those of a control group of non-IVR users and to baseline measurements. The research team concluded that IVR use, including long-term IVR use, does increase the incidence of subtle and reversible changes in the vaginal mucosa compared with non-IVR use, but the clinical significance of these findings, specifically in terms of susceptibility to STIs and HIV-1, is not known.⁹³ The most common findings in the study of IVR users were petechiae,⁹³ which are known to occur in healthy women who do not use the IVR.⁹⁴

While the clinical evaluation is currently pending, the TFV and TFV/LNG IVRs in development by CONRAD are specifically designed to have dimensions and mechanical properties similar to the NuvaRing and to maximize the discreteness of the IVR compared with the bulkier silicone rings.

Emergence of ARV resistance with preventative ARV regimens

There is an ongoing concern that individuals who use either oral or topical PrEP products, once exposed to HIV-1, will be more likely to acquire ARV-resistant strains.⁹⁵ Studies with microbicide vaginal gels support that ARVs delivered vaginally achieve higher levels in the genital tissue than in serum or peripheral blood mononuclear cells.^{38,96} HIV prevention trials utilizing vaginal ARV gels have not demonstrated, in the short term, the acquisition of ARV-resistant strains among the active drug users who acquired HIV.^{4,97} Low systemic levels and high genital compartment concentrations are the PK target for ARV-containing IVRs.¹⁹ We believe that locally delivering effective concentrations of ARVs will significantly reduce the systemic side effects while maintaining protection against mucosal HIV-1 acquisition.

Subclinical safety

Alteration of vaginal microflora

Some studies have associated alterations of vaginal flora, specifically, intermediate vaginal flora or bacterial vaginosis, with HIV-1 acquisition.^{98–100} Increases in anaerobic vaginal bacteria have been shown to increase local genital tract inflammation, which is reversed with normalization of the flora.¹⁰¹ There are data to support that the NuvaRing improves the vaginal flora from baseline measurements;^{102,103} however, the methods used in these studies are less sensitive,¹⁰² and there exists a gap in our knowledge of how IVRs alter the vaginal microbiome, as assessed by more detailed

methods, such as 16S ribonucleic acid (rRNA) sequencing, which is considered the state of the art in assessing the mucosal microbiome.¹⁰⁴ In the bacteria-rich vagina, biofilm forms on the IVR, as would form on any foreign body located in a nonsterile environment. In vitro data support that common vaginal yeast isolates adhere to the NuvaRing.¹⁰⁵ A small study, using scanning electron microscopy of NuvaRing segments from one woman after 28 days of use, supports that bacteria and mucus do not penetrate or erode the IVR surface and are easily washed away with water.⁷⁰ A biofilm develops on contraceptive IVR segments after 28 days of use in nonhuman primates.⁶⁹ Early clinical studies of future microbicide and MPT IVRs will need to include assessments of the changes in the vaginal microbiome with IVR use, to confirm that no adverse changes occur with chronic IVR use.

Effect on vaginal mucosa – local safety endpoints

A major issue in hormonal contraception, which is particularly relevant to MPTs, is the possible link between systemic contraceptive hormones (mainly the intramuscular depot medroxyprogesterone acetate [DMPA]) and the incidence of HIV.^{106–110} One pivotal analysis showed that women in serodiscordant relationships who used DMPA not only had a higher risk of acquiring HIV, but also had an increased risk of transmitting the virus to seronegative male partners.¹¹⁰

Theories as to how exogenous progestins might increase genital tissue susceptibility to HIV-1 infection center on epithelial thinning and the alteration of local mucosal immunity. A decrease in the number of epithelial cell layers or the density of intercellular junction proteins potentially enhances the exposure of cervicovaginal mucosal target cells to HIV-1. In nonhuman primate models of HIV infection, high-dose progestin administration has been found to cause a dramatic atrophy of the vaginal epithelium.^{111–116} However, the data regarding the effect of exogenous progestins on epithelial thickness in the human vagina are mixed, ranging from no change to either increased or decreased thickness,^{117–120} in short-term (3–6 months)^{117,119} and long-term (2–3 years)¹¹⁸ DMPA users.

Exogenous contraceptive hormones might also affect the mucosal susceptibility to HIV-1 infection, through alterations of the cervicovaginal mucosal immune response. This theory will likely continue to be tested as new contraceptive and MPT IVR products are developed. Although the cervix and vagina are likely the initial sites of entry of HIV-1 in women,^{121–123} the effects of exogenous estrogen

and progesterone on the local immune environment of the lower genital tract have not been clearly elucidated,^{120,124,125} with most data focusing on biologic mechanisms within the endometrium.¹²⁶ Our group recently published a study showing that DMPA administration resulted in a significant increase in activated lymphocytes (cluster of differentiation [CD]45, CD3, CD8, CD68, human leukocyte antigen [HLA]-DR, and CCR5) in the vaginal tissues compared with baseline samples obtained in the follicular and luteal phases of the menstrual cycle.¹²⁷ This alteration of local immune response could increase mucosal susceptibility to HIV. Other groups have shown an association between increases in the concentration of systemic (cytokine) and natural killer (NK) cells in the genital compartment and an increased incidence of HIV-1, specifically, in the CAPRISA 004 cohort.¹²⁸ An increase in genital tract inflammatory mediators has also been linked to higher viral loads and lower systemic CD4 counts in HIV-1 positive women.¹²⁹

We hypothesize that the microdose concentrations of hormones, particularly of LNG, being proposed in MPTs would not have the same effect on the vaginal mucosa as do the high-dose injectable progestins, like DMPA. In particular, women using DMPA for contraception have serum estradiol levels similar to those of menopausal women (range 15–40 pg/mL)^{40,130,131} and endogenous progesterone concentrations that are suppressed, at 3–5 nmol/L (1–2 ng/mL), secondary to anovulation.^{40,132} On the contrary, serum progestin (LNG) levels are lower, at approximately 0.6–2.2 nmol/L (0.187–0.682 ng/mL) in women using the 20 µg/d LNG IVR,^{60,63,82} while serum estradiol levels remain well above menopausal levels, at 50–110 pg/mL.⁸² In fact, the systemic levels of LNG found in the previously tested 20 µg/d LNG IVR users were in the same low range as the levels measured among users of the 20 µg/d LNG intrauterine system (IUS) (Mirena) (0.47–1.37 nmol/L [0.147–0.482 ng/mL])^{61–63} and the 14 µg/d LNG IUS (Skyla) (0.06–0.19 ng/mL [0.19–0.61 nmol/L]).⁶⁴

The data support that the mucosal effects seen with DMPA are more a reflection of the hypoestrogenic milieu seen exclusively in DMPA users. For example, it is known that hypoestrogenic states, such as menopause, pregnancy, and lactation, result in cervical and vaginal atrophy,^{133,134} an elevated vaginal pH,¹³⁵ and an altered vaginal microbiome,¹³⁶ with a preponderance of anaerobic bacteria and a decrease in lactobacilli.^{137,138} The in vitro data indicate that epithelial tight junction proteins are altered by estrogen levels.¹³⁹

Based on these theories regarding the effect of exogenous hormones on mucosal susceptibility to HIV, each objective

endpoint (eg, vaginal pH, microbiome, vaginal immune cells, or epithelial tight junction proteins) would be important safety endpoints to test in early Phase I studies of MPT IVRs.

Market demand

A good generalization regarding the currently available contraceptive IVRs is that few women use them, but those who do, report high acceptability. Although there has been a steady increase in the use of the NuvaRing since its release in the USA and other European countries in 2002, the current data indicate that approximately only 1.3% of reproductive-age women in the USA report using the NuvaRing as their current contraceptive method.¹⁴⁰ Worldwide use of the NuvaRing is low, with large databases not subcategorizing the IVR as a separate category.¹⁴¹ This may be due to cost of the IVR, which ranges from \$50–\$120 per month without insurance coverage. The US patent for the NuvaRing expires in April of 2018, and this will allow the development of cheaper generic versions and likely an increase in the global market demand. However, an improved introduction of IVRs to regions of the world hit hardest by the HIV pandemic, particularly sub-Saharan Africa, is required for microbicide and MPT IVRs uptake to be successful in these regions.

Many of the contraceptive/microbicide MPTs currently in development utilize LNG as the contraceptive component. LNG has been endorsed by the Initiative for Multipurpose Prevention Technologies Scientific Advisory Working Group as the contraceptive hormone of choice to use in MPTs, as it is well characterized, inexpensive, generic, and has a good safety profile.

Conclusion

IVRs can successfully deliver contraceptive hormones in a highly efficient, discreet, and acceptable manner. Studies to date also show the potential of IVRs to deliver microbicides. Successfully combining the delivery of the two in a single, long-acting IVR has the potential to be a game changer, as this would provide women across the globe with an easy, discreet method of protecting themselves from both pregnancy and HIV or other STIs. With approximately 41% of global pregnancies unplanned¹³ and 2.5 million new HIV infections per year,¹ an MPT could potentially prevent thousands of unintended/mistimed pregnancies and HIV infections, ultimately saving countless women's lives.

Disclosure

The authors report no conflicts of interest in this work.

References

- UNAIDS. *Global Report. UNAIDS Report on the Global AIDS Epidemic*. Geneva: UNAIDS; 2012. Available from: http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2012/gr2012/20121120_UNAIDS_Global_Report_2012_en.pdf. Accessed August 24, 2013.
- Marrazzo J, Ramjee G, Nair G, et al. Pre-exposure prophylaxis for HIV in women: daily oral tenofovir, oral tenofovir/emtricitabine, or vaginal tenofovir gel in the VOICE study (MTN 003). Presented at: the 20th Conference of Retroviruses and Opportunistic Infections (CROI 2013); March 3–6; 2013; Atlanta, GA.
- van der Linde I. HIV/AIDS in South Africa: At last the glass is half full. Presented at: the 6th SA AIDS Conference; June 18–21; 2013; Durban, South Africa.
- Karim QA, Abdool Karim SS, Frohlich JA, et al; CAPRISA 004 Trial Group. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science Express*. 2010;329(5996):1168–1174.
- Grant RM, Lama JR, Anderson PL, et al; iPrEx Study Team. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *N Engl J Med*. 2010;363(27):2587–2599.
- Dobard C, Sharma S, Martin A, et al. Durable protection from vaginal simian-human immunodeficiency virus infection in macaques by tenofovir gel and its relationship to drug levels in tissue. *J Virol*. 2012;86(2):718–725.
- Baeten JM, Donnell D, Ndase P, et al; Partners PrEP Study Team. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *N Engl J Med*. 2012;367(5):399–410.
- Denton PW, Othieno F, Martinez-Torres F, et al. One percent tenofovir applied topically to humanized BLT mice and used according to the CAPRISA 004 experimental design demonstrates partial protection from vaginal HIV infection, validating the BLT model for evaluation of new microbicide candidates. *J Virol*. 2011;85(15):7582–7593.
- Karim SS, Kashuba AD, Werner L, Karim QA. Drug concentrations after topical and oral antiretroviral pre-exposure prophylaxis: implications for HIV prevention in women. *Lancet*. 2011;378(9787):279–281.
- Dai JY, Gilbert PB, Hughes JP, Brown ER. Estimating the efficacy of preexposure prophylaxis for HIV prevention among participants with a threshold level of drug concentration. *Am J Epidemiol*. 2013;177(3):256–263.
- World Health Organization; UNICEF; UNFPA; World Bank. *Maternal Mortality in 2005*. Geneva: World Health Organization; 2007. Available from: http://whqlibdoc.who.int/publications/2007/9789241596213_eng.pdf. Accessed July 15, 2010.
- Singh S, Wulf D, Hussain R, Bankole A, Sedgh G, Institute G. *Abortion Worldwide: A Decade of Uneven Progress*. New York, NY: Guttmacher Institute; 2009. Available from: <http://www.guttmacher.org/pubs/Abortion-Worldwide.pdf>. Accessed August 24, 2013.
- Singh S, Sedgh G, Hussain R. Unintended pregnancy: worldwide levels, trends, and outcomes. *Stud Fam Plann*. 2010;41(4):241–250.
- Stuart JE, Secura GM, Zhao Q, Pittman ME, Peipert JF. Factors associated with 12-month discontinuation among contraceptive pill, patch, and ring users. *Obstet Gynecol*. 2013;121(2 Pt 1):330–336.
- Friend DR, Doncel GF. Combining prevention of HIV-1, other sexually transmitted infections and unintended pregnancies: Development of dual-protection technologies. *Antiviral Res*. 2010;88 Suppl 1: S47–S54.
- World Health Organization. *Figure 3-1 Comparing Typical Effectiveness of Contraceptive Methods*. Geneva: World Health Organization; 2007. Available from: <http://www.contraceptivetechnology.org/table.html>. Accessed August 24, 2013.

17. Seidman DS. Non-contraceptive benefits of hormonal contraception: time for renewed awareness. *Eur J Contracept Reprod Health Care*. 2011;16(6):407–408.
18. Padian NS, van der Straten A, Ramjee G, et al; MIRA Team. Diaphragm and lubricant gel for prevention of HIV acquisition in southern African women: a randomised controlled trial. *Lancet*. 2007;370(9583):251–261.
19. Johnson TJ, Clark MR, Albright TH, et al. A 90-day tenofovir reservoir intravaginal ring for mucosal HIV prophylaxis. *Antimicrob Agents Chemother*. 2012;56(12):6272–6283.
20. Macht DI. On the absorption of drugs and poisons through the vagina. *J Pharmacol Exp Ther*. 1918;10(7):509–522.
21. Dziuk PJ, Cook B. Passage of steroids through silicone rubber. *Endocrinology*. 1966;78(1):208–211.
22. Mishell DR, Talas M, Parlow AF, Moyer DL. Contraception by means of a silastic vaginal ring impregnated with medroxyprogesterone acetate. *Am J Obstet Gynecol*. 1970;107(1):100–107.
23. Mishell DR, Lumkin ME. Contraceptive effect of varying dosages of progesterone in silastic vaginal rings. *Fertil Steril*. 1970;21(2):99–103.
24. World Health Organization; Task Force on Long-Acting Systemic Agents for Fertility Regulation. Intravaginal and intracervical devices for the delivery of fertility regulating agents. *J Steroid Biochem*. 1979;11(1B):461–467.
25. Koetsawang S, Ji G, Krishna U, et al. Microdose intravaginal levonorgestrel contraception: a multicentre clinical trial. I. Contraceptive efficacy and side effects. World Health Organization. Task Force on Long-Acting Systemic Agents for Fertility Regulation. *Contraception*. 1990;41(2):105–124.
26. Koetsawang S, Ji G, Krishna U, et al. Microdose intravaginal levonorgestrel contraception: a multicentre clinical trial. II. Expulsions and removals. World Health Organization. Task Force on Long-Acting Systemic Agents for Fertility Regulation. *Contraception*. 1990;41(2):125–141.
27. Bounds W, Szarewski A, Lowe D, Guillebaud J. Preliminary report of unexpected local reactions to a progestogen-releasing contraceptive vaginal ring. *Eur J Obstet Gynecol Reprod Biol*. 1993;48(2):123–125.
28. Weisberg E, Fraser IS, Baker J, et al. A randomized comparison of the effects on vaginal and cervical epithelium of a placebo vaginal ring with non-use of a ring. *Contraception*. 2000;62(2):83–89.
29. Mulders TM, Dieben TO, Bennink HJ. Ovarian function with a novel combined contraceptive vaginal ring. *Hum Reprod*. 2002;17(10):2594–2599.
30. Brache V, Payán LJ, Faundes A. Current status of contraceptive vaginal rings. *Contraception*. 2013;87(3):264–272.
31. Malcolm K, Woolfson D, Russell J, Andrews C. In vitro release of nonoxonyl-9 from silicone matrix intravaginal rings. *J Control Release*. 2003;91(3):355–364.
32. Van Damme L, Ramjee G, Alary M, et al; COL-1492 Study Group. Effectiveness of COL-1492, a nonoxonyl-9 vaginal gel, on HIV-1 transmission in female sex workers: a randomised controlled trial. *Lancet*. 2002;360(9338):971–977.
33. Kiser PF, Johnson TJ, Clark JT. State of the art in intravaginal ring technology for topical prophylaxis of HIV infection. *AIDS Rev*. 2012;14(1):62–77.
34. Gupta KM, Pearce SM, Poursaid AE, et al. Polyurethane intravaginal ring for controlled delivery of dapivirine, a nonnucleoside reverse transcriptase inhibitor of HIV-1. *J Pharm Sci*. 2008;97(10):4228–4239.
35. Malcolm RK, Woolfson AD, Toner CF, Morrow RJ, McCullagh SD. Long-term, controlled release of the HIV microbicide TMC120 from silicone elastomer vaginal rings. *J Antimicrob Chemother*. 2005;56(5):954–956.
36. Nel A, Smythe S, Young K, et al. Safety and pharmacokinetics of dapivirine delivery from matrix and reservoir intravaginal rings to HIV-negative women. *J Acquir Immune Defic Syndr*. 2009;51(4):416–423.
37. Woolfson AD, Malcolm RK, Morrow RJ, Toner CF, McCullagh SD. Intravaginal ring delivery of the reverse transcriptase inhibitor TMC 120 as an HIV microbicide. *Int J Pharm*. 2006;325(1–2):82–89.
38. Hendrix CW, Chen BA, Guddera V, et al. MTN-001: randomized pharmacokinetic cross-over study comparing tenofovir vaginal gel and oral tablets in vaginal tissue and other compartments. *PLoS ONE*. 2013;8(1):e55013.
39. Thurman A, Kimble T, Hall P, Schwartz JL, Archer DF. Medroxyprogesterone acetate and estradiol cypionate injectable suspension (Cyclofem) monthly contraceptive injection: steady-state pharmacokinetics. *Contraception*. 2013;87(6):738–743.
40. Mishell DR. Pharmacokinetics of depot medroxyprogesterone acetate contraception. *J Reprod Med*. 1996;41(Suppl 5):S381–S390.
41. van den Heuvel MW, van Bragt AJ, Alnabawy AK, Kaptein MC. Comparison of ethinylestradiol pharmacokinetics in three hormonal contraceptive formulations: the vaginal ring, the transdermal patch and an oral contraceptive. *Contraception*. 2005;72(3):168–174.
42. Timmer CJ, Mulders TM. Pharmacokinetics of etonogestrel and ethinylestradiol released from a combined contraceptive vaginal ring. *Clin Pharmacokinet*. 2000;39(3):233–242.
43. Magnúsdóttir EM, Bjarnadóttir RI, Onundarson PT, et al. The contraceptive vaginal ring (NuvaRing) and hemostasis: a comparative study. *Contraception*. 2004;69(6):461–467.
44. Nahoul K, Dehennin L, Jondet M, Roger M. Profiles of plasma estrogens, progesterone and their metabolites after oral or vaginal administration of estradiol or progesterone. *Maturitas*. 1993;16(3):185–202.
45. Coutinho EM, Coutinho EJ, Gonçalves MT, Barbosa IC. Ovulation suppression in women following vaginal administration of oral contraceptive tablets. *Fertil Steril*. 1982;38(3):380–381.
46. Kobayashi K, Mimura N, Fujii H, et al. Role of human cytochrome P450 3A4 in metabolism of medroxyprogesterone acetate. *Clin Cancer Res*. 2000;6(8):3297–3303.
47. Tseng A, Hills-Niemenen C. Drug interactions between antiretrovirals and hormonal contraceptives. *Expert Opin Drug Metab Toxicol*. 2013;9(5):559–572.
48. Van Damme L, Corneli A, Ahmed K, et al; FEM-PrEP Study Group. Preexposure prophylaxis for HIV infection among African women. *N Engl J Med*. 2012;367(5):411–422.
49. Rosenberg MJ, Waugh MS, Long S. Unintended pregnancies and use, misuse and discontinuation of oral contraceptives. *J Reprod Med*. 1995;40(5):355–360.
50. Blumenthal PD, Voedisch A, Gemzell-Danielsson K. Strategies to prevent unintended pregnancy: increasing use of long-acting reversible contraception. *Hum Reprod Update*. 2011;17(1):121–137.
51. Massai R, Quinteros E, Reyes MV, et al. Extended use of a progesterone-releasing vaginal ring in nursing women: a phase II clinical trial. *Contraception*. 2005;72(5):352–357.
52. Gilliam ML, Neustadt A, Kozloski M, Mistretta S, Tilmon S, Godfrey E. Adherence and acceptability of the contraceptive ring compared with the pill among students: a randomized controlled trial. *Obstet Gynecol*. 2010;115(3):503–510.
53. Alexander NJ, Baker E, Kaptein M, Karck U, Miller L, Zampaglione E. Why consider vaginal drug administration? *Fertil Steril*. 2004;82(1):1–12.
54. Romano J, Variano B, Coplan P, et al. Safety and availability of dapivirine (TMC120) delivered from an intravaginal ring. *AIDS Res Hum Retroviruses*. 2009;25(5):483–488.
55. Malcolm RK, Fetherston SM, McCoy CF, Boyd P, Major I. Vaginal rings for delivery of HIV microbicides. *Int J Womens Health*. 2012;4:595–605.
56. Fetherston SM, Boyd P, McCoy CF, et al. A silicone elastomer vaginal ring for HIV prevention containing two microbicides with different mechanisms of action. *Eur J Pharm Sci*. 2012;48(3):406–415.
57. Clark JT, Johnson TJ, Clark MR, et al. Quantitative evaluation of a hydrophilic matrix intravaginal ring for the sustained delivery of tenofovir. *J Control Release*. 2012;163(2):240–248.

58. Smith J, Rastogi R, Teller RS, et al. A tenofovir disoproxil fumarate intravaginal ring completely protects against repeated SHIV vaginal challenge in nonhuman primates. Presented at: the 20th Conference of Retroviruses and Opportunistic Infections (CROI 2013); March 3–6; 2013; Atlanta, GA.
59. Sahota J, Barnes PM, Mansfield E, Bradley JL, Kirkman RJ. Initial UK experience of the levonorgestrel-releasing contraceptive intravaginal ring. *Adv Contracept*. 1999;15(4):313–324.
60. Landgren BM, Johannisson E, Masironi B, Diczfalussy E. Pharmacokinetic and pharmacodynamic investigations with vaginal devices releasing levonorgestrel at a constant, near zero order rate. *Contraception*. 1982;26(6):567–585.
61. Lockhat FB, Emembolu JE, Konje JC. Serum and peritoneal fluid levels of levonorgestrel in women with endometriosis who were treated with an intrauterine contraceptive device containing levonorgestrel. *Fertil Steril*. 2005;83(2):398–404.
62. Seeber B, Ziehr SC, Gschließer A, et al. Quantitative levonorgestrel plasma level measurements in patients with regular and prolonged use of the levonorgestrel-releasing intrauterine system. *Contraception*. 2012;86(4):345–349.
63. Hidalgo MM, Hidalgo-Regina C, Bahamondes MV, Monteiro I, Petta CA, Bahamondes L. Serum levonorgestrel levels and endometrial thickness during extended use of the levonorgestrel-releasing intrauterine system. *Contraception*. 2009;80(1):84–89.
64. Bayer Healthcare. *Highlights of Prescribing Information* [Package Insert for Skyla Intrauterine System]. Wayne, NJ: Bayer HealthCare Pharmaceuticals; 2013. Available from: http://labeling.bayerhealthcare.com/html/products/pi/Skyla_PI.pdf. Accessed August 24, 2013.
65. Natavio MF, Taylor D, Lewis RA, et al. Temporal changes in cervical mucus after insertion of the levonorgestrel-releasing intrauterine system. *Contraception*. 2013;87(4):426–431.
66. Lähteenmäki P, Rauramo I, Backman T. The levonorgestrel intrauterine system in contraception. *Steroids*. 2000;65(10–11):693–697.
67. Munuce MJ, Nascimento JA, Rosano G, Faundes A, Bahamondes L. Doses of levonorgestrel comparable to that delivered by the levonorgestrel-releasing intrauterine system can modify the in vitro expression of zona binding sites of human spermatozoa. *Contraception*. 2006;73(1):97–101.
68. Moss JA, Malone AM, Smith TJ, et al. Simultaneous delivery of tenofovir and acyclovir via an intravaginal ring. *Antimicrob Agents Chemother*. 2012;56(2):875–882.
69. Gunawardana M, Moss JA, Smith TJ, et al. Microbial biofilms on the surface of intravaginal rings worn in non-human primates. *J Med Microbiol*. 2011;60(Pt 6):828–837.
70. Miller L, MacFarlane SA, Materi HL. A scanning electron microscopic study of the contraceptive vaginal ring. *Contraception*. 2005;71(1):65–67.
71. Hazan Z, Zumeris J, Jacob H, et al. Effective prevention of microbial biofilm formation on medical devices by low-energy surface acoustic waves. *Antimicrob Agents Chemother*. 2006;50(12):4144–4152.
72. Pruthi V, Al-Janabi A, Pereira BM. Characterization of biofilm formed on intrauterine devices. *Indian J Med Microbiol*. 2003;21(3):161–165.
73. Creinin MD, Meyn LA, Borgatta L, et al. Multicenter comparison of the contraceptive ring and patch: a randomized controlled trial. *Obstet Gynecol*. 2008;111(2 Pt 1):267–277.
74. Dieben TO, Roumen FJ, Apter D. Efficacy, cycle control, and user acceptability of a novel combined contraceptive vaginal ring. *Obstet Gynecol*. 2002;100(3):585–593.
75. Novák A, de la Loge C, Abetz L, van der Meulen EA. The combined contraceptive vaginal ring, NuvaRing: an international study of user acceptability. *Contraception*. 2003;67(3):187–194.
76. Oddsson K, Leifels-Fischer B, de Melo NR, et al. Efficacy and safety of a contraceptive vaginal ring (NuvaRing) compared with a combined oral contraceptive: a 1-year randomized trial. *Contraception*. 2005;71(3):176–182.
77. Sivin I, Mishell DR Jr, Alvarez F, et al. Contraceptive vaginal rings releasing Nestorone and ethinylestradiol: a 1-year dose-finding trial. *Contraception*. 2005;71(2):122–129.
78. van der Straten A, Montgomery ET, Cheng H, et al. High acceptability of a vaginal ring intended as a microbicide delivery method for HIV prevention in African women. *AIDS Behav*. 2012;16(7):1775–1786.
79. Jung-Hoffman C, Kuhl H. Pharmacokinetics and pharmacodynamics of oral contraceptive steroids: factors influencing steroid metabolism. *Am J Obstet Gynecol*. 1990;163(6 Pt 2):2183–2197.
80. Koetsawang S, Ji G, Krishna U, et al. Microdose intravaginal levonorgestrel contraception: a multicentre clinical trial. IV. Bleeding patterns. World Health Organization. Task Force on Long-Acting Systemic Agents for Fertility Regulation. *Contraception*. 1990;41(2):151–167.
81. Brache V, Mishell DR, Lahteenmaki P, et al. Ovarian function during use of vaginal rings delivering three different doses of Nestorone. *Contraception*. 2001;63(5):257–261.
82. Xiao BL, Zhang XL, Feng DD. Pharmacokinetic and pharmacodynamic studies of vaginal rings releasing low-dose levonorgestrel. *Contraception*. 1985;32(5):455–471.
83. Landgren BM, Johannisson E, Masironi B, Diczfalussy E. Pharmacokinetic and pharmacodynamic effects of small doses of norethisterone released from vaginal rings continuously during 90 days. *Contraception*. 1979;19(3):253–271.
84. Johannisson E, Brosens I, Cornillie F, et al. Morphometric study of the human endometrium following continuous exposure to levonorgestrel released from vaginal rings during 90 days. *Contraception*. 1991;43(4):361–374.
85. Van de Perre P, Segondy M, Foulongne V, et al. Herpes simplex virus and HIV-1: deciphering viral synergy. *Lancet Infect Dis*. 2008;8(8):490–497.
86. Greenblatt RM, Lukehart SA, Plummer FA, et al. Genital ulceration as a risk factor for human immunodeficiency virus infection. *AIDS*. 1988;2(1):47–50.
87. Wasserheit JN. Epidemiological synergy. Interrelationships between human immunodeficiency virus infection and other sexually transmitted diseases. *Sex Transm Dis*. 1992;19(2):61–77.
88. Royce RA, Seña A, Cates W, Cohen MS. Sexual transmission of HIV. *N Engl J Med*. 1997;336(15):1072–1078.
89. Thurman AR, Doncel GF. Herpes simplex virus and HIV: genital infection synergy and novel approaches to dual prevention. *Int J STD AIDS*. 2012;23(9):613–619.
90. Nath A, Sitruk-Ware R. Progesterone vaginal ring for contraceptive use during lactation. *Contraception*. 2010;82(5):428–434.
91. Sivin I, Díaz S, Croxatto HB, et al. Contraceptives for lactating women: a comparative trial of a progesterone-releasing vaginal ring and the copper T 380A IUD. *Contraception*. 1997;55(4):225–232.
92. Roumen FJ, Apter D, Mulders TM, Dieben TO. Efficacy, tolerability and acceptability of a novel contraceptive vaginal ring releasing etonogestrel and ethinyl oestradiol. *Hum Reprod*. 2001;16(3):469–475.
93. Fraser IS, Lacarra M, Mishell DR, et al. Vaginal epithelial surface appearances in women using vaginal rings for contraception. *Contraception*. 2000;61(2):131–138.
94. Fraser IS, Lähteenmäki P, Elomaa K, et al. Variations in vaginal epithelial surface appearance determined by colposcopic inspection in healthy, sexually active women. *Hum Reprod*. 1999;14(8):1974–1978.
95. Mayer KH, Venkatesh KK. Antiretroviral therapy as HIV prevention: status and prospects. *Am J Public Health*. 2010;100(10):1867–1876.
96. Schwartz JL, Rountree W, Kashuba ADM, et al. A multi-compartment, single and multiple dose pharmacokinetic study of the vaginal candidate microbicide 1% tenofovir gel. *PLoS One*. 2011;6(10):e25974.
97. Abdool Karim SS. CAPRISA 004 two years on: Ten key lessons and their implications. Presented at: Microbicides 2012; April 12, 2012; Sydney, Australia.
98. Martin HL, Richardson BA, Nyange PM, et al. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. *J Infect Dis*. 1999;180(6):1863–1868.

99. Laga M, Manoka A, Kivuvu M, et al. Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. *AIDS*. 1993;7(1):95–102.
100. Myer L, Denny L, Telerant R, Souza MD, Wright TC Jr, Kuhn L. Bacterial vaginosis and susceptibility to HIV infection in South African women: a nested case-control study. *J Infect Dis*. 2005;192(8):1372–1380.
101. Valore EV, Wiley DJ, Ganz T. Reversible deficiency of antimicrobial polypeptides in bacterial vaginosis. *Infect Immun*. 2006;74(10):5693–5702.
102. Davies GC, Feng LX, Newton JR, Dieben TO, Coelingh-Bennink HJ. The effects of a combined contraceptive vaginal ring releasing ethinylloestradiol and 3-ketodesogestrel on vaginal flora. *Contraception*. 1992;45(5):511–518.
103. De Seta F, Restaino S, De Santo D, et al. Effects of hormonal contraception on vaginal flora. *Contraception*. 2012;86(5):526–529.
104. Ravel J, Gajer P, Abdo Z, et al. Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A*. 2011;108 Suppl 1: S4680–S4687.
105. Camacho DP, Consolaro ME, Patussi EV, Donatti L, Gasparetto A, Svidzinski TI. Vaginal yeast adherence to the combined contraceptive vaginal ring (CCVR). *Contraception*. 2007;76(6):439–443.
106. Ungchusak K, Rehle T, Thammapornpilap P, Spiegelman D, Brinkmann U, Sirapaprasiri T. Determinants of HIV infection among female commercial sex workers in northeastern Thailand: results from a longitudinal study. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1996;12(5):500–507.
107. Martin HL Jr, Nyange PM, Richardson BA, et al. Hormonal contraception, sexually transmitted diseases, and risk of heterosexual transmission of human immunodeficiency virus type 1. *J Infect Dis*. 1998;178(4):1053–1059.
108. Baeten JM, Benki S, Chohan V, et al. Hormonal contraceptive use, herpes simplex virus infection, and risk of HIV-1 acquisition among Kenyan women. *AIDS*. 2007;21(13):1771–1777.
109. Morrison CS, Chen PL, Kwok C, et al. Hormonal contraception and HIV acquisition: reanalysis using marginal structural modeling. *AIDS*. 2010;24(11):1778–1781.
110. Heffron R, Donnell D, Rees H, et al; Partners in Prevention HSV/HIV Transmission Study Team. Use of hormonal contraceptives and risk of HIV-1 transmission: a prospective cohort study. *Lancet Infect Dis*. 2012;12(1):19–26.
111. Marx PA, Spira AI, Gettie A, et al. Progesterone implants enhance SIV vaginal transmission and early virus load. *Nat Med*. 1996;2(10):1084–1089.
112. Smith SM, Baskin GB, Marx PA. Estrogen protects against vaginal transmission of simian immunodeficiency virus. *J Infect Dis*. 2000;182(3):708–715.
113. Jiang Y, Tian B, Agy MB, Saifuddin M, Tsai CC. Macaca fascicularis are highly susceptible to an RT-SHIV following intravaginal inoculation: a new model for microbicide evaluation. *J Med Primatol*. 2009;38 Suppl 1:S39–S46.
114. Pal R, Nuttall J, Galmin L, Weiss D, Chung HK, Romano J. Characterization of vaginal transmission of a simian human immunodeficiency virus (SHIV) encoding the reverse transcriptase gene from HIV-1 in Chinese rhesus macaques. *Virology*. 2009;386(1):102–108.
115. Sallé B, Brochard P, Bourry O, et al. Infection of macaques after vaginal exposure to cell-associated simian immunodeficiency virus. *J Infect Dis*. 2010;202(3):337–344.
116. Veazey RS, Shattock RJ, Pope M, et al. Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120. *Nat Med*. 2003;9(3):343–346.
117. Miller L, Patton DL, Meier A, Thwin SS, Hooton TM, Eschenbach DA. Depomedroxyprogesterone-induced hypoestrogenism and changes in vaginal flora and epithelium. *Obstet Gynecol*. 2000;96(3):431–439.
118. Bahamondes L, Trevisan M, Andrade L, et al. The effect upon the human vaginal histology of the long-term use of the injectable contraceptive Depo-Provera. *Contraception*. 2000;62(1):23–27.
119. Mauck CK, Callahan MM, Baker J, et al. The effect of one injection of Depo-Provera on the human vaginal epithelium and cervical ectopy. *Contraception*. 1999;60(1):15–24.
120. Ildgruben AK, Sjöberg IM, Hammarström ML. Influence of hormonal contraceptives on the immune cells and thickness of human vaginal epithelium. *Obstet Gynecol*. 2003;102(3):571–582.
121. Haase AT. Early events in sexual transmission of HIV and SIV and opportunities for interventions. *Annu Rev Med*. 2011;62:127–139.
122. Pudney J, Quayle AJ, Anderson DJ. Immunological microenvironments in the human vagina and cervix: mediators of cellular immunity are concentrated in the cervical transformation zone. *Biol Reprod*. 2005;73(6):1253–1263.
123. Hladik F, Sakchalathorn P, Ballweber L, et al. Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. *Immunity*. 2007;26(2):257–270.
124. Patton DL, Thwin SS, Meier A, Hooton TM, Stapleton AE, Eschenbach DA. Epithelial cell layer thickness and immune cell populations in the normal human vagina at different stages of the menstrual cycle. *Am J Obstet Gynecol*. 2000;183(4):967–973.
125. Wieser F, Hosmann J, Tschugguel W, Czerwenka K, Sedivy R, Huber JC. Progesterone increases the number of Langerhans cells in human vaginal epithelium. *Fertil Steril*. 2001;75(6):1234–1235.
126. Wira CR, Fahey JV, Ghosh M, Patel MV, Hickey DK, Ochiel DO. Sex hormone regulation of innate immunity in the female reproductive tract: the role of epithelial cells in balancing reproductive potential with protection against sexually transmitted pathogens. *Am J Reprod Immunol*. 2010;63(6):544–565.
127. Chandra N, Thurman AR, Anderson S, et al. Depot medroxyprogesterone acetate increases immune cell numbers and activation markers in human vaginal mucosal tissues. *AIDS Res Hum Retroviruses*. 2013;29(3):592–601.
128. Naranbhai V, Abdool Karim SS, Altfeld M, et al; CAPRISA004 TRAPS team. Innate immune activation enhances hiv acquisition in women, diminishing the effectiveness of tenofovir microbicide gel. *J Infect Dis*. 2012;206(7):993–1001.
129. Roberts L, Passmore JA, Mlisana K, et al. Genital tract inflammation during early HIV-1 infection predicts higher plasma viral load set point in women. *J Infect Dis*. 2012;205(2):194–203.
130. Clark MK, Sowers M, Levy BT, Tenhundfeld P. Magnitude and variability of sequential estradiol and progesterone concentrations in women using depot medroxyprogesterone acetate for contraception. *Fertil Steril*. 2001;75(5):871–877.
131. Fotherby K, Saxena BN, Shrimanker K, et al. A preliminary pharmacokinetic and pharmacodynamic evaluation of depot-medroxyprogesterone acetate and norethisterone oenanthate. *Fertil Steril*. 1980;34(2):131–139.
132. Ortiz A, Hirol M, Stanczyk FZ, Goebelsmann U, Mishell DR. Serum medroxyprogesterone acetate (MPA) concentrations and ovarian function following intramuscular injection of depo-MPA. *J Clin Endocrinol Metab*. 1977;44(1):32–38.
133. Wisniewski PM, Wilkinson EJ. Postpartum vaginal atrophy. *Am J Obstet Gynecol*. 1991;165(4 Pt 2):1249–1254.
134. Nilsson K, Risberg B, Heimer G. The vaginal epithelium in the postmenopause – cytology, histology and pH as methods of assessment. *Maturitas*. 1995;21(1):51–56.
135. Roy S, Caillouette JC, Roy T, Faden JS. Vaginal pH is similar to follicle-stimulating hormone for menopause diagnosis. *Am J Obstet Gynecol*. 2004;190(5):1272–1277.
136. Petricevic L, Domig KJ, Nierscher FJ, et al. Characterisation of the oral, vaginal and rectal Lactobacillus flora in healthy pregnant and postmenopausal women. *Eur J Obstet Gynecol Reprod Biol*. 2012;160(1):93–99.
137. Pabich WL, Fihn SD, Stamm WE, Scholes D, Boyko EJ, Gupta K. Prevalence and determinants of vaginal flora alterations in postmenopausal women. *J Infect Dis*. 2003;188(7):1054–1058.

138. Gondo F, da Silva MG, Poletti J, et al. Vaginal flora alterations and clinical symptoms in low-risk pregnant women. *Gynecol Obstet Invest*. 2011;71(3):158–162.
139. Gorodeski GI. Estrogen modulation of epithelial permeability in cervical-vaginal cells of premenopausal and postmenopausal women. *Menopause*. 2007;14(6):1012–1019.
140. Jones J, Mosher W, Daniels K; Division of vital statistics. Current contraceptive use in the United States, 2006–2010, and changes in patterns of use since 1995. *Natl Health Stat Report*. 2012;(60):1–26. Available from: <http://www.cdc.gov/nchs/data/nhsr/nhsr060.pdf>. Accessed August 24, 2013.
141. un.org [homepage on the Internet]. World contraceptive use 2010. United Nations, Department of Economic and Social Affairs, Population Division; 2010 [updated February 2011; cited July 15, 2011]. Available from: http://www.un.org/esa/population/publications/wcu2010/Data/UNPD_WCU_2010_Contraceptive_prevalence_method.xls. Accessed August 24, 2013.

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