

# PROGRAM & ABSTRACTS



# M2010

MICROBICIDES:  
Building Bridges  
in HIV Prevention

MAY 22–25, 2010

David L. Lawrence Convention Center  
Pittsburgh, Pennsylvania, USA

# 2010 International Microbicides Conference

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The background of the image is a soft-focus, abstract representation of biological structures. It features various green and white, translucent, rounded shapes that suggest the form of cells, leaves, or perhaps microscopic organisms. These shapes are scattered across the frame, with some appearing in the foreground and others in the background, creating a sense of depth and organic movement.

Abstracts

Sunday  
May 23, 2010

# ORAL SESSIONS

## SESSION 2

### Oral Abstracts (OA1): Pregnancy and HIV Risk in Microbicide Trials

**Moderators:** Jeanne Marrazzo, Heather Watts

Sunday, May 23, 9:30 am–11:15 am

Ballroom

## 3

### Pregnancy Incidence and Outcomes in Women Using Candidate Vaginal Microbicides PRO 2000 and BufferGel: Results of the HPTN 035 Trial

L.A. Maslankowski<sup>\*1</sup>, B. Richardson<sup>2</sup>, C. Kelly<sup>3</sup>, B. Makanani<sup>4</sup>, C. Reid<sup>5</sup>, L. Chinula<sup>6</sup>, A. Pathir<sup>7</sup>, E. Chigutsa<sup>8</sup>, A. Coletti<sup>9</sup>, L. Soto-Torres<sup>10</sup>, S.S. Abdool Karim<sup>11</sup>

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**BACKGROUND:** HPTN 035 was a four-arm Phase II/IIb randomized controlled trial of 0.5% PRO 2000 and BufferGel, with two control arms, HEC placebo gel and no gel. Pregnancy in microbicide trials is an important study design issue that can dilute the expected effectiveness of a microbicide since product is generally held during pregnancy. Pregnancy rates in microbicide studies have been as high as 64 per 100 woman-years (WYs), so pregnancy rates need to be accurately factored into sample size calculations and minimized if product is to be held during pregnancy. In HPTN 035, we assessed the impact of PRO 2000, BufferGel and HEC placebo gel on pregnancy rates and outcomes.

**METHODS:** 3099 eligible African and U.S. women who were not planning pregnancies were randomly assigned to one of the 4 study arms (1:1:1:1). At screening, enrollment and at each monthly visit, women had a urine pregnancy test (Quidel QuickVue One-Step hCG). Contraception counseling and access to highly effective contraceptive methods were provided throughout the study, along with condoms and condom promotion counseling. Women testing positive for pregnancy had gel use temporarily discontinued. Self-reported data on gel, condom and contraceptive use were collected at baseline and quarterly visits. All pregnancies were followed to outcome. Pregnancy incidence rates are based on time to first positive pregnancy test result.

**RESULTS:** 3069 women (HIV-negative at baseline) were followed for 4866 WYs. 55% of women were using highly effective contraception at baseline. 613 pregnancies occurred in 551 women (18.0% ever pregnant) with a pregnancy incidence rate of 11.32 per 100 WYs overall (range 8.82–17.13 across sites). Pregnancy rates did not differ across study arms (10.94, 12.16, 9.87, and 12.34 for BufferGel, PRO2000, HEC placebo gel, and no gel, respectively; (PRO2000 vs. HEC placebo, p=0.09; HEC vs. no gel, p=0.08). Product hold led to 5.9% of follow-up time off product overall with no difference between arms. Pregnancy was the main reason for product hold (82% of time on product hold). Pregnancy outcomes were full-term live birth (65%), premature live birth (4%), still birth (3%), spontaneous abortion (20%) and therapeutic abortion (8%) with no difference between arms.

**CONCLUSIONS:** 0.5% PRO 2000, BufferGel and HEC placebo gel had no impact on pregnancy incidence or outcomes. Follow-up time on product hold was low, largely due to the low overall pregnancy rate.

## 4

### Pregnancy Among Women in HIV-discordant Relationships Enrolled in a HIV Prevention Trial in Kisumu, Kenya

J.B. Odoyo<sup>\*1</sup>, B. Rono<sup>1</sup>, F. Onchir<sup>1</sup>, C.R. Cohen<sup>2</sup>, E.A. Bukusi<sup>1</sup>

<sup>1</sup>Kenya Medical Research Institute; <sup>2</sup>University of California, San Francisco

**BACKGROUND:** Pregnancy in HIV discordant couples is common and points to unprotected sex which is associated with high risk of HIV transmission. Factors driving this may include desire for children coupled with poor access to contraception. We evaluated factors influencing incident pregnancy among women in HIV discordant relationships who were enrolled into a randomized placebo-controlled trial of HSV-2 suppression to prevent HIV transmission (the Partners in Prevention HSV/HIV Transmission Study).

**METHODS:** A total of 287 HIV positive and 252 HIV-negative women in HIV discordant relationships were followed for up to two years at the Kisumu, Kenya site; HIV+ women were encouraged to avoid pregnancy. Socio-demographic and clinical data were collected at baseline and then at monthly intervals for HIV+ and quarterly for HIV- participants. Pregnancy test was done at every visit or as indicated for the HIV+ women and only on request for the HIV negative women. A year later, the study started offering combined oral contraceptives (COC) and depomedroxyprogesterone acetate (DMPA) in addition to condoms. Multiple regression analysis was done to examine predictors of incident pregnancy.

**RESULTS:** Women had a mean age of 29.4 years, (SD 8.6), an average of 2 children and 63% had less than 8 years of education. During the 767 woman-years of follow up, 160 pregnancies were reported for an overall incidence of 20.85/100wyr 95% CI (17–24). There was no association between incidence of pregnancy and HIV status, marital status, living with a study partner, number of living children or income. Women aged 18–24years had a pregnancy incidence of 36.2/100wyr compared to 24.3/100wyr among 25–34 year olds and 1.8/100wyr for those aged 35–44; p =0.0001.

Pregnancy incidence was 18.6/100wyr, 24.5/100wyr and 12.6/100wyr among women with no education, 1–8 years of education and over 8 years of education respectively; p= 0.005. 14.1% and 9.9% of the women used condom and DMPA respectively, the incidence of pregnancy was 22.2/100wyr among the condom users compared to 11.0/100wyr among DMPA users; p= 0.0284.

**CONCLUSIONS:** Use of DMPA was associated with reduced risk of pregnancy suggesting greater efficacy of user-independent contraception. Dual contraception for discordant couples may reduce unplanned pregnancies while providing protection against HIV transmission. Further research is required to ascertain the pregnancy intention of discordant couples at diagnosis.

# ORAL SESSIONS

SESSION 2  
Sunday, May 23, 2010

## 5

### Improving Contraceptive Uptake and Reducing Pregnancy Rates in a Microbicide Trial

S. Sibeko\*, C. Baxter, N. Yende, L. Mthongana, Q. Abduol Karim, S.S. Abduol Karim on behalf of the CAPRISA 004 Team

CAPRISA University of KwaZulu Natal

**BACKGROUND:** Microbicide trials have been plagued by high pregnancy rates ranging from 15 to 50 per 100 person years. Such high pregnancy rates impact negatively on the statistical power of the study in that person years of exposure to study product are reduced by placing these participants on product hold for the duration of the pregnancy. In order to circumvent this problem, CAPRISA 004 introduced stringent inclusion criteria and a supportive and continuous contraceptive counselling programme.

**METHODS:** The objective of this paper was to assess whether strict inclusion criteria with a structured contraception counselling and on-site contraceptive provision is able to achieve a pregnancy rate below 5 per 100 person-years in a microbicide trial. The inclusion/exclusion criteria to reduce pregnancy rates were 1) family planning intentions, 2) planning to fall pregnant anytime over the study duration, and 3) willingness to be on a non-barrier form of contraception. Eligible women were only enrolled into the study once they had been commenced on a contraceptive method. Contraceptives were provided on-site and contraceptive information was entered into a contraceptive log which was reviewed at each subsequent monthly visit in order to provide more contraceptives and to assess adherence to the method of their choice. Contraceptive logs were reviewed to establish contraceptive uptake at enrolment, subsequent defaulting rates and pregnancy rates. Descriptive analysis of contraceptive and pregnancy data was undertaken.

**RESULTS:** A total of 900 women were enrolled in CAPRISA 004 between May 2007 and January 2009 with all women being on a contraceptive method at enrolment. Only 27% had been on a contraceptive method (besides condom use) prior to study participation. At 12 months of follow up, 1.5% were not on any method due to pregnancy while 4.8% were recorded as not being on method due to missed visits. At completion of follow up the pregnancy rate was 3.95 per 100 women years. Pregnancies, when they did occur, were associated with use of an oral contraceptive rather than the injectable method, missing study visits and therefore missing contraceptive doses, receiving contraceptive outside study site, and switching between methods.

**CONCLUSIONS:** Stringent inclusion criteria together with a structured contraception program which includes on-site provision of methods led to low pregnancy rates in this microbicide trial setting. These results indicate that a comprehensive approach to contraception for participants can contribute to the preservation of study power and study integrity by reducing the time off product within the trial.

## 6

### Predictors of Pregnancy Among HPTN 035 Participants in Lusaka, Zambia

S.T. Roberts<sup>\*1,2</sup>, C. Kelly<sup>3</sup>, C. Reid<sup>1,2</sup>, M.B. Guffey<sup>1,2</sup>

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**BACKGROUND:** Pregnancy during microbicide trials may lead to time off study products, reduced statistical power, and potential bias. Identifying predictors of pregnancy could improve pregnancy prevention strategies and potentially reduce pregnancy incidence in ongoing and future trials. We hypothesized that a woman's reproductive history and contraceptive choices were likely to predict in-trial pregnancy.

**METHODS:** Demographics, reproductive history, contraceptive choices, and condom use were collected from the 320 participants enrolled in HPTN 035 in Lusaka, Zambia. These variables were compared for women who became pregnant during the study (PW) and women who did not become pregnant during the study (NPW) using univariate and multivariate analyses. Contraceptive methods were grouped into Effectiveness Categories (ECs): In Lusaka, EC1 included surgical sterilization, intrauterine devices, implants and injections; EC2 included only oral contraceptive pills (OCPs); and EC3 included male and female condoms, natural and traditional methods.

**RESULTS:** Pregnancy occurred in 51 women (16%). PW had fewer children (1.5 vs. 2.1, p=0.001) and 22% had never been pregnant compared to only 7% of NPW (p=.002). The age of the youngest living child of PW was older than NPW (25.5 vs. 19.8 months, p=0.04). At study enrollment, 72% of NPW were on EC1 methods versus 45% of PW, and 41% of PW were on OCPs versus 14% of NPW (p=0.0001). At enrollment, PW were less likely than NPW to have used condoms at the last sexual act (65% vs. 79%, p=0.02). During the study, PW spent less time than NPW on EC1 methods (33% vs 74%, p<0.0001) and more time on OCPs (56% vs. 20.4%, p<0.0001). 75% of PW were on OCPs at conception. In multivariate analysis, the odds of pregnancy in-study decreased by 35% for each additional living child the woman had at study enrollment (p=.0037). The odds of pregnancy for women on OCPs at enrollment were 4.2 times higher than for women on EC1 methods (p<0.0001) and 2.9 times higher than for women on EC3 methods (p=0.052). Women reporting condom use at last vaginal sex were also less likely to become pregnant during the study (OR=0.48, p=0.038).

**CONCLUSIONS:** Having fewer children, use of OCPs at enrollment compared with other contraceptive options, and lack of condom use at last sexual act were predictors of in-trial pregnancy. Careful consideration of these factors during participant screening may reduce the incidence of pregnancy during future HIV microbicide trials.

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### Impact of Becoming HIV+ on Contraceptive Use in the MIRA Trial

K. Blanchard<sup>\*1</sup>, K. Otis<sup>1</sup>, A. Bostrom<sup>2</sup>, A. van der Straten<sup>3</sup>, G. Ramjee<sup>4</sup>, G. de Bruyn<sup>5</sup>, T. Chipato<sup>6</sup>, E.T. Montgomery<sup>6</sup>, N.S.Padian<sup>3</sup>

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<sup>3</sup>RTI International, San Francisco, CA, USA; <sup>4</sup>South African Medical Research Council, Durban, South Africa; <sup>5</sup>Perinatal HIV Research Unit, Johannesburg, South Africa; <sup>6</sup>University of Zimbabwe-UCSF Collaborative Research Programme, Harare, Zimbabwe

**BACKGROUND:** Research on the reproductive health needs of HIV+ women is critical to improve quality of and access to services and counseling, yet few studies have documented the contraceptive practices of women who seroconvert. We examined the effect of becoming HIV+ on contraceptive practices in the context of a phase III multisite randomized controlled trial of the diaphragm for HIV prevention (the MIRA trial).

**METHODS:** Women enrolled in the trial were HIV-, non-pregnant, sexually-active, and willing to be randomized to use a diaphragm with lubricant gel, in addition to receiving condoms, safer sex counseling and curable STI testing and treatment. Data on contraceptive use were collected at baseline and quarterly visits; contraceptive use was coded into seven categories based on the most effective method reported. We compared demographic characteristics and effectiveness of method used at baseline and last visit between women who did and did not become HIV+ during the trial (using t-tests, Chi square and Kruskal-Wallis tests as appropriate). We compared changes in most effective method used from baseline to last visit and calculated the percentage of women that moved to a more or less effective method or stayed the same (using Chi square test). We also examined immediate changes in contraceptive use after learning HIV+ status.

**RESULTS:** 4645 women remained HIV- and 309 women became HIV+ during the trial. There were significant (but small) demographic differences between the two groups but median effectiveness of contraceptive use was similar both at baseline ( $p=0.33$ ) and last visit ( $p=0.50$ ). 26-28% of women in both groups reported use of a more effective contraceptive method, half did not change methods and 21-22% of women changed to a less effective method. The pattern of change was similar between the two groups ( $p=0.81$ ). Overall few women reported using long-acting methods. Within the HIV+ group, among 243 women with pre- and post-seroconversion data, shorter-term changes in contraceptive effectiveness were similar to longer term changes between baseline and last visit though somewhat more women used the same method at the visit after seroconversion.

**CONCLUSIONS:** Becoming HIV+ did not appear to significantly change patterns of use of effective contraceptives or the probability of switching to a more or less effective method. Long-acting methods should receive more attention and be offered as part of routine contraceptive care for all women.

# ORAL SESSIONS

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## Pregnancy is Associated with an Increased Risk for HIV Transmission Among African HIV-1 Serodiscordant Couples

N. Mugo<sup>\*1,2</sup>, R. Heffron<sup>2</sup>, D. Donnell<sup>3</sup>, E. Were<sup>4</sup>, J. Kiarie<sup>1,2</sup>, K. Kayitenkore<sup>5</sup>, C. Cohen<sup>6</sup>, C. Celum<sup>2</sup>, A. Wald<sup>2</sup>, J. Baeten<sup>2</sup> for the Partners in Prevention HSV/HIV Transmission Study Team

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**BACKGROUND:** Physiologic, immunologic and behavioral changes of pregnancy may alter HIV-1 susceptibility and infectiousness. Some epidemiologic studies have found pregnancy increased women's HIV-1 acquisition risk, and HIV-1 shedding in genital secretions is increased in HIV-1 infected pregnant women, suggesting increased risk to male partners.

**METHODS:** Analysis of a prospective study of African HIV-1 serodiscordant couples was done to evaluate the effect of pregnancy on risk of male-to-female and female-to-male HIV-1 transmission, after adjusting for sexual behavior and other potentially confounding factors. Participants were followed up to 24 months.

**RESULTS:** 3321 HIV-1 serodiscordant couples from 7 African countries were enrolled, 1085 (32.7%) with HIV-1 susceptible female partners and 2236 (67.3%) with HIV-1 susceptible male partners. There were 823 pregnancies (320 in HIV-1 susceptible women, 503 in HIV-1 infected women). 64 women acquired HIV-1 (incidence 3.8 per 100 person-years), of which 17 (26.6%) occurred during pregnancy, and 57 men acquired HIV-1 from their female partners (incidence 1.7 per 100 person-years), of which 12 (21.1%) occurred during their partners' pregnancy. Pregnancy was associated with increased male-to-female HIV-1 transmission risk (hazard ratio [HR] 2.1, 95% confidence interval [CI] 1.2-3.7, p=0.009). The risk was attenuated and no longer statistically significant (adjusted HR 1.53, 95% CI 0.84-2.77, p=0.2) after adjusting for female partner age, unprotected sex, and hormonal contraceptive use. Pregnancy in female HIV-1 infected partners was associated with increased female-to-male HIV-1 transmission risk (HR 2.21, 95% CI 1.17-4.19, p=0.02) and this effect was not attenuated in adjusted analysis (adjusted HR 2.28, 95% CI 1.16-4.46, p=0.02). Further adjustment for other factors, including plasma HIV-1 levels, CD4 count, and male circumcision, did not substantially change the findings.

**CONCLUSIONS:** Among heterosexual HIV-1 serodiscordant couples, pregnancy was associated with increased risk of male-to-female and female-to-male HIV-1 transmission. In adjusted analysis, the risk of male-to-female transmission appeared to be largely explained by behavioral and other factors. This is the first study to show pregnancy increased the risk of female-to-male HIV-1 transmission, which was not fully attributable to confounding factors and may reflect biological changes of pregnancy that may increase HIV-1 infectiousness.

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## Maternal Single-Dose Pharmacokinetics and Placental Transfer of Tenofovir 1% Vaginal Gel Among Healthy Term Gravidas

R. Beigi<sup>\*1</sup>, L. Noguchi<sup>2</sup>, I. Macio<sup>1</sup>, R.P. Kunjara<sup>2</sup>, C. Hendrix<sup>3</sup>, B. Mâsse<sup>4</sup>, M. Valentine<sup>5</sup>, D.H. Watts<sup>6</sup>, J. Piper<sup>7</sup>

<sup>1</sup>Magee-Womens Hospital of UPMC, <sup>2</sup>Microbicide Trials Network—Magee-Womens Research Institute, <sup>3</sup>Johns Hopkins University School of Medicine, <sup>4</sup>Statistical Center for HIV/AIDS Research and Prevention (SCHARP), <sup>5</sup>Family Health International, <sup>6</sup>Unicef Kennedy Shriver National Institute of Child Health and Human Development, <sup>7</sup>National Institute of Allergy and Infectious Diseases, Division of AIDS

**BACKGROUND:** Topical microbicides (TM) represent a promising approach for prevention of sexual HIV transmission. Young reproductive-age women are a primary target population for TM but they also frequently become pregnant. Data suggests pregnancy may heighten risk of HIV acquisition while physiologic changes of pregnancy may alter the absorption of vaginally administered medications when compared to non-pregnant women. The current investigation assessed the pharmacokinetics and placental transfer of tenofovir 1% vaginal gel in term pregnancy.

**METHODS:** Healthy pregnant women scheduled to undergo planned cesarean delivery at term gestation (>37 weeks) were enrolled. Women received a single application of 4 grams of tenofovir 1% vaginal gel (40 mg PMPA) in the pre-operative suite. Maternal blood for serum drug concentrations was collected at time 0,1,2,4,6,8,12, and 24 hours. Specimens of amniotic fluid, fetal cord blood, placenta and endometrium were collected during surgery. Maternal and neonatal adverse events were also collected.

**RESULTS:** Twelve women have received tenofovir 1% gel to date (12/16, 75% of target) and 11 (91.6%) have at least one detectable serum tenofovir level (Figure 1). Seven of the 12 infants (58.3%) have detectable levels in fetal cord blood. The median maternal Cmax and fetal Cmax tenofovir levels were 4.3 and 1.9 ng/ml, respectively. The median cord:maternal blood ratio was 0.47 (consistent with oral dosing). No serious adverse events among mothers or neonates were judged to be related to tenofovir gel exposure.

**CONCLUSIONS:** Single application of tenofovir 1% vaginal gel in term pregnancy produces low overall serum levels consistent with levels reported in non-pregnant women. While tenofovir does appear to cross the placenta, absolute fetal exposure after vaginal dosing is low with a similar cord:maternal ratio noted after oral dosing. These findings support ongoing investigation of tenofovir 1% vaginal gel in pregnant women.

## SESSION 3

### Mini-Symposia (MS1): Measuring Adherence in Prevention Studies

**Moderators:** Benoît Mâsse, Neetha Morar

Sunday, May 23, 9:30 am–11:00 am

Rooms 301–304

## 10

### Use of Telephone and Web-based Technology to Monitor Adherence

Ana Ventuneac

HIV Center for Clinical and Behavioral Studies: New York State Psychiatric Institute, Columbia University, USA

Accurate measures of adherence to product use are crucial in microbicide trials. Self-reported data are often susceptible to social desirability and recall biases. Interactive voice response (IVR) systems are web-based technologies that offer inexpensive ways to program applications that collect coital diary data by telephone, thereby reducing time and effort on the part of study staff and participants. Participants enrolled in a Phase 1 clinical trial conducted through a unique collaboration between the MTN and ATN to examine safety and acceptability of a vaginal microbicide (MTN-004 and ATN-062) called a toll-free number to respond to questions about gel use and sexual behavior during the 14-day period of product use. A repeated measurements analytic approach with GEE methodology to account for within-subject correlation was used to analyze daily self-reports of gel use as a function of timeline, day of the week, study condition, and acceptability ratings. Our findings indicate that it is feasible to implement such methods in a microbicide trial and that participants found the IVR system acceptable, but that design issues need to be carefully considered to minimize confusion that may result to erroneous reports. We will report design features and considerations to improve these methods for future trials.

## 11

### Advances in Adherence Monitoring

Steven Asimwe  
*Kids in Need, Uganda*

In the absence of an effective vaccine, HIV continues to spread worldwide, emphasizing the need for new biomedical interventions to limit its transmission. Appreciation of the challenges that HIV has to face to initiate an infection mucosally has spurred interest in evaluating the use of antiretroviral drugs to prevent infection. Pre-exposure prophylaxis to prevent HIV infection (PrEP) is a potentially safe and intermittent intervention for very high-risk people, and several clinical trials to evaluate this preventive strategy are underway. This is particularly a promising new HIV prevention method for women in high-risk groups like discordant partnerships where we are currently assessing objective adherence measures to PrEP. If efficacious, the public health impact of PrEP can be substantial. However, this impact may be undermined, diminished, or even reversed, by changes in risk behavior and poor adherence. This presentation discusses the progress to-date, challenges and opportunities in PrEP adherence monitoring within the context of a clinical trial.

## 12

### Pharmacokinetic Assessment of Adherence

Craig Hendrix  
*John Hopkins University School of Medicine, USA*

The inability to make objective and quantitative assessments of drug adherence in clinical trials limits one's ability to rule out poor adherence as a cause of drug failure, potentially resulting in discontinuation of an effective drug in development. Unfortunately, most of the measures used to measure adherence are either subjective, qualitative, or both. To improve our ability to provide quantitative and objective measures of adherence to HIV prevention regimens, measurement of drug concentrations has been proposed as a potential adherence measure. In this method, blood or other clinical samples of interest are sampled at specified times and the resultant "observed" drug concentrations are compared to the "expected" drug concentrations based either on data from the individual or data from a population estimate. The ability of these pharmacological methods to provide accurate results depends on numerous factors including the intra- and inter-individual variability in drug pharmacokinetics, dose-proportionality of drug pharmacokinetics at and below concentrations associated with 100% adherence, assay sensitivity, and the accumulation index of the drug in the prevention regimen. This pharmacological approach to assessment of adherence is currently under study in several clinical trials where it will be compared to existing adherence metrics.

# ORAL SESSIONS

## SESSION 4

### Oral Abstracts (OA2): Identifying Participants for HIV Prevention Trials

**Moderators:** Lynn Paxton, Nomita Chandhiok

Sunday, May 23, 9:30 am–11:00 am

Rooms 319–321

**13**

#### Willingness to Participate in a Vaginal Microbicide Trial Among Women in Kisumu, Kenya

H. Awuoche\*, Dr. S. Gitome,  
Dr. M. Adudans, Dr. B. Njoroge, Dr. E. Bukusi  
*Centre for Microbiology Research, Kenya Medical Research Institute*

**BACKGROUND:** In preparation for a phase I/II vaginal microbicide safety and acceptability trial, we went into the community to find out if the women would be willing to participate as well as the reasons why they would not be willing to participate, thus helping us in planning our trial.

**METHODS:** Door-to-door informal interviews were conducted in two proposed recruitment areas during which we assessed willingness to participate in a microbicide trial among women aged between 18–40 years.

**RESULTS:** Of 4860 women interviewed, 3718 (77%) reported willingness to participate in a vaginal microbicide trial and 1142 were unwilling to participate in the trial. Willingness to participate was high (56%) among women between 18–27 years old compared to 17% among women 28–37 years old and 3% among those 38 years old and above. Data on reasons for unwillingness were available for 78% of the women who were not willing to participate. These reasons were: needing more time to think over participation (21%), no time for participation (17%), reasons related to product use, i.e., fears of side effects and of inserting objects into the vagina (11%), unsure of male partners' consent (9%), other reasons (31%) and no reason (11%).

**CONCLUSIONS:** Our findings suggest that participation in a vaginal microbicide trial is acceptable to the majority of women surveyed between 18–40 years in Kisumu. Continued microbicide education may improve microbicide knowledge in the women who were willing to participate in the trial and help to ensure that they make informed choices when the trial begins. Microbicide education may also improve the microbicide knowledge of the women who were not willing to participate in the trial and hopefully encourage them to participate in the trial. Re-visiting the women who needed more time to think about trial participation will enable us find out if they have made any decisions on participating in the trial and provide an opportunity for additional microbicide education. Male involvement during the preparation stage and also during the trial may encourage male support of their female partners' participation. All of these activities may facilitate timely recruitment and subsequent follow-up of well informed participants.

**14**

#### Willingness and Covert Use of Vaginal Microbicides by Male Partners of Women Participating in a Cross-sectional Survey

R. Kohli<sup>1</sup>, S. Deshpande<sup>1</sup>, B. Tolley<sup>2</sup>,  
S. Mehendale<sup>1</sup>

<sup>1</sup>National AIDS Research Institute, Pune, India,  
<sup>2</sup>Family Health International, USA

**BACKGROUND:** In male-dominated societies like India, mere availability of new woman-initiated HIV prevention options may not ensure its access and use by women. Therefore, we sought to explore factors which might affect willingness of men to support use of vaginal microbicide by their partners and their attitudes towards its covert use by their partners.

**METHODS:** Between Sept and Oct 2005, 151 consenting married, sexually active men were invited to participate in a survey through their spouses, who themselves participated in microbicide willingness survey. Most women were recruited from the referral clinics of National AIDS Research Institute [NARI] and its NGO network.

**RESULTS:** Relationships of socio-demographic and behavioral factors was assessed with two dependent variables; willingness to use a microbicide with a partner and support for covert use by the partner. Behavioral domains such as Couple Harmony, Partner Infidelity, HIV Risk-Perception and Protection Efficacy were evaluated in multivariate logistic regression analysis.

Mean age of men was 34 years (SD 6.6). Most men had completed ten years of schooling (71.5%) and 96.7% were employed. Nearly 58% men reported using any contraceptive method. Nearly 50% men reported high-risk behavior and 26 men were sero-positive for HIV/STIs. Self-risk perception was low as nearly three-fourths of men did not perceive the risk of HIV acquisition despite almost half of them having had multi partner relationships. Most men (90.1%) reported willingness to support use of a microbicide with their partner while more than half (58.1%) expressed concern about the covert use of a microbicide by their spouses. Younger men [below 34 years] [AOR: 7.92, 95% CI: 1.31, 47.87] and those reporting higher couple harmony [AOR: 6.7, 95% CI: 1.64, 27.65] were more willing to use microbicides compared to older men and less harmonious relationships. Men practicing high-risk behavior were twice as likely [AOR: 1.98, 95% CI 1.00, 3.92] and those with a history of previous contraceptive use were almost three times [AOR: 2.93, 95% CI 0.99, 8.78] more likely not to mind covert use of microbicides by their partners as compared to low risk men and with no history of contraceptive use.

**CONCLUSIONS:** Careful attention needs to be paid to socio-cultural and familial context of potential users and the marketing strategies should also address men in order to increase their acceptability among married women in real life setting.

## 15

### Assessing Yields of Recruitment Strategies for an HIV Prevention Trial Among HIV Discordant Couples in Kenya

P. Michira<sup>\*</sup>, K. Ngure<sup>1</sup>, E. Irungu<sup>1</sup>, D. Irungu<sup>1</sup>, J. Nyokabi<sup>1</sup>, N. Mugo<sup>1,2</sup>

<sup>1</sup>Kenyatta National Hospital,

<sup>2</sup>University of Washington

**BACKGROUND:** HIV incidence among HIV discordant couples who are not aware of their discordance status is estimated at up to 14% per year, and thus HIV discordant couples are an important target population for HIV prevention research. In a population with low uptake for Couples HIV Counseling and Testing (CHCT), recruitment of HIV discordant couples for clinical trials can be challenging and expensive. This paper assesses recruitment strategies at Thika, Kenyan site for the Partners in Prevention HSV/HIV randomized placebo controlled transmission study among HIV discordant couples.

**METHODS:** We used a three-prong approach to mobilize the community: (1) Community education; development of education & communication (IEC) materials and hosting of radio talk shows, (2) Capacity building of HIV counselors; training on CHCT and providing continuous psychological support supervision and (3) Community mobilization, including skits, dramas, music and dances. Data was collected at the study site on where the participants learnt about the research facility. Activities were assessed regularly versus the total number of couples enrolled from the particular strategy. Staff time was calculated as a percentage of time spent per strategy.

**RESULTS:** A total of 587 HIV discordant couples were referred to the study site over a ten- month period of which 213 were enrolled into the trial.(with a monthly average of 21.3 couples). A total of 84 HIV counselors received a five- day formal training with an additional 80 counselors receiving a two-hour monthly CHCT sensitization. Over 12000 IEC materials were distributed during the recruitment phase, while 3 radio talk shows were conducted. A total of 80 street theatres shows were conducted throughout the enrolment period. High yielding recruitment strategy was HIV counselor referrals with 194/213 (91%).Low yielding strategies were radio shows and IEC materials with only a small number of couples (0.5%) reporting exposure to these recruitment strategies. HIV counselor referrals were labour intensive taking up to 50% of recruitment staff time.

**CONCLUSIONS:** The highest yielding strategy was referral by HIV counselors. An established referral system among HIV counselors made it the most effective strategy. Direct HIV discordant couple recruitment strategies from the community yielded less couples. Regular analysis of yield per strategy is important for optimum recruitment in clinical trials. A multi-prong approach is key to successful recruitment of study participants.

## 16

### Recruiting and Retaining Participants in the Durban MDP 301 Trial Sites: Challenges and Strategies

Philisiwe Buthelezi\*, Victoria Zuma, Sarita Naidoo, Sharika Gappoo, Yukteshwar Sookrajh, Gita Ramjee and the MDP Trial Team

HIV Prevention Unit, Medical Research Council, Durban, South Africa

**INTRODUCTION:** The MDP 301 trial was conducted at the Tongaat, Verulam and Isipingo centres in Durban from November 2005 to September 2009. Although these communities were previously exposed to research conducted in these areas and were not research naïve, there were still challenges encountered with recruiting and retaining women for the trial. The purpose of this study is to describe the recruitment and retention strategies and the challenges experienced in the three Durban centres participating in the MDP 301 trial.

**METHODS:** Recruitment was mainly done in semi rural and township areas. Education sessions on HIV/STIs and the MDP 301 trial were conducted in the target communities. During these sessions recruitment of potential participants was conducted. Recruitment strategies included use of peer educators, door-to-door education and recruiting at public centres. Recruitment and information sessions were done at local and mobile clinics, women's groups and pension pay points. During education sessions and follow up visits participants verbalized concerns regarding participation in the study. Peer educators also informed study staff of the rumours regarding the trial within the communities.

**RESULTS:** A total of 2391 HIV negative women were enrolled at the three Durban centres and a retention rate of at least 90% was maintained by each centre. Challenges faced during recruitment included fear of HIV testing, fear of stigmatization by neighbours and relatives because of misconceptions or rumours about the study, partners and parents not allowing potential participants to come to the trial sites for screening and fear of losing partners if they discovered trial participation. Challenges encountered during retention included relocation of participants due to job hunting and gaining employment, participants being discouraged by long clinical procedures or pelvic exams and inadequate money for transport to the clinic. Some of the strategies used to overcome these challenges included involving extensive education and involvement of male partners, suggestion boxes at the clinics to address rumours and misconceptions, and weekend clinics were held to accommodate participants who were employed.

**CONCLUSION:** Recruitment and retention strategies were constantly monitored and discussed with the clinic staff. Feedback from the suggestion box together with a combination of innovative strategies contributed to achieving successful recruitment and a high retention rate.

## 17

### Impact of Community Education on Accrual in the VOICE Study: Zimbabwe Experience

C. Mashoko\*, C. Chasakara, N. Mgodi, T. Magure, M. Mlingo, Z.M. Chirenje

UZ-UCSF Research Programme

**BACKGROUND:** Stringent inclusion and exclusion criteria for VOICE pose important and complex accrual challenges. Among the study entry criteria, women are required to be 18–35 years, at high risk of HIV, HIV negative, sexually active, not highly mobile, not pregnant or planning to become pregnant within the next two years, willing to use an effective contraceptive method and not breastfeeding. Women's limited power and autonomy along with fear of using ARV drugs as prevention, may negatively affect women's decision to join the study. To mitigate these accrual challenges intensive and sustained community education is critical. Community education is imperative not only for accrual purposes, but it is also an ethical obligation of researchers to enlighten members of the community about potential prevention methods.

**METHODS:** We intensified community sensitization by expanding to non-traditional recruitment sources, previously not used as primary recruitment sources. These include, market places, and newly resettled high density areas, and homes. 371 sensitization and 80 recruitment meetings were conducted; approximately 580 men and 2680 women attended. We promoted male involvement by engaging men in these meetings and encouraged women to disclose their intention to participate to their partners.CAB members, community stakeholders, former research trial participants and community volunteers, were mobilized to gather community members to participate in sensitization and recruitment meetings.

**RESULTS:** Non-traditional recruitment sources contributed 80 % of women screened for VOICE. As of week 15, we screened 245 women, and enrolled 67. Screening to enrolment ratio met the target of 3:1. Monthly accrual targets for the first three months (10, 20 and 30) were met; we enrolled 10, 23 and 34 participants respectively. To date, no concerns or social harms have arisen from the community, including participants' partners.

**CONCLUSIONS:** Intensified and sustained community education makes accrual exceedingly achievable in a trial with very stringent inclusion and exclusion criteria. A number of factors may be contributing to our accrual success thus far; however, we attribute to a greater extent the critical role of community education efforts.

# ORAL SESSIONS

## 18

### Identifying High- and Low-Risk MSM for HIV Prevention Trials

J. Maynard\*

*Fenway Institute at Fenway Health*

**OVERVIEW:** As a group that is disproportionately affected by HIV, MSM participation in HIV prevention trials is key to developing new biomedical interventions but identifying and recruiting cohorts can be challenging. This presentation will be a practical guide to venue based and online outreach and recruitment of both high- and low-risk MSM. Topics will include: identifying and supervising culturally competent recruiters, targeting print and on-line media marketing, and tailoring venue based outreach to MSM subgroups. Special emphasis will be given to utilizing current and emerging MSM social networking sites or what has been called the "new cruise bars" of the internet generation.

## SESSION 5

### Oral Abstracts (OA3): Preclinical Studies of New Molecules for HIV Prevention

**Moderators:** Jim Turpin, Stephen Becker

Sunday, May 23, 9:30 am–11:00 am

Rooms 403–405

## 19

### L'644, a Cholesterol Derivatized Version of the gp41 Fusion Peptide C34, Provides Superior Activity in Preclinical Microbicide Assays

S. Harman<sup>\*1</sup>, C. Herrera<sup>1</sup>, N. Armanasco<sup>1</sup>, P. Fletcher<sup>1</sup>, J. Nuttall<sup>2</sup>, J. Romano<sup>2</sup>, R. Shattock<sup>1</sup>

<sup>1</sup>*St. George's, University of London, London, UK*, <sup>2</sup>*International Partnership for Microbicides, MD, USA*

**BACKGROUND:** Topical blockade of fusogenic protein gp41 is one possible strategy by which microbicides could prevent HIV infection, working early against infection, prior to integration. L'644 is a cholesterol derivatized version of the fusion peptide C34 (also known as C34-Chol) that shows dramatically increased antiviral potency against primary isolates. L'644 has been licensed to the International Partnership for Microbicides for microbicide development. We present a preclinical evaluation of L'644, using TZM-bl cells and *ex vivo* genital and colorectal tissue to determine its suitability as an anti-HIV microbicide.

**METHODS:** L'644 was assessed for biocompatibility and efficacy (against wild type and ARV resistant isolates) in TZM-bl cells and in human genital and colorectal tissue explants. Its activity was assessed following pulse (2 hours), continuous and delayed addition and compared to related fusion inhibitors enfuvirtide T20 and T1249. L'644 toxicity and stability in biological fluids were determined.

**RESULTS:** Using TZM-bl cells, L'644 demonstrated sub-nanomolar activity that was not reduced following pretreatment with cervical mucus or seminal plasma. L'644 was more potent against HIV<sub>BaL</sub> than T1249 or T20 in TZM-bl cells. In time of addition studies, only L'644 demonstrated activity when cells were pre-treated with compound for 1 hour, then compound subsequently removed by washing. In experiments to evaluate the potential for preventing HIV-1 infection of *ex vivo* tissue explants, L'644 was the most active against infection of genital tissue explants and was able to inhibit dissemination by migratory cells that emigrate from explant tissue in culture. The relative potency of fusion inhibitors against infection of mucosal tissue explants was L'644>T1249>T20.

**CONCLUSIONS:** The results demonstrate that L'644 was able to inhibit HIV-1 infection of genital and colorectal tissue cultured *ex vivo*, with higher potency than other fusion inhibitors, which could be further increased through sustained delivery. This suggests that L'644 is a good candidate for development as a microbicide.

**20****Searching for New Inhibitors Disrupting gp41 Function as Microbicides for the Prevention of HIV Infection**S. Pikul<sup>1</sup>, J. Romano<sup>\*2</sup>, E. Springman<sup>3</sup><sup>1</sup>Princeton API Services, LLC, Princeton, NJ, USA;  
<sup>2</sup>International Partnership for Microbicides, Silver Spring, MD, USA; <sup>3</sup>Locus Pharmaceuticals, Inc., Blue Bell, PA, USA

**BACKGROUND:** The lack of detailed structural information on small molecules binding to HIV gp41 and lack of a validated structure-activity hypothesis has inhibited the design of such drugs. We attempted to identify small molecules that target HIV gp41 for microbicide development.

**METHODS:** Biochemical testing of gp41 inhibition was performed using Size Exclusion Chromatography (SEC) and Gel Electrophoresis (GE). Additionally, Fluorescence Resonant Energy Transfer (FRET) assays were used. Compounds were evaluated using a cell fusion inhibitory activity assay, and their binding to gp41 was investigated by X-ray co-crystallography.

**RESULTS:** Fragment-based computational design was carried out to identify several new chemical series as candidates for gp41 binding. Representative probe compounds (>100) were synthesized and tested using SEC and GE. Several exhibited inhibitory activity and as a result, 7 chemical series were identified for further evaluation. The most interesting screening hits were tested using HIV cell fusion assay. This identified 10 molecules with  $IC_{50} < 10 \mu\text{M}$  and 4 with activity below 1  $\mu\text{M}$ . Additional analogs of the lead series of molecules were synthesized to establish structure activity relationships (SAR).

A new biochemical screen for gp41 functional inhibition was developed using FRET coupled with proteolytic cleavage of the gp41 C-peptide (HR-2). In this assay, C-peptide sequestered in the hexameric complex with gp41 N-peptide (HR-1) is protected from proteolytic cleavage and no signal is detected. Conversely, unbound C-peptide is digested, releasing the FRET signal. Inhibitors of gp41 hexamer complex formation elicit a strong positive fluorescence response. This assay has significantly higher throughput compared to SEC and GE and is suitable for routine screening in support of drug discovery.

**CONCLUSIONS:** Series of gp41 binding small molecules were discovered using computational fragment-based design. Analysis of initial compounds showing best cell-cell fusion inhibitory activity did not correlate with those showing best HIV gp41 binding inhibition activity. The lack of correlation between these two functional measures suggests limited value in further development of these compounds.

**21****Anti-HIV Activity of the Candidate Microbicide Maraviroc, a CCR5 Receptor Antagonist**P. Fletcher<sup>\*1</sup>, C. Herrera<sup>1</sup>, N. Armanasco<sup>1</sup>, J. Nuttall<sup>2</sup>, J. Romano<sup>2</sup>, R. Shattock<sup>1</sup><sup>1</sup>St. George's University of London, London, UK;  
<sup>2</sup>International Partnership for Microbicides, Silver Spring, MD, USA

**BACKGROUND:** Topical blockade of CCR5 is one possible strategy by which microbicides could prevent HIV infection. Maraviroc, a CCR5 receptor antagonist, is currently in development as a microbicide. We present a preclinical evaluation of maraviroc, using cellular models and *ex vivo* genital and colorectal tissue, to determine its suitability as an anti-HIV microbicide.

**METHODS:** Maraviroc was assessed for biocompatibility following overnight exposure in TZM-bl cells and human genital/colorectal tissue. Efficacy against direct infection (HIV-1<sub>BaL</sub>) of TZM-bl was determined in the continued presence of maraviroc in the absence/presence biological fluids (12.5% seminal plasma (SP) or synthetic cervical mucus (CM)). Efficacy against infection (HIV-1<sub>BaL</sub>) of human genital and colorectal tissue explants and against transfer of infection from HIV-1<sub>BaL</sub>-infected cells (PBMC or dendritic cells (DC)) to uninfected PM-1 T cells was determined following pulsed (3 hours), overnight or continuous exposure to maraviroc. Cultures were maintained in the absence or presence of maraviroc accordingly for the duration of the assay.

**RESULTS:** Maraviroc was biocompatible in *ex vivo* tissue explants up to concentrations of 100 M, with a  $CC_{50}$  of 114  $\mu\text{M}$  in TZM-bl cells. Using TZM-bl cells, maraviroc demonstrated an  $IC_{50}$  of 11.2±8.9nM, and retained activity in the presence of CM and SP. Maraviroc was also active against transfer of infection from both infected DC and PBMC to PM-1 T cells when maraviroc was present for the entire culture period. In experiments to evaluate the potential for preventing HIV-1 infection of *ex vivo* tissue explants, maraviroc was most active against infection of colorectal tissue ( $IC_{50}$  values of 109.9±84.5nM, 22.1±13.8nM and 2.2±1.1nM for a 3-hour pulse, overnight or continuous exposure respectively). In genital tissue explants, maraviroc was most active during continuous exposure.

**CONCLUSIONS:** The results demonstrate that the potency of maraviroc against infection of cells and genital/colorectal tissue can be enhanced through continuous exposure. This data suggests that maraviroc is a good candidate for development as a microbicide to be used in sustained release delivery systems.

**22****Combinations of Maraviroc and Reverse Transcriptase Inhibitors as Potential Microbicides**C. Herrera<sup>\*1</sup>, N. Armanasco<sup>1</sup>, P. Fletcher<sup>1</sup>, J. Nuttall<sup>2</sup>, J. Romano<sup>2</sup>, R. Shattock<sup>1</sup><sup>1</sup>Division of Cellular & Molecular Medicine, St George's University of London, UK; <sup>2</sup>International Partnership for Microbicides, Silver Spring, MD, USA

**BACKGROUND:** Multiple drug combinations are highly effective in HAART and may also be more effective as microbicides against HIV-1 than single drugs. The aim of this study was to assess the activity of combinations based on compounds with different HIV-1 inhibitory mechanisms.

**METHODS:** The antiviral efficacy of an entry inhibitor, maraviroc, and three reverse transcriptase inhibitors (RTIs): a nucleotide reverse transcriptase inhibitor (NRTI), tenofovir (PMPA), and two non-nucleoside RTIs (NNRTI), UC-781 and dapivirine (TMC120), used in double combinations, was assessed in cellular (luciferase reporter TZM-bl cells, T cells and dendritic cells (DC) isolated from peripheral blood mononuclear cells) and colorectal explant models. Pre-incubation of cells or tissue with the drugs individually or in combination, for one hour was followed by addition of an R5-tropic virus, BaL. Infection was determined by measurement of luciferase expression (in TZM-bl cells) or virion protein (p24 antigen) in culture supernatants.

**RESULTS:** Dual combinations of maraviroc with any of the RTIs tested against BaL in TZM-bl cells showed an increase of activity compared to the drugs used alone, with an average 56% reduction of the  $IC_{50}$  value of each drug used in combination. When double combinations were tested in colorectal explants a higher shift in the dose response curves was detected when maraviroc was combined with tenofovir or dapivirine. These two double combinations (maraviroc-tenofovir and maraviroc-dapivirine) were further investigated for their activity in cell-associated virus transmission in two models: DC to T cell, and T cell to T cell. Both combinations were also positive in these two models of cell-to-cell transmission.

**CONCLUSIONS:** The increased activity of combinations of drugs inhibiting HIV transmission at different steps of the viral replication cycle (entry and reverse transcription), when compared with the activity of each drug alone, suggests these combinations have greater potential as microbicides.

# ORAL SESSIONS

**23**

## Protection Against Repeated Vaginal SHIV Exposures in Macaques by a Topical Gel with an Integrase Inhibitor

C. Dobard\*, S. Sharma<sup>1</sup>, A. Martin<sup>1</sup>, D. Hazuda<sup>2</sup>, D. Hanson<sup>1</sup>, J. Smith<sup>1</sup>, R.A. Otten<sup>1</sup>, F. Novembre<sup>3</sup>, G. Garcia-Lerma<sup>1</sup>, W. Heneine<sup>1</sup>

<sup>1</sup>CDC, Atlanta, GA, USA; <sup>2</sup>Merck, North Wales, PA, USA; <sup>3</sup>Yerkes Primate Research Center and Emory University, Atlanta, GA, USA

**BACKGROUND:** HIV continues to spread, primarily through heterosexual sex. Topical gels containing antiretroviral drugs are currently under evaluation as a prevention strategy against HIV acquisition. Previous work has focused on entry and reverse transcriptase inhibitors. Here we assess an integrase inhibitor representing a new class of drugs for topical prophylaxis, using a repeat-challenge macaque model that resembles human vaginal transmission.

**METHODS:** We formulated the integrase inhibitor L-870812 into a stable 0.2% gel with hydroxyethyl cellulose. We performed 14 intravaginal SHIVSF162p3 challenges twice-weekly with a low-dose (10-TCID<sub>50</sub>) inoculum. Female pig-tailed macaques were assigned to a placebo gel (n=1) or the integrase inhibitor gel (n=3). Three mLs of gel were applied vaginally 30 min before challenge. Infection was monitored by serology and PCR of SHIV sequences in plasma. Drug absorption was determined 30 min after gel application by measuring plasma drug levels using liquid chromatography-mass spectrometry. Drug resistance was monitored by sequencing the integrase region from plasma viral RNA.

**RESULTS:** The realtime placebo macaque was infected after 3 challenges, consistent with historical controls (median 4 challenges, range: 2-11). In contrast, 2/3 macaques receiving L-870812 gel remained uninfected after 14 challenges, demonstrating that L-870812 provided significant protection ( $p<0.05$ ; Fisher's exact test). Low levels of L-870812 (median=15.5 ng/ml; range: 6-140) were detected in plasma suggesting rapid absorption. The single breakthrough infection showed a wild-type virus genotype and no evidence of drug resistance emergence despite continued twice-weekly gel use for 15 weeks post infection. Plasma viremia in this animal was similar to controls.

**CONCLUSIONS:** Topically applied gel with an integrase inhibitor protects from repeated vaginal challenges. Systemic absorption of L-087812 did not impact viremia or select for drug resistance. These data identify a novel strategy for topical prophylaxis with integrase inhibitors and support further evaluation of this class of drugs.

**24**

## Protease Inhibitors Darunavir, Lopinavir and Ritonavir as Potential Microbicides

A. Evans\*, P. Fletcher, C. Herrera, R. Shattock

St George's, University of London, London, UK

**BACKGROUND:** Protease inhibitors (PI) darunavir (DRV), ritonavir (RTV) and lopinavir (LPV) inhibit cleavage of HIV Gag-Pol by the protease enzyme, preventing formation of mature infectious virions. Dapivirine (DPV; also known as TMC120), a non-nucleoside reverse transcriptase inhibitor, has exhibited potent anti-HIV-1 activity in cellular and human genital tissue models of infection. We have investigated the potential of DRV, RTV and LPV to inhibit HIV-1 in cellular and human genital tissue models of infection to evaluate these compounds as possible microbicide candidates. Further, anti-HIV-1 activity of DRV and DPV in combination was assessed.

**METHODS:** Anti-HIV-1 activity of PIs was investigated using PM-1 T cells and primary monocyte-derived dendritic cells (DC), individually and in a model of DC to T cell viral transmission with continual exposure to compounds. Human cervical tissue co-cultured with PM-1 T cells was exposed to DRV (2 and 24 hour pulse) to evaluate the compound's capacity for anti-maturational activity against newly formed virions. DRV and DPV were combined using an IC<sub>50</sub>:IC<sub>50</sub> ratio and assessed for anti-HIV-1 activity in continually drug exposed PM-1 T cells. Results were obtained by measurement of p24 using ELISA. The effect of varying concentrations of tested PIs on cell and tissue viability was evaluated using MTT assay with Nonoxynol-9 as a control.

**RESULTS:** The assessed PIs demonstrated inhibition of HIV-1<sub>BaL</sub> in PM-1 T cells, DC and DC to T cell transfer of infection, with DRV exhibiting greatest efficacy in all cell types. DRV showed antiviral activity in cervical tissue co-cultured with PM-1 T cells when pulsed for 24 hours. There was no reduction in cell or tissue viability at inhibitory concentrations of DRV. On PM-1 T cells a combination of DRV and DPV demonstrated enhanced efficacy against HIV-1 with a 70% reduction in IC<sub>50</sub> compared to DPV alone, and a 47% reduction in IC<sub>50</sub> compared to DRV alone.

**CONCLUSIONS:** DRV, LPV and RTV demonstrate good antiviral activity in cellular assays, with DRV the most potent PI. DRV inhibited HIV-1 infection in cervical tissue when co-cultured with PM-1 T cells. Inhibitory levels of DRV did not compromise cell and tissue viability. Furthermore, the anti-HIV-1 activity of DRV combined with DPV in PM-1 T cells was greater than that of each compound individually in the same model. These data indicate DRV may be a potential candidate microbicide, particularly in combination with DPV.

## SESSION 6

### Oral Abstracts (OA4): Learning from 1st Generation Products: N-9, PRO 2000, and Cellulose Sulfate

Moderators: Sheena McCormack, Henry Gabelnick

Sunday, May 23, 11:30 am–1:00 pm

Ballroom

## 25

### Inflammatory Response in the Lower Genital Tract Does Not Reflect Vaginal Drug Exposure or Impact

C.A. Schreiber<sup>\*1</sup>, H.I. Su<sup>2</sup>, C. Fay<sup>3</sup>, K.T. Barnhart<sup>4</sup>

<sup>1</sup>Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA; <sup>2</sup>Department of Reproductive Medicine, University of California San Diego, San Diego, CA 92130, USA; <sup>3</sup>Center for Research of Reproduction and Women's Health, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA; <sup>4</sup>Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

**BACKGROUND:** The vagina and endometrium are both entry portals for pathogens. We lack valid, measurable markers of disruption of the local immune system in the female genital tract. We sought to determine whether vaginal epithelial integrity, endometrial mucosal integrity, and/or cytokine levels are surrogate markers of vaginal drug safety.

**METHODS:** We conducted a randomized, assessor-blinded, crossover trial to test the effect of nonoxynol-9 gel (Gynol II) and placebo gel (hydrocellulose acetate [HEC]) on epithelial integrity and inflammatory markers in the endometrium and vagina. Eighteen subjects underwent colposcopy, vaginal lavage (VL), endometrial lavage (EL) and endometrial biopsy at baseline evaluation without gel exposure and up to two more evaluations after exposure to two possible conditions: 3 days of Gynol II or HEC gel. Eight pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, IL-8, MCP-1, MIP-1 $\alpha$ , MIP-1 $\beta$ , RANTES, and TNF- $\alpha$ ; three anti-inflammatory cytokines (IL-1 $\alpha$ , IL-10, Slp-1) were assayed in all VL and EL specimens.

**RESULTS:** In 46 completed cycles, vaginal colposcopy and endometrial histopathology results were no different among baseline, Gynol II and HEC cycles. Multiple cytokines differed between the vagina and endometrium. Uniform differences in pro- or anti-inflammatory cytokines were not observed: of pro-inflammatory cytokines, IL-1 $\beta$  ( $p=0.001$ ) was significantly higher, while RANTES ( $p<0.001$ ) was significantly lower in the vagina. Of anti-inflammatory markers, IL-1 $\alpha$  ( $p<0.001$ ) was higher, while IL-10 ( $p<0.001$ ) and Slp-1 ( $p<0.001$ ) were significantly lower in the vagina than in the endometrium. HEC exposure was associated with significant changes in endometrial cytokines.

**CONCLUSIONS:** Gross inspection of the vagina and histology of the endometrium are not sensitive for detecting differences in response to Gynol II, HEC and no gel exposure. Both placebo gel and Nonoxynol-9 containing gels appear to modulate cytokines in the vagina and endometrium. Our findings suggest that immune response varies by different sites of the lower reproductive tract. However, we see no common trend in pro- and anti-inflammatory cytokines between these sites to support their use as markers of vaginal drug safety. Differences in measures of inflammation may reflect independent immune responses at the two sites, or, alternatively, that vaginal immune responses are a product of mixed vaginal and endometrial immune response.

## 26

### Assessing the Safety of PRO 2000 Vaginal Gel in the MDP 301 Phase III Microbicide Trial

S. McCormack<sup>\*1,2</sup>, M. Chisembele<sup>3</sup>, C. Gilbert<sup>2</sup>, M. Gafos<sup>4</sup>, R. Hayes<sup>5,6</sup>, U. Jentsch<sup>7</sup>, A. Kamali<sup>8</sup>, C. Lacey<sup>9</sup>, G. Ramjee<sup>10</sup>, H. Rees<sup>10</sup>

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**BACKGROUND:** PRO 2000 is a synthetic naphthalene sulphonated polymer. MDP 301 is a Phase III randomised placebo-controlled trial to assess the safety and effectiveness of 0.5% and 2% PRO 2000 gels. This presentation describes the detailed safety results from this trial.

**METHODS:** Healthy women were randomised to receive 0.5%, 2% or placebo gel for 12m (up to 24m in Uganda) to be applied pre-sex at 6 centres in East and Southern Africa. All women received condoms and safe sex counselling. The 2% gel was discontinued in Feb 2008 on the recommendation of the independent Data Monitoring Committee and analyses for this group versus placebo were censored at this time. The primary safety endpoint was a grade 3 or higher adverse event and the local toxicity endpoint was any grade of genital itching, burning, internal epithelial disruption, internal erythema or internal oedema. Systemic toxicity was assessed from routine laboratory parameters which were collected in a cohort of participants enrolled in Durban, Johannesburg and Masaka (n=1840). The main safety analysis included all events reported throughout follow-up. A planned sub-group analysis of the local and systemic toxicity endpoints was conducted according to gel exposure, derived from the number of returned used applicators, with the median used to classify women as high or low gel users.

**RESULTS:** 15818 women were screened and 9385 enrolled. 97% of women contributed to the safety analysis. Overall there were 423 primary safety endpoints experienced by 398 participants, with non-menstrual bleeding the most commonly reported event. 282 were included in the comparison of 0.5% and placebo, incidence 4.6 (154/3349) and 3.9 (128/3317)/100wyrs respectively. The HR for 0.5%:placebo was 1.18 (95% CI 0.93–1.49). 16% experienced at least one local toxicity event, HR=1.05 (95% CI 0.93–1.19). For systemic toxicity, 34% experienced at least one event, HR=0.96 (95% CI 0.79–1.16). In the comparison of 2% to placebo, the incidence of primary safety events was 5.9 (116/1956) and 5.8 (111/1911)/100wyrs respectively; HR=1.00 (95% CI 0.77–1.30); for local toxicity, HR=1.01 (95% CI 0.87–1.17); systemic toxicity HR=0.98 (95% CI 0.76–1.26). There was no evidence that high gel users of either 0.5% or 2% concentration experienced more local or systemic toxicity events.

**CONCLUSIONS:** Both concentrations of PRO 2000 were found to be safe in terms of primary safety, local and systemic toxicity endpoints.

# ORAL SESSIONS

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## Bridging the Gap Between Preclinical Studies and the Performance of Microbicides in Large Scale Clinical Trials: A Reanalysis of Cellulose Sulfate

T.J. Segarra<sup>\*1</sup>, E. Fakioğlu<sup>1</sup>, S. Wilson<sup>1</sup>, H. Boukari<sup>1</sup>, G.F. Doncel<sup>2</sup>, B.C. Herold<sup>1</sup>

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**BACKGROUND:** Despite the finding of significant protection in preclinical studies including non-human primate models, no microbicide to date has shown significant efficacy in clinical trials and an unanticipated trend towards increased HIV acquisition has been observed in some studies. These results highlight the need to modify preclinical tests with the goal of identifying assays that may prove more predictive of clinical trial outcomes.

**METHODS:** We evaluated 4 blinded samples (DE-018-021) provided by CONRAD, including the 6% cellulose sulfate formulation and the hydroxyethylcellulose-placebo gels used in the clinical trials in an expanded murine safety and efficacy model. Mice were treated intravaginally with the 4 CONRAD formulations, 3.5% nonoxynol-9 (N9), or no gel and the ability of microbicides to prevent HSV infection following intravaginal challenge (efficacy) or on susceptibility to HSV following low dose viral challenge (safety) determined. In addition, epithelial cell architecture, junctional proteins and inflammation were assessed.

**RESULTS:** Only DE-018 and 020 significantly protected mice from genital herpes when HSV-2 was introduced in buffer 15 minutes following gel application ( $p<0.05$ ). Vaginal washes collected 12 h after 7 daily doses of DE-018, but not any of the other compounds, inhibited HSV-2 plaque formation in vitro, indicating persistence of drug within the genital tract. However, mice treated with 7 daily doses of DE-018 or N9, were significantly ( $p<0.001$ ) more susceptible to be infected with low doses of HSV-2 when challenged 12 h after the 7th dose. The increased susceptibility was not associated with increases in inflammatory cytokines or chemokines or with NF- $\kappa$ B activation. Confocal studies to determine possible disruption of the epithelium are in progress.

**CONCLUSIONS:** Despite the protective effects of DE-018, the findings of increased susceptibility to HSV in the murine safety model suggest that this formulation could compromise the mucosal barrier to infection. Unblinding of the samples will help determine if this expanded murine safety model provides a biomarker for evaluation of future microbicides.

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## Evaluation of 6% Cellulose Sulfate and 0.5% PRO 2000 Gel Microbicide Efficacy in a Single-Dose Intravaginal R5+X4 SHIV Infection Model in Rhesus Macaques Pre-treated with Depo-Provera

M. Saifuddin<sup>\*1</sup>, L. Tsai<sup>2</sup>, A. Gettie<sup>2</sup>, R. Bohm<sup>3</sup>, G. Doncel<sup>4</sup>, C. Cheng-Mayer<sup>2</sup>

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**BACKGROUND:** Nonhuman primate models play critical role in preclinical evaluation of topical microbicides. We have shown that 6% cellulose sulfate (CS) gel prevents the development of viremia and seroconversion in rhesus macaques challenged weekly with 100 TCID<sub>50</sub> of R5 and X4 SHIV (3:1 ratio) (R5+X4). The current study was aimed at comparing CS efficacy in Depo-Provera treated animals infected by a single dose of 300 TCID<sub>50</sub> of R5+X4 SHIV.

**METHODS:** Animals (n=30) were treated intravaginally with 2 ml of active or placebo gel (using their clinical formulations) and challenged 30 min later with 300 TCID<sub>50</sub> of R5+X4 SHIV. Infection was monitored by measuring plasma viremia, antibody seroconversion and proviral DNA. Results were analyzed with Fisher's exact test.

**RESULTS:** The study was undertaken as two experiments of 12 monkeys each (n=6 per group). The combined outcome showed a statistically significant protection of CS gel compared to placebo (1/12 vs 7/12 animals were free of systemic infection in the placebo and CS group, respectively;  $P = 0.0272$ ). As in the low-dose repeated challenge model, proviral DNA was occasionally detected in PBMC and lymphoid cells of the aviremic animals. PRO2000 (0.5%) gel, included in the second experiment, protected 4/6 animals in this model ( $P=0.0217$ ).

**CONCLUSIONS:** CS prevented systemic viremia in all animals challenged weekly with 100 TCID<sub>50</sub> R5+X4 SHIV and in about 60% of Depo-treated animals challenged with a single dose of 300 TCID<sub>50</sub>. PRO2000 also protected about 60% of these animals. Given the differences in the models, these results are consistent and demonstrate the preclinical efficacy of these gels. The fact that these results do not predict the outcome of clinical trials may be related to multiple reasons including definition of protection, cutoff for predictive value and issues related to consistent and proper use of the gels by humans.

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## Assessing Markers of Inflammation after Vaginal Product Use: Nonoxynol-9, Cellulose Sulfate, and HEC Placebo Comparative Double-Blind Phase I Trial

M. Saifuddin<sup>\*1</sup>, L. Tsai<sup>2</sup>, A. Gettie<sup>2</sup>, R. Bohm<sup>3</sup>, G. Doncel<sup>4</sup>, C. Cheng-Mayer<sup>2</sup>

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**BACKGROUND:** Cellulose sulfate (CS) did not prevent and may have increased the risk of HIV infection in one trial. The present study was done to investigate established and new vaginal Phase I safety markers in an effort to better understand Phase III results and determine which markers may be most useful in future studies.

**METHODS:** Sixty women were randomized to use nonoxynol-9 (N9), CS, or hydroxyethylcellulose placebo (HEC) vaginal gel twice daily for 2 weeks. Endpoints were assessed before use, after 1 week, and 8–18h and 58–66h after the last dose. Established endpoints included adverse events, colposcopic findings, microflora changes, and soluble markers in cervicovaginal lavage (CVL). Exploratory endpoints included cellular markers in CVL and biopsy, histopathology, and anti-HIV activity of CVL. Natural-log-transformed soluble marker data were modeled by individual clinical variables, gel, center, and visit using mixed models (visit as repeated measure). Due to baseline variability, change-from-baseline scores were calculated.

**RESULTS:** CS was associated with decreased MPO and IL-1RA levels compared with HEC, controlling for clinical variables, center, and visit. CD45+ and CD68+ cell counts in CVL pellets were lower with CS than HEC. Change scores for IL-8, MPO, and SLPI were lower for CS than HEC and change scores for *E.coli* and *Enterococcus* were higher. Anti-HIV activity of CS was maintained during dosing. N9 was associated with increased IL-1 $\alpha$ , IL-1 $\beta$ , IL-8, and MPO levels compared with HEC, controlling for most clinical variables, center, and visit. Change scores for IL-1 $\alpha$ , IL-1 $\beta$ , IL-1RA, IL-8, MPO, and anaerobic gram negative rods were higher for N9 than HEC, and SLPI was lower. Among the 2 women with inflammation seen by biopsy, levels of IL-1 $\alpha$ , IL-1 $\beta$ , IL-1RA, and MPO were 9–23 times higher than for women without, controlling for gel, center, and visit. Colposcopic epithelial disruption or findings >1cm were associated with 2–6 times higher levels of IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, and MPO and lower SLPI levels.

**CONCLUSIONS:** N9 was associated with an inflammatory pattern of results, but CS was not. CS exposed to the vaginal environment retained its anti-HIV activity. The importance of the microflora changes seen with CS is unclear. Certain markers obtained via CVL may indicate inflammation seen using biopsies which are more invasive. Epithelial disruption and large colposcopic findings also appear to be associated with increased inflammatory markers.

## SESSION 7

### Oral Abstracts (OA5): But Did They Like It?—An Update on Product Acceptability

**Moderators:** Ariane van der Straten, Alex Carballo-Dieguez

Sunday, May 23, 11:30 am–1:00 pm

Rooms 301–304

# 30

### The LINK between Gel Properties and User Perceptions: Implications for Rational Design of Microbicides

K. Morrow<sup>\*1</sup>, P. Kiser<sup>2</sup>, R. Rosen<sup>3</sup>, D. Katz<sup>4</sup>

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**BACKGROUND:** Vaginal gel acceptability is likely integral to consistent use, both in effectiveness trials and in post-market use. Data from effectiveness trials have noted inconsistent gel use. How a gel “behaves” in the vaginal environment derives from its rheological and physicochemical properties. Women’s ability to perceive product-specific behavioral differences, and the meanings women ascribe to those different sensations, may impact a candidate microbicide’s success. We propose that differences in rheological and other gel properties—and their relationships to each other—create specific gel “behavior” changes that are perceived and evaluated by users.

**METHODS:** Behavioral and biophysical studies evaluated 2 gels (HEC; Replens). 121 women rated gel characteristics and “behaviors” along dimensions of leakage, application, covert use and sexual pleasure (blinded, cross-over design). Gel rheological properties were measured, undiluted and diluted with vaginal fluid simulant, and input to biophysical computations of gel stresses and spreading rates—in applicators, on the hand/fingers, and vaginal surfaces.

**RESULTS:** Psychometric analyses identified 11 user rating scales that measured significant differences perceived between the 2 gels: on the hands and in the vagina; and during/after ambulation and simulated coitus. Biophysical analyses predicted these significant differences between gels per scale, in vitro and in vivo. Tradeoffs amongst gel properties are salient for these predictions: yield stress vs. (viscosity vs. shear rate), and changes in those properties due to dilution. Different gel properties dominated in different dimensions of women’s perceptions: e.g., yield stress acted to contain Replens in the applicator and the fornix, while HEC flowed more immediately out of the applicator and along the vaginal canal; the sense of lubrication was associated with greater viscosity at intermediate shear rates.

**CONCLUSIONS:** Initial results are promising and iterative studies are ongoing. An objective framework is being developed to understand and design vaginal gels that can be both behaviorally acceptable and biologically functional as drug delivery systems. Women’s perceptions of gels in vivo and imaging studies of gel vaginal distribution complement each other, both validating the biophysical analyses. Gel yield stress appears to be an important property, and results suggest that gel dilution was not significant during the brief exposure times of this study.

# 31

### Acceptability of 0.5% PRO 2000 Gel and BufferGel in the HPTN 035 Microbicide Trial

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**BACKGROUND:** Effectiveness of a vaginal microbicide will depend on consistent and correct use, a key component of which is acceptability to both the woman and her sexual partner(s). The purpose of this study was to assess acceptability data from women enrolled in a large multi-site microbicide trial.

**METHODS:** HPTN 035 was a Phase II/IIB randomized controlled trial of two candidate vaginal gels (PRO 2000 and BufferGel) compared to two controls (placebo and no gel); 3099 women were enrolled. Self-reported acceptability was assessed through interviewer-administered questionnaires at month 3 and study exit visits.

**RESULTS:** At month 3 and study exit, virtually all women responded that they would use the study gels if the gels were found to be effective in preventing HIV (99.0% in BufferGel arm, 98.6% in PRO 2000 arm, 99.0% in placebo gel arm, 98% in no gel arm). Characteristics such as protection against HIV/STIs, ease of use, no sexual interruption, and increase in sexual pleasure were the main qualities women found acceptable at both assessments. Characteristics that were challenging to women were remembering to use the product and messiness during sex. Few women reported the partner’s dislike of the gel or lack of disclosure of gel use to the partner. Among women in the three gel arms, increasing levels of high adherence were seen as women’s age increased. Married women were significantly more likely to be high gel users. Higher level of education for both participant and partner was significantly related to gel use. Women who reported using a male condom with the last act of vaginal sex prior to enrolment, and women reporting douching before and/or after sex, were significantly higher gel users compared to those women who did not report this.

**CONCLUSIONS:** The positive response to BufferGel and PRO 2000/5 Gel over the duration of the study bodes well for the acceptability and use of similar gel products in the future. The assessment of microbicide acceptability, including factors that lead to increased or decreased acceptability and correlation with adherence, throughout a clinical trial is important. Hence, better measures of acceptability are needed to distinguish those with high levels of acceptability from those with lower levels of accountability.

# ORAL SESSIONS

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## Microbicides Acceptability: The Influence of Social and Cultural Norms, Interpersonal Relations and Sexual Socialization

S. Abbott<sup>\*1</sup>, N. Morar<sup>1</sup>, S. Madiba<sup>2</sup>, L. Katzen<sup>3</sup>, J. Phillips<sup>1</sup>, M. Mokgatle-Nthabu<sup>2</sup>, B. Friedland<sup>3</sup>

<sup>1</sup>Medical Research Council, HIV Prevention Research Unit (HPRU); <sup>2</sup>University of Limpopo—Medunsa Campus; <sup>3</sup>Population Council, New York, NY, USA

**BACKGROUND:** Participants enrolled in clinical trials for vaginal microbicides are women of childbearing age. Trials endeavor to offer comprehensive services for women who seroconvert, including services for sexual and reproductive health. However, much is still unknown about the needs of women affected by the virus. This study explores the fertility intentions of newly HIV infected women, their experiences in public and private sector clinics, and their changing reproductive health needs.

**METHODS:** In-depth interviews (IDIs) were conducted with 39 women recently diagnosed with HIV. Respondents had been enrolled in one of two clinical studies in South Africa sponsored by the Population Council at the time they tested positive for HIV. This qualitative study was conducted at the University of Cape Town (UCT) Empilisweni Centre for Wellness Studies and the University of Limpopo/Medunsa campus (Medunsa), Setshaba Research Centre.

**RESULTS:** HIV diagnosis produced a major shift in sexual and reproductive desires, with the respondents almost unanimously reporting that they no longer wished for children. This can be attributed to five reoccurring themes: fear of orphaning their children, blame, guilt, concerns that their own health would be jeopardized by a pregnancy, and fear of community stigmatization. Less than half of the sample was aware of PMTCT programs. Difficulties negotiating safer sexual practices combined with depression and anxiety resulted in a loss of sexual desire. Respondents reported a number of challenges in accessing services, including long queues, insensitive practitioners, and a fear of loss of confidentiality. The presence of ongoing support group at the Medunsa site provided women with immediate care, which they credited with an easier acceptance and ability to disclose.

**CONCLUSIONS:** Meeting the unique reproductive and sexual needs and rights of newly diagnosed women requires effective referral systems to ensure continuity of care and support. In addition to HIV services, HIV+ women require counselling and information on effective contraceptive options, including emergency contraception and access to abortion, as well as PMTCT programs for those wishing to conceive. Ongoing care in HIV prevention trials must include family planning counseling and methods. Ultimately, a lack of continuity of care can lead to missed opportunities to provide health care and endangers the realization of sexual and reproductive rights.

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## Factors Affecting Acceptability and Adherence of a Candidate Microbicide Gel Among High-Risk Women in Africa and India

E. Greene<sup>\*1</sup>, G. Batona<sup>2</sup>, J. Hallad<sup>3</sup>, S. Johnson<sup>4</sup>, S. Neema<sup>5</sup>, E.E. Tolley<sup>1</sup>

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**BACKGROUND:** Inter-personal and contextual factors that shape microbicide adherence and use are critical issues to understand. Phase III effectiveness trials can provide valuable insights into the individual, interpersonal, social and environmental factors affecting microbicide acceptability and adherence. We examine qualitative, post-trial, in-depth interview data from a Phase III clinical trial of 6% Cellulose Sulfate microbicide gel in order to better understand factors that influence the relationship between microbicide acceptability and adherence.

**METHODS:** Eligible participants of a closed Phase III clinical trial of 6% Cellulose sulfate microbicide gel were selected non-randomly and agreed to participate in post-trial in-depth interviews about their experiences using gel as part of a separate Behavioral and Social Sciences study. Fifty-two high-risk women from four sites (Benin; Uganda; Chennai, India; and Bagalkot, India) participated in one interview each. NVivo8 was used to code and analyze the interview transcripts; excel matrices were developed to track, quantify, and compare themes and sub-themes. Analysis focused on 1) participants' acceptance of the study gel, and 2) their adherence to the study gel during the trial. An adaptation of the socio-ecological model provided an organizing framework.

**RESULTS:** While there were variations both between and within countries, most women found the gel to be highly acceptable for its lubricant qualities and/or perceived protective benefits, but adherence and consistent use were more dependent on contextual and partner-related factors. Women found adherence to be relatively easy with partners with whom there were no expectations of fidelity, in situations where private space was accessible, and at times when sexual intercourse was expected. Gel adherence was more difficult with primary partners than casual partners due to decreased perceptions of risk, inconvenience, or fear of partner disapproval. The gel's physical properties often prevented true covert use, particularly with regular partners, but many participants found ways of negotiating use that were not threatening to fidelity, trust, or power dynamics within relationships.

**CONCLUSIONS:** Microbicide gel acceptance does not necessarily coincide with adherence. Relationship dynamics and contextual factors play a critical role in the consistent and sustained use of microbicides, and ultimately, in the effectiveness of microbicides in preventing HIV.

## SESSION 8

### Mini-Symposia (MS2): For Whom and How?—Partnerships in Prevention

**Moderators:** Polly Harrison, Yasmin Halima

Sunday, May 23, 11:30 am–1:00 pm

Rooms 319–321

**34**

#### Developing Models of HIV Prevention Advocacy

Jim Pickett

*International Rectal Microbicide Advocates (IRMA), Chicago, USA*

We've been fighting back and fighting AIDS since the early days of "gay cancer." And we've worked hard, really hard, to ethically intervene in risky behaviors to stop onward HIV transmission. For a couple of decades, prevention advocacy has centered on clean needles, male and female condoms, VCT, behavior change, and more recently, biomedical interventions like MTCT and male circumcision. Organized advocacy around these biomedical interventions, as well as new strategies still in development (vaccines, microbicides, and PrEP), is absolutely critical if we are to have products that are safe, effective, acceptable, and accessible. Focused advocacy around NPTs is in its infancy compared to the long history of advocacy for treatment research and access. With the formerly stark lines between treatment and prevention becoming more and more blurry, a variety of organizations and networks have arisen to bridge the gap and create a smart, savvy, and strategic NPT advocacy movement. This talk will look at several groups doing this work.

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#### Creating Research Partnerships with the Community—A Site Perspective

Jorge Sanchez

*Asociacion Civil IMPACTA Salud y Educacion, Peru*

Investigators from the IMPACTA Peru Clinical Trials Unit (CTU) have been working in men who have sex with men (MSM) research since 1994, creating partnerships with community-based organizations and the Ministry of Health of Peru.

Since 1998, the Ministry of Health of Peru has provided the environment to improve visibility for MSM communities throughout gay friendly clinics and a system of peer educators. Several MSM community-base organizations (CBOs) have been funded by peer educators previously working at the Ministry of Health of Peru. These CBOs have representation at our Community Advisory Boards (CABs). This relationship model provides continuous feedback between the community and investigators enhancing community knowledge in research and foster participation in clinical trials.

Currently the Community Education & Involvement Resource Unit of the IMPACTA Peru CTU ensures community input to science generation and research implementation. Activities include: a) development and implementation of population-targeted community education plans including community mobilization; b) development of Recruitment and Retention Plans; c) Production of Information, Education and Communication (IEC) materials; d) maintenance and promotion of a STD/HIV/AIDS Prevention Clearinghouse; e) liaison with Lima and Iquitos' CABs; f) media relations and advocacy issues; and g) training of peer educators.

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#### Research Literacy for the Community and Community Literacy for Researchers

Tabita Mahlangeni

*Aurum Institute for Health Research, South Africa*

Research literacy empowers community members with all the necessary knowledge they need to make a truly informed decision whether or not to take part in a research study. Our community programme was first exposed to research issues specific to microbicide trials by undergoing intensive training on microbicides trials from the Global Campaign on Microbicides; and our Community Advisory Group took part in microbicides research literacy training offer by the site Community education manager, Tabita Mahlangeni. The microbicides research literacy education was first received with caution by school teachers, especially male teachers as they expressed concerns about safety of a gel on the penis and whether the gel would make them infertile or decrease their sexual performance. However, in the end, male teachers stated that they would support their partners if they were to be in the study. In contrast, women were more receptive to research information, but still had concerns on how their partners would respond to this information and felt could not guarantee that their partners would support them.

Continuous research literacy education is necessary before, during and after a research trial to facilitate participant recruitment and accrual.

# ORAL SESSIONS

## SESSION 9

### Oral Abstracts (OA6): Pharmacokinetics

**Moderators:** Craig Hendrix, Angela Kashuba

Sunday, May 23, 11:30 am–1:00 pm

Rooms 403–405

# 37

### Pre-treatment with Depo-Provera Modifies the Pharmacokinetics of CMPD167 in Rhesus Macaques Following Vaginal Ring Administration

K. Malcolm<sup>\*1</sup>, D. Lowry<sup>1</sup>, L. Green<sup>2</sup>, R. Shattock<sup>3</sup>, M. Mitchnick<sup>4</sup>, L. Geer<sup>4</sup>, P. Klasse<sup>5</sup>, J. Moore<sup>5</sup>, R. Veazey<sup>2</sup>

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<sup>4</sup>Particle Sciences Inc., Bethlehem, PA, USA; <sup>5</sup>Weill Medical College of New York, Cornell University, NY, USA

**BACKGROUND:** Vaginal ring devices are being developed to provide sustained release of HIV microbicides. To date, only limited pharmacokinetic data is available from animal or human studies. Here we report the effect of Depo-Provera (DP) pre-treatment, commonly used to thin the vaginal epithelium in challenge experiments, on the pharmacokinetic profile of CMPD167 (a small molecule CCR5 co-receptor antagonist) in rhesus macaques following vaginal ring administration.

**METHODS:** A single 400mg CMPD167 silicone elastomer vaginal ring was inserted into each of twelve female rhesus macaques. Six macaques were treated with (DP) 30 days before ring placement; the other six macaques were untreated. Blood, vaginal fluid and vaginal biopsies were collected prior to and at various times during 28 days of ring placement and assayed for CMPD167 levels by HPLC. Rings were assayed for residual CMPD167 at the end of the study and the calculated amount of CMPD167 released in vivo compared with in vitro release data.

**RESULTS:** Vaginal fluid, plasma and tissue levels of CMPD167 were detectable throughout ring placement. Significant differences were observed in mean daily vaginal fluid levels between the DP-treated (16–56 mcg/mL) and untreated groups (48–181 mcg/mL). Plasma CMPD167 levels were significantly higher peaking at 4 ng/mL and maintaining levels of 1–2 nM throughout the 14 days of testing in animals pre-treated with DP compared to non DP-treated macaques (<1 ng/mL maintained). Tissue levels were varied between 2–10 g/mL CMPD167 with no significant difference between the DP-treated and untreated macaques.

**CONCLUSIONS:** The study demonstrates that clinically relevant, and possibly protective doses of CMPD167 are released in the vaginal vault of rhesus macaques from vaginal rings through 28 days duration. DP is known to induce vaginal epithelial thinning and lower vaginal fluid levels, which accounts for the increased plasma levels of CMPD167. In contrast, macaques not treated with DP had minimal absorption into plasma compartments and significantly higher levels of CMPD167 in the vagina, similar to those previously shown to be protective against vaginal challenge.

# 38

### Temporal Association of Protection by Carraguard-based Gels Containing MIV-150 After Single Versus Repeated Vaginal Application in Macaques

M. Aravantinou<sup>\*1</sup>, J. Kenney<sup>1</sup>, R. Singer<sup>1</sup>, A. Gettie<sup>2</sup>, J. Lifson<sup>3</sup>, M. Piatak Jr.<sup>3</sup>, J. Fernandez-Romero<sup>1</sup>, T. Zydowsky<sup>1</sup>, J. Blanchard<sup>4</sup>, M. Robbiani<sup>1</sup>

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<sup>3</sup>AIDS and Cancer Virus Program, SAIC-Frederick, Inc., NCI Frederick, Frederick, MD, USA; <sup>4</sup>Tulane National Primate Research Center, Covington, LA, USA

**BACKGROUND:** In Phase 3 testing Carraguard (CG) was shown to be safe and acceptable for use in women, yet exhibited limited anti-HIV activity. Using CG as a delivery vehicle, we tested the novel NNRTI, MIV-150, for its ability to prevent vaginal infection with SHIV-RT (SIVmac239 and HIV-1 RT) in rhesus macaques. Initial studies revealed that CG and CG containing 500 M of MIV-150 (PC-817) exhibited comparable protection against vaginal challenge, due to the barrier effect of CG when given just prior to exposure. Herein we examined challenging at later time points after single versus repeated gel application.

**METHODS:** Animals were treated with Depo-Provera 5 weeks before challenge with 103 TCID50 SHIV-RT. PC-815 (50 M MIV-150 in CG), PC-817 (500 M MIV-150 in CG), and methyl cellulose (MC) placebo were tested. Single gel applications (3ml) were administered 4 or 24h pre-challenge. Alternatively, animals received 2ml of gel per day for 2 weeks before being challenged 8 or 24h after the last gel was applied. We measured virus DNA in PBMCs, plasma virus RNA, serology, and IFN-γ ELISPOT.

**RESULTS:** Nine of 16 animals (56.3%) that received a single dose of MC became infected, whereas 1 of 7 (14.3%, p=0.09 compared to MC) and 3 of 6 (50%) became infected after a single application of PC-817 when given 4 and 24h pre-challenge, respectively. 85.7% (12 of 14) of animals became infected after repeated MC application, while 71.4% (5 of 7 animals) and 57.1% (4 of 7 animals) became infected after repeated CG application followed by challenge 8 and 24h later. We then compared repeated application of PC-815 and PC-817, challenging 8h after the last gel. Two of 7 PC-815-treated animals (28.6%, p=0.017 when compared to MC) and 3 of 7 PC-817-treated animals (42.9%, p=0.12 when compared to MC) became infected. Further analysis of repeated PC-815 administration, revealed that the partial protective effect was lost when the animals were challenged 24h after the last gel (5 of 7 infected, 71.4%). T and B cell responses were typically observed in infected animals.

**CONCLUSIONS:** Repeated gel dosing led to diminished but not complete protection from SHIV-RT challenge compared to single doses. Efficacy was greatest when the animals were challenged within 8h of the last gel administration.

# ORAL SESSIONS

SESSION 9  
Sunday, May 23, 2010

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## Protection by TFV Gel Against Vaginal SHIV Infection in Macaques Three Days after Gel Application and Its Relationship to Tissue Drug Levels

C.W. Dobard<sup>\*1</sup>, S. Sharma<sup>1</sup>, A. Holder<sup>1</sup>, Z. Kuklenyik<sup>1</sup>, D.L. Hanson<sup>1</sup>, J. Smith<sup>1</sup>, R. Otten<sup>1</sup>, F.J. Novembre<sup>2</sup>, G. Garcia-Lerma<sup>1</sup>, W. Heneine<sup>1</sup>

<sup>1</sup>Centers for Disease Control and Prevention, Atlanta, GA, USA; <sup>2</sup>Yerkes Primate Center/Emory University, Atlanta, GA, USA

**BACKGROUND:** HIV continues to spread, primarily through heterosexual sex. Topical gels containing antiretroviral drugs are currently under evaluation as a prevention strategy against HIV acquisition. Previous work has focused on entry and reverse transcriptase inhibitors. Here we assess an integrase inhibitor representing a new class of drugs for topical prophylaxis, using a repeat-challenge macaque model that resembles human vaginal transmission.

**METHODS:** We formulated the integrase inhibitor L-870812 into a stable 0.2% gel with hydroxyethyl cellulose. We performed 14 intravaginal SHIVSF162p3 challenges twice-weekly with a low-dose (10-TCID50) inoculum. Female pig-tailed macaques were assigned to a placebo gel (n=1) or the integrase inhibitor gel (n=3). Three mLs of gel were applied vaginally 30 min before challenge. Infection was monitored by serology and PCR of SHIV sequences in plasma. Drug absorption was determined 30 min after gel application by measuring plasma drug levels using liquid chromatography-mass spectrometry. Drug resistance was monitored by sequencing the integrase region from plasma viral RNA.

**RESULTS:** The realtime placebo macaque was infected after 3 challenges, consistent with historical controls (median 4 challenges, range: 2-11). In contrast, 2/3 macaques receiving L-870812 gel remained uninfected after 14 challenges, demonstrating that L-870812 provided significant protection ( $p<0.05$ ; Fisher's exact test). Low levels of L-870812 (median=15.5 ng/ml; range: 6-140) were detected in plasma suggesting rapid absorption. The single breakthrough infection showed a wild-type virus genotype and no evidence of drug resistance emergence despite continued twice-weekly gel use for 15 weeks post infection. Plasma viremia in this animal was similar to controls.

**CONCLUSIONS:** Topically applied gel with an integrase inhibitor protects from repeated vaginal challenges. Systemic absorption of L-870812 did not impact viremia or select for drug resistance. These data identify a novel strategy for topical prophylaxis with integrase inhibitors and support further evaluation of this class of drugs.

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## The Pharmacokinetics of Tenofovir Following Intravaginal and Intrarectal Administration of Tenofovir Gel to Rhesus Macaques in a Microbicide Trial

J. Nuttall<sup>\*1</sup>, A. Kashuba<sup>2</sup>, R. Wang<sup>2</sup>, N. White<sup>2</sup>, P. Allen<sup>3</sup>, J. Roberts<sup>3</sup>, J. Romano<sup>1</sup>

<sup>1</sup>International Partnership for Microbicides, Silver Spring, MD, USA; <sup>2</sup>University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; <sup>3</sup>Valley Biosystems, West Sacramento, CA, USA

**BACKGROUND:** Tenofovir gel is being developed as a vaginal and rectal microbicide for the prevention of HIV infection. This study was conducted to determine the pharmacokinetics of tenofovir in macaques following intravaginal and intrarectal administration.

**METHODS:** Groups of 6 Rhesus macaques were pretreated with depomedroxyprogesterone acetate (30 mg i.m.) 30 days prior to tenofovir administration to synchronize menstrual cycles and thin the vaginal mucosa. Gel containing 0.2, 1 or 5% tenofovir was administered intravaginally at a dose volume of 0.6 mL/kg. Plasma and vaginal and rectal fluid samples were collected pre-dose and at 0.25, 1, 4, 8 and 24h after dosing for analysis of tenofovir concentrations by validated LC-UV or LC-MS/MS methods. At 24 hours, biopsies from the vaginal wall, cervix and rectum were collected for analysis of tenofovir and tenofovir diphosphate concentrations. After a 3-week washout period, the same gel doses were applied intrarectally to the same animals, with samples collected as outlined above. Pharmacokinetic parameters were generated using noncompartmental analysis (WinNonlin 5.1).

**RESULTS:** Following vaginal and rectal administration, tenofovir was detectable by 0.25h in all matrices distal to where the dose was administered. At least 5/6 macaques in the 1% and 5% groups had detectable tenofovir concentrations in all matrices 24 h after dosing. At all doses, concentrations at the dosing site were typically 1-2 logs higher than in the opposite compartment, and 4-5 logs higher than in the plasma. Cmax and AUC0-24 values in vaginal fluid after vaginal dosing were 58-82% lower than in rectal fluid after rectal dosing, but plasma Cmax and AUC0-24 values were 1-2 fold greater after vaginal dosing than after rectal dosing. In all matrices, tenofovir exposure increased with increasing dose. Concentrations of tenofovir diphosphate were unquantifiable in all tissue samples, but this may have been due, at least in part, to technical constraints associated with the samples and assays used.

**CONCLUSIONS:** The intravaginal administration of tenofovir gel to macaques resulted in rapid distribution of the drug to the rectum. Similarly, intrarectal administration of tenofovir resulted in rapid distribution to the vagina. Based on these data, it is recommended that evaluation of tenofovir gel as an HIV prevention technology should include pharmacokinetic studies using a variety of dosing strategies in humans.

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## Pilot Clinical Pharmacokinetic and Pharmacodynamic Study of UC781 Vaginal Gel

J. Schwartz<sup>\*1</sup>, G. Doncel<sup>2</sup>, S. Asin<sup>3</sup>, A. Thurman<sup>2</sup>, T. Kimble<sup>2</sup>, N. Chandra<sup>2</sup>, C. Rollenhagen<sup>3</sup>, J. Cooper<sup>2</sup>, S. Ju<sup>1</sup>, D. Archer<sup>1</sup>

<sup>1</sup>CONRAD, Arlington, VA, USA; <sup>2</sup>CONRAD, Norfolk, VA, USA; <sup>3</sup>Dartmouth Medical School, Lebanon, NH

**BACKGROUND:** *In vivo* application of microbicides followed by tissue sampling and *ex vivo* HIV challenge of the explants is a promising way to assess pharmacodynamics (PD). Several technical issues, however, remain to be resolved, e.g., site and size of biopsy and method of transport (frozen vs. medium). In addition, tissue sampling to assess drug concentrations in the female genital tract is critical to the clinical evaluation of antiretroviral candidate microbicides but method development is challenging and requires sensitive assays. This pilot study was designed to address some of these technical issues. A pharmacokinetic (PK), PD safety study of UC781gel in women is planned.

**METHODS:** This was a prospective study in which 15 women were enrolled in three groups of five to undergo three genital tract biopsies (cervical and/or vaginal) at the baseline visit and again four hours after intravaginal application of 0.1% or 0.25% UC781 (3.5 mL) at the treatment visit. Genital tract tissue was analyzed for UC781 levels and for HIV infectivity following *ex vivo* exposure of tissue explants to HIV-1<sub>BaL</sub>. Explant infection was measured by *in vitro* supernatant p24 levels and UC781 tissue levels were measured by LC/MS/MS. The results of each group of 5 were used to inform the procedures in the following group.

**RESULTS:** All baseline biopsies (40) showed productive HIV infection 7 days after an *ex vivo* explant challenge. Replication in cervical and vaginal tissues was not statistically different, with p24 medians normalized per tissue weight of 187 and 137 pg/mg, respectively. There appeared to be no significant differences between frozen tissue and tissue transported in medium or half versus whole biopsies (n=5). Median UC781 concentrations were 633 ng/g in vaginal biopsies (n=14) and 395 ng/g in cervical biopsies (n=15), and were higher with exposure to 0.25% compared to 0.1% UC781. Data on infectivity of UC781-exposed tissues are being collected.

**CONCLUSIONS:** These pilot data showed that it is feasible to freeze cervical and vaginal biopsies from human subjects prior to *ex vivo* HIV challenge and that viral replication results are similar with both transport methods. A recently validated assay for vaginal and cervical tissue concentrations of UC781 showed tissue levels orders of magnitude higher than its *in vitro* effective concentration, following a single dose of UC781 gel. The established methodology will be used in the upcoming expanded safety study of UC781 gel.

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**SPL7013 Gel (VivaGel®) Retains Potent Antiviral Activity Against HIV-1 and HSV-2 Following Vaginal Administration in Humans**

C.F. Price<sup>\*1</sup>, C. Sonza<sup>2</sup>, D. Tyssen<sup>2</sup>, J.R.A. Paull<sup>1</sup>, T.R. Moench<sup>3</sup>, A. Humberstone<sup>4</sup>, S. Evans<sup>1</sup>, A. Davie<sup>1</sup>, P. Hodzman<sup>4</sup>, G.R. Lewis<sup>1</sup>, S. Xia<sup>1</sup>, T. Spelman<sup>2</sup>, G. Tachedjian<sup>2</sup>

<sup>1</sup>Starpharma Pty Ltd, Melbourne, Australia; <sup>2</sup>Burnet Institute, Melbourne, Australia; <sup>3</sup>ReProtect, Inc., Baltimore, MD, USA; <sup>4</sup>Nucleus Network, Melbourne, Australia

# ORAL SESSIONS

**BACKGROUND:** SPL7013 Gel (VivaGel) is a microbicide in development for prevention of HIV and HSV. This open-label, 5-period crossover study assessed retention and duration of antiviral activity following vaginal administration of 3.5g of 3% SPL7013 Gel in 12 healthy women.

**METHODS:** Participants received 5 single doses of product with ≥5 days between doses. A cervicovaginal fluid sample (CVS) was collected using a SoftCup™ prior to dosing, then at one of the following times after each dose: immediately (2-10min), 1, 3, 12 or 24h. The sequence of times was randomly assigned. CVS was recovered from the SoftCup in 20mL saline. Anti-HIV-1 and -HSV-2 activities of CVS diluted 1:2 and 1:50 respectively, were determined in cell culture assays (data not corrected for dilution). Antiviral activity in the presence of seminal plasma (SP) was also tested. Mass and concentration of SPL7013 in CVS was determined and corrected for dilution. Safety was assessed by reporting of adverse events. Statistical analysis was performed using the Wilcoxon rank-sum test with Bonferroni adjustment; p≤0.003 was significant.

**RESULTS:** Eleven participants completed the study. Median (interquartile range) inhibition of HIV-1 and HSV-2 by pre-dose CVS was 1.9% (0,5.6) and 0.6% (0,1.5) respectively. CVS obtained immediately after dosing almost completely inhibited HIV [96% (95,97)] and HSV [86% (85,94)], and activity was maintained in all women at 3h (HIV [96% (95,98) p=0.9]; HSV [94% (91,97) p=0.004]). At 24h, >90% of initial HIV and HSV-2 inhibition was maintained in 6/11 women. A mean of 48mg SPL7013 was recovered in CVS obtained at baseline (46% of 105mg dose). At 3 and 24h, 22mg and 4mg SPL7013 respectively, was recovered. Over 90% and 80% inhibition for HIV and HSV respectively, was observed if there was >10mg SPL7013 in CVS, and >70% if there was >0.5mg. High levels of antiviral activity were retained in the presence of SP. VivaGel was safe and well tolerated with no signs or symptoms of vaginal, vulval or cervical genital irritation reported.

**CONCLUSIONS:** We demonstrated potent antiviral activity against HIV-1 and HSV-2 immediately following vaginal administration of VivaGel, with activity maintained for at least 3h post-dose. Antiviral activity was retained in the presence of SP, and >0.5% of the original SPL7013 dose gave high levels of antiviral activity. The data provide evidence of antiviral activity in a clinical setting, and suggest VivaGel could be administered up to 3h before coitus.

# POSTER SESSIONS

SESSION 10  
Sunday, May 23, 2010

## SESSION 10

### Poster Discussion (PD1): Rectal Microbicides—From Development to Implementation

Moderators: Victor Garcia-Martinez, Alex Carballo-Dieguex

Sunday, May 23, 3:00 pm–4:00 pm

Ballroom

**159**

#### Using Drug Combinations to Design Effective Colorectal Microbicides: Where is the Limit?

C. Herrera<sup>\*1</sup>, M. Cranage<sup>1</sup>, I. McGowan<sup>1</sup>, P. Anton<sup>3</sup>, R. Shattock<sup>1</sup>

<sup>1</sup>Division of Cellular & Molecular Medicine, St George's University of London, UK; <sup>2</sup>Magee-Women Research Institute, University of Pittsburgh Medical School, USA; <sup>3</sup>Center for HIV and Digestive Disease, David Geffen School of Medicine, UCLA, USA

**BACKGROUND:** Receptive anal intercourse is associated with the highest probability for sexual HIV infection. The aim of the study was to assess the importance of reverse transcriptase inhibitor (RTI) combinations in the design of colorectal microbicides and the number of drugs required to obtain maximum efficacy against wild type isolates and RTI-escape mutants.

**METHODS:** The antiviral efficacy of two nucleos(t)ides reverse transcriptase inhibitors (NRTI) PMPA and FTC, and two non-NRTIs (NNRTI) UC-781 and TMC120, used in double, triple and quadruple combinations, was assessed in colorectal explants. Pre-incubation with the drugs individually or in combination, for one hour was followed by addition of virus. Infection was determined by measurement of virion protein (p24 antigen) in colorectal explants supernatants.

**RESULTS:** All combinations inhibited the isolates tested in colorectal explants, and produced, for at least one of the compounds, a change in the dose response curve. Double and triple combinations incrementally augmented activity, even against RTI escape mutants, whereas quadruple combinations conferred little further advantage. The colorectal explant model is key to identification of the best candidate molecules and their combinations at the preclinical stage.

**CONCLUSIONS:** Triple combinations based on RTIs have potential as colorectal microbicides to prevent the transmission of wild type and resistant isolates.

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#### PC-815 Blocks Rectal Transmission of SHIV-RT When Applied 4 Hours Prior to Virus Challenge

R. Singer<sup>\*1</sup>, J. Kenney<sup>1</sup>, M. Aravantinou<sup>1</sup>, A. Gettie<sup>2</sup>, J. Lifson<sup>3</sup>, M. Piatak Jr.<sup>3</sup>, J. Fernandez-Romero<sup>1</sup>, T. Zydowsky<sup>1</sup>, J. Blanchard<sup>4</sup>, M. Robbiani<sup>1</sup>

<sup>1</sup>Population Council, New York, NY, USA; <sup>2</sup>Aaron Diamond AIDS Research Center, New York, NY, USA; <sup>3</sup>AIDS and Cancer Virus Program, SAIC-Frederick, Inc., NCI Frederick, Frederick, MD, USA; <sup>4</sup>Tulane National Primate Research Center, Covington, LA, USA

**BACKGROUND:** Testing anti-HIV microbicides in rectal transmission models is important due to the higher efficiency of rectal relative to vaginal transmission. Earlier research revealed that Carraguard (CG) is safe when applied rectally, unlike commonly used lubricants. In addition, CG has been shown to be safe after repeated usage in women, although the anti-HIV effects were limited. We evaluated the rectal efficacy of a topical microbicide, PC-815, comprising CG to deliver an NNRTI, MIV-150, in rhesus macaques. PC-815 has shown partial efficacy against vaginal transmission in rhesus macaques. To evaluate the durability of protection, we challenged either 0.5 or 4h after gel application.

**METHODS:** Rhesus macaques were treated rectally with 5ml of PC-815 (n=8) or methyl cellulose (MC) placebo (n=4) gel either 0.5 or 4h prior to rectal challenge with 1000 TCID<sub>50</sub> of SHIV-RT (SIVmac239 with HIV RT). Infection was assessed by testing for plasma virus RNA, PBMC virus DNA, IFN-γ ELISPOT, and seroconversion. Statistical significance was determined with Fisher's exact test.

**RESULTS:** All PC-815-treated animals (4 of 4 in each group) were protected from rectal SHIV-RT infection when gel was applied either 0.5 or 4h prior to rectal challenge, while 100% of the control MC-treated animals (4 of 4) became infected. Thus, PC-815 afforded statistically significant protection against rectal challenge, even when administered 4h prior to exposure ( $p=0.029$ ). This study utilized animals recycled from other studies where they had been previously exposed to SHIV-RT. While the animals were evenly distributed between the test and control groups, we examined whether the uninfected animals might be inherently resistant to SHIV-RT infection. Activated PBMCs from the uninfected animals were all susceptible to SHIV-RT infection *in vitro* (additional controls of PBMCs from animals that resisted both *in vivo* and *in vitro* infection were included). Analysis of PBMCs confirmed that the uninfected animals were also negative for SIV DNA. All infected animals had normal viremia and developed SIV-specific T and B cell responses.

**CONCLUSIONS:** PC-815 blocked rectal SHIV-RT infection when applied up to 4h prior to virus challenge ( $p<0.03$ ). These data are promising for the development of NNRTI-containing gels for the prevention of rectal transmission. Future studies are needed to determine how long this protective effect lasts and to ensure that repeated application of the gels remains safe.

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#### In Vivo Detection of Microbicide-Induced Damage in Small Animal Rectal Epithelium Using Confocal Microendoscopy

G. Vargas<sup>\*1,2</sup>, J.K. Rebecca<sup>2</sup>, K. Vincent<sup>3</sup>, M. Motamedi<sup>1,4</sup>, N. Bourne<sup>3</sup>

<sup>1</sup>Center for Biomedical Engineering; <sup>2</sup>Department of Neuroscience and Cell Biology; <sup>3</sup>Department of Pediatrics; <sup>4</sup>Department of Ophthalmology The University of Texas Medical Branch

**BACKGROUND:** Microbicide safety has primarily been evaluated in the vagina but use of microbicides rectally necessitates effective preclinical methods to detect toxic effects of candidate agents on rectal epithelium. Real-time noninvasive imaging for assessment of epithelial disruption could provide unique information regarding microbicide effects. In this feasibility study, confocal microendoscopy was evaluated for *in vivo* visualization of the mouse rectal epithelium treated with either PBS or benzalkonium chloride (BZK) to determine if acute (20 minutes) injury could be detected.

**METHODS:** Ten mice were given 50μl of 0.2% BZK or PBS rectally for 20 minutes. The rectal epithelium was then labeled with the nuclear staining fluorescent dye acriflavine orange (0.05%) for 5 minutes and rinsed. *In vivo* imaging was performed using the Cell-Vizio confocal microendoscope having a 1.5mm diameter imaging probe. Twelve images per mouse were obtained then mice were sacrificed and rectal tissue removed for histological processing. Images were evaluated for features associated with PBS or BZK treatment including surface topography, presence of surface debris, and presence and shape of crypts. Using criteria established in a five mouse training subset prior to this study, images from the mice treated with 0.2% BZK or PBS were classified (blinded assessment) and a determination of treatment made.

**RESULTS:** PBS controls had smooth surface topography with one of two appearances: 1) areas in which crypts appeared as dark circular regions with smooth edges and continuous transition between crypts and 2) highly uniform fields where crypts were not evident but the surface appeared smooth. The most prominent feature associated with BZK treatment was presence of crypts retaining a generally circular shape but with highly rugated edges and distinct gaps between crypts. Surface topography was nonuniform and textured. Surface debris was present in both groups. Histology revealed loss of surface columnar cells in BZK animals and areas of disrupted superficial crypt structure. Visual classification resulted in the correct identification of 4/5 BZK and 4/5 PBS cases.

**CONCLUSION:** These preliminary *in vivo* results are highly promising and indicate that confocal microendoscopy may provide a sensitive surface assessment of rectal epithelial integrity in small animal models for evaluating microbicide effects.

# POSTER SESSIONS

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### Twenty-Eight Day Repeat Dose Toxicology Studies of UC781 Vaginal Gel in Sprague-Dawley Rats and New Zealand White Rabbits Following Rectal Administration

T.J. McCormick<sup>\*1</sup>, P. Anton<sup>2</sup>, R. O'Meara<sup>3</sup>, S. Murphy<sup>3</sup>

<sup>1</sup>CONRAD, West Chester, PA, USA; <sup>2</sup>David Geffen School of Medicine, University of California, Los Angeles, USA; <sup>3</sup>Pacific Biolabs, Hercules, CA, USA

**BACKGROUND:** The objective of these studies was to determine the potential toxicity of UC781 vaginal gel following single rectal administration daily for 28 days to male and female rats and rabbits. These animal studies were used to support the first Phase I rectal safety study of the vaginal formulation of UC781 microbicide gel (supported by DAIDS IPCP #AI060614).

**METHODS:** Rats (5 animals/sex/group) and rabbits (4 animals/sex/group) were assigned to six treatment groups and received a single daily rectally administered dose (0.1 mL for rats and 1.0 mL for rabbits) of test article, placebo, or control for 28 days. The six treatment groups included: three test article groups of UC781 vaginal gel (0.25%, 0.75%, and 2.5% w/w), one vehicle placebo gel group, and a sham control group. Cageside observations were performed daily and body weights were recorded weekly. Blood samples for assessment of clinical pathology parameters were collected just prior to necropsy on Day 29. Blood for pharmacokinetic analysis was collected at 0 (pre-dose), 1, 2, 4, 8, and 24 hours on Days 1 and 28. On Day 29, animals were euthanized and gross necropsy was performed. Selected organs were weighed and absolute and relative weights were recorded. Also, specified tissues (rectum, anus, and descending colon) were collected for histological evaluation.

**RESULTS:** No mortalities were reported during the course of these studies. No test-article related findings were observed in clinical observations, body weights, food consumption, or clinical pathology following 28 days of rectal administration of UC781 vaginal gel. Also, no significant findings were noted upon necropsy or histological evaluation of specified tissues. Analysis of the Day 1 and 28 rabbit plasma samples and the Day 1 rat plasma samples indicated that UC781 was either not detected or was below the lower limit of quantitation (LLOQ) of 25 ng/mL. For the Day 28 rat plasma samples UC781 was detected in the plasma of nine rats at concentrations ranging from 26–89 ng/mL. However, these findings appear to be toxicologically irrelevant and no clear dose response was observed.

**Conclusions:** Based upon the results of these studies, the apparent no-observable-adverse-effect-level (NOAEL) of UC781 vaginal gel is 2.5% (w/w) in Sprague-Dawley rats and New Zealand White rabbits treated once daily via the rectal route of administration for 28 days.

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### Sexual Practices of MSM in Nigeria and Interest in Microbicides

I. Nnaji\*, M. Ukpong, O. Ezech, T. Owolabi

AIDS CONTROL MoH Enugu, Nigeria HIV/Microbicides Advocacy Society, Nigeria Institute for Medical Research, Lifeline Plus Foundation

**BACKGROUND:** To assess the sexual practices of MSM and their acceptability of rectal microbicide

**METHODS:** Questionnaire based cross-sectional study among persons with same sex practice in Eastern Nigeria on sexual behaviour, knowledge and attitude on microbicides. The data on MSM was extracted from the entire data for reporting. Data with STATA version 10.0

**RESULTS:** A questionnaire was administered to 498 persons of same practice in Ebonyi and Enugu states of Nigeria. There were 293 (58.8%) female and 205 male (41.2%) respondents. Respondents age ranged from 16–43 years with mean of 25.4 years. 359(72.7%), were unmarried, 110 (22.3%) were married and 25 (5.0%) are planning to get married. Only 55 (27.4%) of the men respondents were married. Four were planning to get married.

Of the 205 male respondent, 107 (52.2%) had had sex before while 98 (47.8%) had not. 101 (49.3%) had engaged in vaginal sex and 51 (24.9%) in anal sex with a woman. 69 (33.7%) males were bisexual, 180 (87.8%) had more than one sexual partner. 80 (41.9%) respondents had a stable female relationship out of which 33 (41.3%) had shared information with their female partners.

Only 4 (0.8%) of respondent use lubricant. Other use lubrications in the form of saliva (27), baby oil and vaseline (187). Four use cotrimozazole cream. Lubricants are often use so as to make penetration easy and reduce pain.

390 (83.7%) persons had learnt of microbicide mostly through health seminars. 250 (73.5%) are willing to use a microbicide when developed. Respondents are willing to insert the microbicide into the anus, vagina and apply it around the penis. 64 would apply to both vagina and anus. Most respondent noted they will want the microbicide to protect them from HIV infection and STIs.

**CONCLUSIONS:** The development of a microbicide for prevention of sexual transmission of HIV infection is of equally high importance to men in Africa. Microbicide development should not be marketed as a gender specific commodity.

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### Preclinical Evaluation of Aptamers as Candidate Rectal Microbicides

L. Mince<sup>\*1,2</sup>, M. Moore<sup>3</sup>, B. Sproat<sup>4</sup>, W. James<sup>3</sup>, I. McGowan<sup>2</sup>

<sup>1</sup>University of Pittsburgh Medical Center and <sup>2</sup>Magee-Womens Research Institute, Pittsburgh, PA, US; <sup>3</sup>Sir William Dunn School of Pathology, University of Oxford, Oxford, UK; <sup>4</sup>Chemconsilium GCV, Boischt, Belgium

**BACKGROUND:** An aptamer is a short RNA nucleotide sequence specific to a target, in this case the HIV gp-120 glycoprotein, for which activity has been seen in a PBMC model. We present our evaluation for efficacy and toxicity of aptamers.

**METHODS:** 3 aptamers at 100nM and 500nM concentrations were evaluated against HIV-1 BaL ( $10^5$  infectious units/mL). Nonoxynol-9 (N-9), tenofovir, TAK-799 and MAbs-12 were used as controls. Most experiments were conducted in triplicate. In the colorectal explant, biopsies obtained by sigmoidoscopy had a 2 to 4-hour exposure to virus and drugs, and were maintained on gel rafts. Supernatant was collected at 4 or 16 hr, 3, 7, 10 and 14 days, and evaluated for p24 by ELISA. TZM-bl cells were incubated at a density of  $10^4$  cells per well, with infection and toxicity quantified by Tat transactivated luciferase gene luminescence and ATP production, respectively. In the transwell model, CaCo2 cells ( $3 \times 10^5$  cells/mL) grown to confluence as measured following the transepithelial electrical gradient (TEER) to a plateau were exposed to aptamers for 18 hr. TEER was measured for up to 7 days afterwards. Additionally, we evaluated the ability of HIV to freely cross the CaCo2 and infect PM-1 cells in the lower chamber (by p24 quantification) at one and 4 days after exposure to aptamers. Fluorescence microscopy was performed to evaluate for integrity by staining for zonula occludens (ZO-1).

**RESULTS:** The explant model showed variable results with a moderate, yet significant ( $p \leq 0.05$ ), mean reduction of infection with aptamers at 100nM concentration at day 14. A significant dose dependent inhibition of infection was noted in the TZM-bl model (up to 90% reduction with 500nM). No toxicity was seen with any aptamer at 100 or 500nM. The TEER remained stable. The architecture was preserved in fluorescence microscopy, and no increased viral translocation was noted in the transwell model. No decrease in ATP production was seen.

**CONCLUSIONS:** Aptamers show moderate efficacy in the TZM-bl and colorectal explant models, although a wide range of activity was noted in the last platform. No toxicity was seen. Additional experiments need to be conducted at higher doses and with newer aptamers. Experiments to evaluate for aptamer-induced inflammation and apoptosis are needed as well.

# POSTER SESSIONS

SESSION 11  
Sunday, May 23, 2010

## SESSION 11

### Poster Discussion (PD2): Thinking Outside the Box—Novel Drugs/Approaches for HIV Prevention

**Moderators:** Lisa Rohan, Gustavo Doncel

Sunday, May 23, 3:00pm–4:00pm

Rooms 301–304

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#### Cationic Cell-Penetrating Peptides Inhibit HIV-1 Infection

S. Keegan\*, S. Passic, B. Wigdahl, F. Krebs  
*Drexel University College of Medicine, Philadelphia, PA*

**BACKGROUND:** In the absence of an effective microbicide that reduces or eliminates the risk of human immunodeficiency type 1 (HIV-1) transmission, the development of new anti-HIV-1 drugs remains a priority. Our efforts in this area suggest that molecules belonging to the family of cell-penetrating peptides (CPP) may inhibit HIV-1 entry. CPPs are short peptides able to cross the plasma membrane, a formidable barrier to many molecules. Because of this ability, CPPs are being studied as delivery vehicles for therapeutic agents that cannot enter the cell. A 10-amino acid (aa) peptide derived from HIV-1 Tat protein has been well studied as a drug delivery agent. Our studies explore the activity of Tat peptide and other CPPs against HIV-1 infection.

**METHODS:** Peptides used in these studies included: (i) Tat peptide, (ii) R-9 (nona-arginine), and (iii) RG-20 (arginine-rich, 20 aa peptide). P4-R5 MAGI reporter cells were infected with cell-free HIV-1 IIIB (X4) or HIV-1 BaL (R5) in the absence or presence of half-log, serially diluted concentrations of peptide for 2 h. Cells were washed twice, harvested 48 h later and assayed for HIV-1 infection. Peptide cytotoxicity was assessed by MTT assay following 2 h exposure to P4-R5 cells.

**RESULTS:** Tat peptide caused a concentration-dependent inhibition of HIV-1 IIIB infection, but was ineffective against HIV-1 BaL. In contrast, R-9, which differs from Tat by a small number of aa residues, was shown to have activity against HIV-1 IIIB and, surprisingly, concentration-dependent activity against BaL. RG-20 was able to inhibit HIV-1 IIIB and BaL infection comparable to R-9. None of the peptides were cytotoxic at the concentrations used.

**CONCLUSIONS:** Differences in amino acid sequence between the Tat peptide and R-9 peptide contribute to co-receptor-dependent anti-HIV-1 activity. These results suggest dissimilar mechanisms of action against viruses using CXCR4 or CCR5 co-receptors. Additionally, increased peptide length does not appear to be a critical determinant of antiviral activity, since R-9 and RG-20 were similarly active. Using Tat peptide and R-9 as starting points, further studies will identify sequence determinants for CPP biological activity and mechanisms of antiviral activity, and explore the potential of these peptides as novel HIV-1 inhibitors.

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#### Development of Flavonoids for Use in a Combination Microbicide

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**BACKGROUND:** Developing a combination microbicide that inactivates multiple sexual pathogens is desirable since genital infections are often polymicrobial and synergistic. The development of a combination microbicide directed against HIV and HSV could reduce HIV transmission both by directly inactivating HIV and by disrupting the biological synergy link between HSV infection and HIV transmission. Flavonoids derived from green (catechins) and black (theaflavins) tea, are on the Food and Drug Administration's GRAS (generally recognized as safe) list of compounds approved for human consumption. The objective of this study was to measure their virucidal activity against HSV and HIV.

**METHODS:** The antiviral activity of catechins from green tea and theaflavins from black tea was measured against panels of HIV-1 and HSV-2 and HIV. Electron microscopy and confocal microscopy were used to determine the effects of flavonoids on HSV and Vero cell morphology. Aggregation of HSV-1 glycoproteins was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

**RESULTS:** Oxidative dimerization of the tea flavonoid (-)-epigallocatechin gallate (EGCG) produces compounds with increased antiviral activity against HSV-1 and HSV-2 at acidic, neutral and alkaline pH when compared to monomeric EGCG, which is only antiviral at neutral and alkaline pH. EGCG inactivated HSV-1 and HSV-2 at pH 8.0 by 1,000–10,000 fold but was ineffective at pH 5.7. However, the EGCG digallate dimers theasinensin A, P2 and theaflavin-3,3'-digallate inactivated both viruses by 1,000–10,000 fold at pH 5.7 and as much as 100,000-fold at pH 8.0. EGCG did not inactivate vesicular stomatitis virus (VSV) in the pH range of 5.7–7.4 and by ≤ 100-fold at pH 8.0 while the digallate dimers theasinensin A and P2 reduced VSV titers by ≥ 100,000-fold from pH 5.7–8.0. Examination of purified HSV-1 virions and Vero cells utilizing electron and confocal microscopy showed that dimerized EGCG (100 M) destroyed HSV-1 virions but did not damage Vero cells. EGCG dimers inactivated cell surface HSV-1 virions as effectively as cell free virus and produced aggregation of HSV-1 glycoprotein B but not glycoprotein D within 1 hour. EGCG dimers decreased the titer of HIV virions to the same or greater degree as the monomer at neutral pH.

**CONCLUSIONS:** EGCG dimers have equivalent or greater anti-HSV activity at pH 7.4–8.0 than the EGCG monomer but have much greater antiviral activity at acidic pHs which is relevant for the vaginal environment. EGCG dimers, both relative to the degree of HSV inactivation and the broad range of effective pH, appear to have excellent potential to be utilized in a topical microbicide in conjunction with an NNRTI such as UC781 in the pH range found vaginally.

# POSTER SESSIONS

## 136

### Design and Synthesis of Variants of the HIV-1 Aptamer, UCLA1 That Binds to gp-120

B. Sproat<sup>1</sup>, W. James<sup>2</sup>, M.D. Moore<sup>2</sup>, I. McGowan<sup>3</sup>

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**BACKGROUND:** We have observed that the HIV-1 gp-120-binding aptamer, UCLA1, is sensitive to degradation by nucleases present in vaginal and rectal lavages, limiting its potential as a microbicide compound. Accordingly, we sought to introduce chemical modifications that would protect the most sensitive cleavage sites: residues A9 and A10, which are found in a region of the aptamer lacking secondary structure.

**METHODS:** The starting point was a version of UCLA1 known as UCLA5v1, which was synthesized on solid phase using optimized 2'-O-TBDMS phosphoramidite chemistry, whereby the 3'-terminus was capped with an inverted thymidine residue to block 3'-exonucleases. All pyrimidines were incorporated as 2'-fluoro-2'-deoxyribonucleotides to give stability to RNases, whilst all purines were ribonucleotides. 6 residues in stem I were 2'-O-dimethylallylribonucleotides to enhance the thermal stability of this stem. The 5'-terminus was Cy5 modified to monitor degradation. Two variants, UCLA5v2 and UCLA5v3, were synthesized in which residues A9 and A10 were 2'-O-methyladenosine, respectively.

**RESULTS:** Hypermodified aptamers were obtained in respectable overall yield and purity by chemical synthesis. Alkaline hydrolysis patterns of UCLA5v2 and v3 confirmed the correct location of the 2'-O-methyladenosines. Relative to the parent, UCLA5v1, some limited protection was achieved by these modifications but the effects were far from dramatic and varied according to the lavage tested. In some cases enhanced cleavage was switched to other sites.

**CONCLUSIONS:** Chemical synthesis permits the individual modification of each of the nuclease-sensitive sites of the HIV-1 aptamer to evaluate the optimal chemical modification pattern compatible with maximum activity and stability for eventual *in vivo* use. Our results here suggest that the aggressive nucleases present in rectal and vaginal lavages are not conventional RNases but more general nucleases. The next step forward based on our results is to synthesize variants of UCLA5v1 in which phosphodiester bonds proximal to sensitive residues are modified by sulfurization.

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### Identification of Anti-lipopolysaccharide Factor (ALF) from the Hemocytes of Mud Crab *Scylla Serrata*: A Promising Molecule Having Anti-STI and Anti-HIV Activities

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**BACKGROUND:** The HIV/AIDS epidemic continues to have a devastating impact on the lives of millions of people worldwide. In the absence of an effective prophylactic anti-HIV therapy, current efforts are aimed at developing safe and effective microbicides.

The bacterial endotoxin also known as lipopolysaccharide (LPS) is a major cause of inflammation regulated by release of pro-inflammatory cytokines. LPS is known to induce TLR4 induced HIV-LTR transactivation. Hence molecules that bind to LPS (ALF) and prevent the release of pro-inflammatory cytokines and inhibit HIV transcription are considered important candidates for microbicide development. This study describes the identification of full length cDNA coding for *Scylla serrata* anti-lipopolysaccharide factor (SsALF) protein having antibacterial and anti-HIV activities.

**METHODS:** Using degenerate and RACE-PCR approaches a full length cDNA coding for SsALF protein has been identified. The recombinant SsALF (rSsALF) has been produced in *E.coli* system and characterized. Initially a microtiter plate based assay was performed to determine the ability of the rSsALF (6.25–200 g ml<sup>-1</sup>) to bind to bacterial LPS. Later the MIC of the rSsALF was evaluated against vaginal pathogens and commensal lactobacilli. The ability of rSsALF to inhibit the LPS mediated release of TNF- $\alpha$  in U937 cells was evaluated by ELISA. The ability of rSsALF to inhibit LPS mediated HIV-LTR activation was evaluated on HeLa cell lines transfected with HIV-LTR-GFP reporter plasmid. Finally the anti-HIV activity of rSsALF was carried out using TZM-bl cell line. Virus was quantitated by the NIH-ELISA kit for p24<sup>gag</sup>.

**RESULTS:** rSsALF protein demonstrated antibacterial activity against vaginal isolates (*E. coli*, *P. aeruginosa*, *S. aureus*, *C. albicans*, *N. gonorrhoeae* and *S. pyogenes*) at concentrations 100–200 g ml<sup>-1</sup>. rSsALF exhibited a significant binding to LPS at 100 g ml<sup>-1</sup> as observed by a microtiter plate assay. Up to 2 folds decrease in TNF- $\alpha$  levels was observed in U937 cells incubated with LPS along with rSsALF (200 g ml<sup>-1</sup>). rSsALF caused a 70–80% decrease in HIV-LTR-GFP promoter activity. rSsALF also exhibited anti-HIV activity.

**CONCLUSION:** From the above studies it is clear that rSsALF can bind to LPS and inhibit TNF- $\alpha$  production and subsequent decrease in LPS regulated HIV infection. Therefore rSsALF can be considered as a promising candidate for microbicide development for the prevention of STIs including HIV / AIDS.

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### Two Medicines That Block HIV-1 Gene Expression and Kill HIV-1-Infected Cells Preferentially, Yet Lack Epithelial Toxicity

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**BACKGROUND:** HIV-1 gene expression and cellular apoptosis involve post-translationally hydroxylated cellular proteins. We hypothesized that protein hydroxylation inhibitors show a novel anti-HIV profile, promoting apoptosis of infected cells by disrupting retroviral control over this innate response. To test our hypothesis, we are using drugs, rationally identified via the active site geometry and/or the catalytic mechanism of protein hydroxylases. We published that two protein hydroxylation-inhibiting drugs, ciclopirox (CPX), the globally available vaginal fungicide, and deferiprone (DEF), a medicinal chelator, interfere with HIV-1 promoter activity and gene expression. We have shown in primary lymphocytes that CPX and DEF block HIV-1 infectivity, kill HIV-infected cells preferentially, and block HIV-1 rebound after drug cessation, an apoptosis-related finding also noted in a pilot DEF trial. We report the cell biology of the drugs' proapoptotic effect and test the epithelial toxicity of DEF.

**METHODS:** Conventional / raft culture of established cell lines; multi-color flow cytometry.

**RESULTS:** HIV-1 infection of CD4+ H9 cells (H9-HIV) markedly stabilized the mitochondrial membrane potential ( $\Delta\Psi$ ), a key endogenous trigger of apoptosis, and significantly reduced apoptosis markers, e.g. the caspase 3-generated 89-kDa fragment of poly (ADP-ribose) polymerase (frag-PARP). CPX and DEF reversed this relation in a dose-dependent manner. Within 24 hours, control-normalized  $\Delta\Psi$  collapse in H9-HIV caused by 30  $\mu$ M CPX was twice that of uninfected H9 ( $P = 0.02$ ); DEF at 200  $\mu$ M gave similar results. CPX increased frag-PARP+ cells by almost tenfold over controls in H9-HIV, but only about twofold in H9. The signal intensity for frag-PARP+ cells was distinctly higher in H9-HIV ( $\geq 1$  log after 24 hours). 30  $\mu$ M CPX did not induce anti-apoptotic Bcl-2; 200  $\mu$ M DEF did so marginally, consistent with a less prominent frag-PARP increase. DEF was as effective as CPX in achieving apoptosis in H9-HIV, measured by annexin / 7-AAD, TUNEL, volume contraction, and cell death. DEF did not degrade the barrier integrity of a tight-junction-linked model mucosa (uterine EEC-1), even after 6 days at 200  $\mu$ M.

**CONCLUSIONS:** CPX and DEF cause preferential apoptotic ablation of HIV-1 infected cells via the mitochondrial pathway. CPX, with its known clinical safety profile as vaginal antifungal, and DEF, which lacks epithelial toxicity in the ECC-1 model, should be considered as candidate microbicides.

# POSTER SESSIONS

SESSION 11/12  
Sunday, May 23, 2010

## 138

### Driving HIV Into Suicide— A New Approach for a Microbicide

K. Moelling\*

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Advanced Studies, Berlin

**BACKGROUND:** We are developing an alternative approach to siRNA, which may be designated as siDNA, small interfering DNA, by using hairpin-loop-structured DNA oligodeoxynucleotides (ODN), targeted to viral or cellular mRNAs. ODNs activate the viral RNase H in retroviral particles and cellular RNases H inside the cell. Also Ago2 may play a role. Other inhibitory mechanisms such as translational arrest may contribute.

**METHODS:** We selected ODNs against various viral and mRNAs of HIV, HSV, Influenza, HCV, HBV, and the terminal repeat of telomeres in malignant melanomas in mice. The ODNs were applied with or without carriers. Furthermore their effects were directly compared to those of single-stranded antisense DNAs and siRNAs to allow comparison of the various efficiencies.

**RESULTS:** The ODNs were most effective in HIV. We are able to induce HIV suicide and inactivate HIV virus particles to prevent infections, inactivate cell-free HIV in the blood from infected individuals, in the vagina of mice, and increase survival time of retroviral-infected mice. We could prevent infection if treated early. 5 out of 6 humanized SCID mice did not get infected with HIV. Furthermore we could reduce other viruses such as HSV. The effects are sequence—and dose—dependent, but the optimal algorithm is not yet known. We are analyzing whether there is a preference for G tracts, which may form higher-ordered structures and enhance uptake.

**CONCLUSIONS:** We can inactivate HIV virus particles before infection without a carrier. Thus HIV is inactivated before infection. The dsODNs are often superior to single-stranded antisense DNA and resemble the effects of siRNAs but with different kinetics. In contrast, we are targeting viral RNA by partially dsDNA. The method may complement existing silencing approaches.

## SESSION 12

### Poster Discussion (PD3): How Safe is Safe?

**Moderators:** Kenneth Mayer, Nyaradzo Mgodi

Sunday, May 23, 3:00 pm–4:00 pm

Rooms 319–321

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### A New Model for Studies on Cervicovaginal Inflammation and Barrier Disruption

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USA; <sup>2</sup>MatTek Corp., Ashland, MA, USA

**INTRODUCTION:** Several microbicide candidates have failed in clinical trials because they induce inflammation and epithelial barrier disruption in the female reproductive tract. Unfortunately, many of the cervicovaginal epithelial models currently used to test microbicides are limited due to poor availability, reproducibility and cost.

**OBJECTIVE:** The purpose of this study was to establish a reliable and cost-effective in vitro ectocervical/vaginal inflammation model, and to further determine the molecular basis for inflammation-induced changes in barrier permeability.

**MATERIALS AND METHODS:** EpiVaginal™ cultures (MatTek Corp., Ashland, MA) were treated with increasing doses of the proinflammatory cytokine TNF- for 24 hours. The culture medium of treated tissues was assessed for IL-6 and IL-8 production by Bioplex Cytokine Immunoassay. Epithelial permeability was determined by measuring transepithelial electrical resistance (TEER). The integrity of the epithelium is regulated by junctional proteins, which were characterized by immunohistochemistry.

**RESULTS:** Treatment with 2ug/ml of TNF- significantly increased secretion of IL-6 and IL-8 into the culture medium, and increased epithelial permeability by 25%. Expression of junctional proteins JAM-A, JAM-C, E-cadherin and ZO-1 in the untreated EpiVaginal model resembled that of normal vaginal tissue, and was reduced following TNF- treatment.

**CONCLUSIONS:** We have developed a new model to characterize molecular events underlying increased permeability induced by proinflammatory cytokines in vaginal epithelial tissue. These findings could provide important insight into how vaginal permeability is regulated in order to guide future microbicide development. Further, the identification of biomarkers to detect barrier dysfunction could advance the efforts to formulate safe and effective microbicides to prevent the transmission of sexually transmitted pathogens.

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### Mechanisms of Cellulose Sulfate Interaction with the Human Bacteria-Colonized Cervicovaginal Epithelium—Use of a New *In Vitro* Model to Assess Microbicide Safety

R. Fichorova<sup>\*1</sup>, H. Yamamoto<sup>1</sup>, V. Tang<sup>1</sup>, H. Yuan<sup>1</sup>, G. Doncel<sup>2</sup>

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**BACKGROUND:** Several anti-HIV microbicides including cellulose sulfate (CS) have passed conventional preclinical evaluation and safety trials, yet ultimately failed to succeed in Phase III trials despite their anti-HIV activity in surrogate models of efficacy. Concerns have been raised that current preclinical algorithms are deficient in addressing the complexity of the human vaginal environment. A limitation of traditional vaginal in-vitro models is that they have lacked the physiologic host-microflora interactions and thus ignored the role of the normal microbial biofilm as a modifier of the epithelial immune function. We applied a novel microflora-colonized cervicovaginal epithelial model to investigate potential interferences of CS with the vaginal barrier function.

**METHODS:** Immortalized and primary cervical and vaginal epithelial cells were colonized with normal and bacterial vaginosis associated microflora (BV) and exposed to a panel of microbicide compounds including CS for up to 24h. Epithelial colonization was assessed by colony forming units of bacteria stably associated with the vaginal epithelial cells in the presence of microbicides and pathogenic bacterial and viral determinants. NF- $\kappa$ B activation was assessed by a luciferase reporter assay. Extracellular and intracellular inflammatory, immunoregulatory, antimicrobial and apoptotic markers were measured. Dose responses were determined and differences between treatments were assessed by ANOVA.

**RESULTS:** At low concentrations (<100 g/mL), CS enhanced NF- $\kappa$ B activation but not cytokine production. In bacteria colonized vaginal cells, low dose CS even decreased cytokine production. At higher doses, CS increased cytokine production by BV-exposed endocervical cells but reduced TLR-mediated cytokine production against viral and bacterial determinants by bacteria-colonized vaginal and endocervical cells. Epithelial responses to viral determinants were suppressed by BV, and CS did not modify this effect. Hydroxyethyl cellulose did not induce any of these changes.

**CONCLUSIONS:** These results suggest that microbicides can differentially modulate vaginal-microflora interactions and suggest that CS may have variable pro- and anti-inflammatory effects depending on drug concentration and variations in the vaginal microflora. Further studies are needed to assess the importance of these interactions regarding the safety of microbicide candidates.

# POSTER SESSIONS

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### Microbicide Excipients that Increase Susceptibility to Genital Herpes

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<sup>4</sup>Starpharma Pty Ltd, Melbourne, Australia

**BACKGROUND:** Prior studies have reported that several microbicide active ingredients cause toxic effects that increase susceptibility to infection in mouse HSV-2 vaginal susceptibility models (Cone et al., BMC Inf Dis 2006;6:90; Galen et al., JID 2007;195:1332). Moreover, these results correlate well with instances of increased HIV transmission observed in microbicide clinical efficacy trials. These mouse models thus appear useful for detecting significant toxic effects prior to Phase III clinical trials. Excipients or "inactive ingredients" used in microbicide formulations might also have toxicities that increase susceptibility, but have not previously been tested in susceptibility models.

**METHODS:** Excipients commonly used in topical products were formulated in a well-studied non-toxic vehicle (the "HEC universal placebo"), or other formulations as specified. One of these excipients, the surfactant/emulsifier glycerol monolaurate (GML), is also being developed as an active microbicide, formulated in K-Y Warming Jelly (KYWJ). Twelve hr after delivering the test agent or PBS control, mice were challenged with 1.0 intravaginal infectious dose50 of HSV-2, and 3 d later were assessed for infection by vaginal lavage culture. Agents were compared to PBS control by two-tail Fisher's exact test.

**RESULTS:** Significantly increased susceptibility to HSV-2 was observed after a single exposure to 5% GML in the extremely hyperosmotic vehicle (osmolality 10,300 mOsm/kg) KYWJ ( $P<0.0001$ ), to KYWJ alone ( $P<0.0001$ ), to each of the neat humectant/solvent components of KYWJ (propylene glycol ( $P<0.006$ ) and PEG-8 ( $P<0.02$ )), and to 5% GML as a colloidal suspension in PBS ( $P<0.006$ ). A trend toward increased HSV-2 transmission was seen after 0.1% disodium EDTA ( $P<0.095$ ), but not 0.0186% disodium EDTA. Increased susceptibility was not observed following exposure to the solvent/humectants 10% glycerin, or 10% propylene glycol, or with the preservatives 0.18% methylparaben plus 0.02% propylparaben, or 1% benzyl alcohol.

**CONCLUSIONS:** As reported with other surfactants, a single exposure to GML markedly increased susceptibility to HSV-2. Consistent with reports that extremely hyperosmotic formulations cause mucosal toxicity, the non-aqueous KYWJ and each of its undiluted humectant/solvent constituents also markedly increased susceptibility. EDTA at high levels caused a trend towards increased susceptibility.

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### In Vitro Evaluation of the Safety Profile of Pharmaceutical Excipients for the Use in Microbicide Formulations

Y. Gali<sup>\*1</sup>, O. Delehay<sup>2</sup>, J. Brouwers<sup>3</sup>, T. Bourlet<sup>2</sup>, B. Pozzetto<sup>2</sup>, H. Hamzeh-Cognasse<sup>2</sup>, P. Augustijns<sup>3</sup>, K. Ariën<sup>1</sup>, G. Vanham<sup>1</sup>

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**BACKGROUND:** The results of the nonoxynol-9 and cellulose sulfate clinical trials have shown an increased risk of HIV infection and an absence of efficiency, respectively. These failures suggest that more extensive *in vitro* strategies must be implemented to anticipate the *in vivo* efficiency/toxicity rate of future candidate microbicide formulations. Not only the safety of the active pharmaceutical ingredient used in the formulation, but also the safety of pharmaceutical excipients is of great importance.

**METHODS:** The safety of excipients belonging to various classes, i.e. preservatives (PRES), co-solvents (CS), surfactants (SF) and cyclodextrins (CD), was evaluated on HEC-1A epithelial layers, grown in a dual chamber setup and on cervical explants cultured in a non-polarized fashion. Upon 24h exposure of the explants and the HEC-1A epithelial layers to pharmaceutically relevant concentrations of the excipients, viability was determined (WST-1 assay). Passage of fluorescent beads (100 nm, similar to an HIV virion) across cell layers was used as a measure of epithelial layer integrity. IL-8 production was assessed as a marker for induction of inflammation by excipients.

**RESULTS:** All tested SF exerted toxic effects on HEC-1A cells and cervical explants at concentrations typically used in formulations (sodium lauryl sulfate > TPGS1000 succinate >Polysorbate 80 >Cremophor® EL). Cell viability and epithelial layer integrity were also compromised by the PRES benzalkoniumchloride and benzylalcohol, but less by sorbic acid, methyl- and propylparaben. Among CD, the following rank order for toxicity was observed: dimethyl-β-CD >α-CD, β-CD >γ-CD >hydroxypropyl-β-CD >sulfobutylether-β-CD >hydroxypropyl-γ-CD (no toxic effects). The CS ethanol and glycerin, propylene glycol and polyethylene glycol 400 and 1000 exerted considerably lower toxic effects. Finally, IL-8 production was differentially affected by the various excipients.

**CONCLUSIONS:** The present study revealed a reduced viability of epithelial cells and cervical explants and impaired epithelial layer integrity upon apical exposure to various pharmaceutical excipients. Identification of these safety issues in early development is of great assistance to formulation scientists. Careful selection of excipients for microbicide formulation seems warranted.

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### Repeated Agent Exposures Affect In Vitro and In Vivo Measures of Microbicide Safety

K. Lozenski<sup>\*1</sup>, T. Kish-Catalone<sup>2</sup>, V. Sanghvi<sup>1</sup>, L. Steel<sup>1</sup>, B. Wigdahl<sup>1</sup>, F. Krebs<sup>1</sup>

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**BACKGROUND:** The disappointing clinical failures of vaginal microbicides have provided new insights into factors that impact microbicide effectiveness, including repeated product application and its association with increased HIV-1 infection. Clinical trials examining the nonoxynol-9 (N-9)-containing product COL1492 determined that daily applications increased the risk of HIV-1 infection. Therefore, despite being an effective spermicidal agent, N-9 was determined to be an ineffective microbicide. This increased risk of HIV-1 infection can be attributed to N-9-induced breaks in the epithelial barrier combined with local inflammation and immune cell recruitment. These results clearly indicate the need to study the impact of multiple applications on microbicide safety and efficacy.

**METHODS:** To investigate the effects of repeated use on microbicide safety, multiple exposure experiments used a mouse model of cervicovaginal toxicity and cervicovaginal cell lines. Toxicity studies using the Swiss Webster mouse model used H&E tissue staining to reveal changes in cervicovaginal epithelial integrity. In *in vitro* experiments, End 1 cells were exposed daily for 4 days to unformulated N-9 and cell viability was measured at 10 minutes, 2, 4, 8, and 24 h daily using the MTT cellular viability assay. For initial *in vitro* cytokine studies, real-time PCR was performed to measure changes in mRNA levels after multiple N-9 applications.

**RESULTS:** In the mouse experiments, the first application of 1% N-9 caused considerable damage to the cervical epithelium. Subsequent daily exposures were characterized by diminished cervical toxicity. Multiple daily exposures also increased the exposure duration required to elicit levels of epithelial damage similar to those seen after the first exposure. The vaginal epithelium was unaffected by single and multiple exposures. However, *in vitro* cytotoxicity experiments conversely demonstrated that End 1 cells became increasingly sensitive to N-9 after multiple exposures. Changes in cytokine expression *in vitro* were both time- and exposure-dependent.

**CONCLUSIONS:** Multiple N-9 exposures do not appear to result in cumulative cervical epithelial damage. In fact, multiple exposures cause changes in the cervical epithelium that increase its tolerance to N-9 exposure. Furthermore, comparisons of *in vitro* and *in vivo* results indicate that that *in vitro* assays designed to assess the effects of multiple exposures may not be predictive of *in vivo* outcomes.

# POSTER SESSIONS

SESSION 13  
Sunday, May 23, 2010

## SESSION 13

### Poster Discussion (PD4): Sex, HIV, and Biomarkers

**Moderators:** James Cummins, Christine Mauck

Sunday, May 23, 3:00pm–4:00pm

Rooms 403–405

**65**

#### Characterization of Seminal Enhancer of Viral Infectivity (SEVI) on HIV-1 Penetration in the Female Genital Tract

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**BACKGROUND:** One mode of human immunodeficiency virus type 1 (HIV-1) transmission occurs via vaginal exposure to the semen of HIV-1 infected men. For a productive infection to occur, HIV-1 within semen must gain access to target cells by penetrating natural mucosal barriers including the cervical epithelium. Seminal enhancer of viral infectivity (SEVI) amyloid fibrils enhance HIV infectivity by sequestering the virus and promoting viral/host cell interactions as shown by Munch et al., 2001. However, these *in vitro* studies were conducted using multiple cell-lines and immune cells isolated from whole blood and may not be optimal for studying the role of SEVI in the context of HIV transmission.

**METHODS:** To characterize the effects of SEVI on HIV-1 penetration of the cervical epithelium, studies were conducted using a more biologically relevant approach: human cervical explants. For *ex vivo* studies, fresh human cervical specimens were dissected into 1cm cubes of endo- and ectocervix and incubated with photo-activatable GFP-Vpr HIV in the presence or absence of SEVI for four hours. Tissues were then removed from inoculum, sectioned, stained and imaged.

**RESULTS:** Results show that in the human ectocervix, SEV/HIV-1 complexes aggregate at the outermost surface of the ectocervical epithelium. Moreover, the number of penetrating virions was decreased three-fold in SEVI treated ecto-cervical tissues although the degree of penetration was similar. The introduction of SEVI to endocervical tissues seemed to modify filamentous SEVI into a spherical conformation. Analysis of penetration in the endocervix illustrates that in both treated and untreated conditions, the number of penetrating virions and the extent of penetration were similar.

**CONCLUSIONS:** Taken together, these results indicate the likelihood of two distinct roles for SEVI. In the ectocervix, SEVI may act to inhibit penetration of the epithelium while in the endocervix, SEVI may shuttle HIV through the epithelium to enhance penetration.

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#### Broad Spectrum Endogenous Antimicrobials in Secretions from Female Reproductive Tract Epithelial Cells in Culture

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**BACKGROUND:** Relatively little is known about the mechanisms through which the female reproductive tract (FRT) is protected against the diverse sexually transmitted infections (STI) that threaten the lives and reproductive health of women. In addition to providing a physical barrier to infectious microorganisms, FRT epithelial cells, which are the sentinel cells to first see potential STI pathogens, actively secrete a spectrum of constitutive and induced factors that inhibit infection by some STI pathogens. To understand the scope of these secreted factors, we hypothesized that epithelial cells secrete innate antimicrobial factors that inhibit a range of potential pathogens of the FRT.

**METHODS:** Epithelial cells were isolated from FRT tissues (Fallopian tube, uterus, endocervix and ectocervix) following hysterectomy and cultured in cell inserts until confluence for lower tract cells or polarization for upper tract cells. Apical secretions from primary cultures from epithelial cells were incubated for one hour with HIV-1, *Neisseria gonorrhoeae*, or *Candida albicans* prior to being tested for their ability to grow and/or infect target cells. Secretions from FRT epithelial cells incubated with *Lactobacillus crispatus* was also tested to determine whether they were inhibitory to commensals found in the lower FRT. Several factors in uterine epithelial secretions were identified by ELISA or Luminex analysis.

**RESULTS:** Epithelial cell secretions from throughout the FRT inhibit, *N. gonorrhoeae* and *C. albicans* infection of target cells. When HIV-1 R5 strains were analyzed, secretions from the uterus, cervix, and ectocervix had HIV neutralizing activity. In contrast, HIV-1 X4 has less activity in secretions from epithelial cells from the upper and lower FRT. Interestingly the commensal *L. crispatus* was not affected by any of the secretions tested. Thus, epithelial cells from throughout the FRT secrete factors with anti-viral, anti-bacterial and anti-fungal activity. Antimicrobial, cytokine and chemokine analysis of uterine secretions revealed several candidate molecules that could account for pathogen inhibition.

**CONCLUSIONS:** These findings provide definitive evidence for the critical role of epithelial cells in protecting the FRT from infections, without comprising the beneficial presence of commensals.

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#### Molecular Mechanisms Mediating Proinflammatory Responses in Human Vaginal Epithelium

T. Joseph\*, I. Zalenskaya, G. Doncel  
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**BACKGROUND:** The cervicovaginal epithelium of the lower female genital tract actively participates in diverse pro-inflammatory responses to external pathogenic stimuli and endogenous vaginal microbiota. These responses usually involve the recognition of certain conserved pathogen-associated molecular patterns by specific receptors called Toll-like receptors (TLRs). Most of these TLRs activate MAP kinases (MAPK) and NF- $\kappa$ B signal transduction pathways. These pathways are also involved in the expression of proinflammatory mediators like the prostaglandin synthase COX-2. In this study, we evaluated key signal transduction mechanisms leading to COX-2 expression by human vaginal cells exposed to TLR ligands, nonoxynol-9 (N-9), TNF- $\alpha$  and tenofovir.

**METHODS:** Human vaginal epithelial cells (VK2/E6E7), grown to 70–80% confluence, were treated with N-9, TNF- $\alpha$  and TLR ligands such as macrophage activating lipopeptide (MALP2), Pam3CSK4 (Pam), zymosan, polyinosine-polycytidylic acid (poly dI:dC) and lipoteichoic acid (LTA), and dose and time response experiments were performed. Cells were also treated with these proinflammatory stimuli in the presence of pathway inhibitors such as Bay11 7082 (NF- $\kappa$ B), SB202190, SB203580 and U0126 (MAPK). Nuclear, cytoplasmic and total cellular proteins were used to assay for p65 (NF- $\kappa$ B) nuclear translocation, MAPK phosphorylation and COX-2.

**RESULTS:** Stimulation of vaginal cells with TLR ligands, N-9 and TNF- $\alpha$  resulted in COX-2 expression in a dose and time-dependent manner. For example, N-9 caused maximum COX-2 expression at 12.5  $\mu$ g/ml for 6h. Phosphorylation of MAPKs and p65 nuclear translocation was observed with most TLR ligands, indicating activation of MAPK and NF- $\kappa$ B pathways. Inhibition of these pathways decreased epithelial COX-2 expression. Unlike the above agents, tenofovir did not stimulate proinflammatory pathways or COX-2 expression.

**CONCLUSIONS:** Multiple TLR ligands, TNF- $\alpha$  and N-9 activate vaginal epithelial MAPK and NF- $\kappa$ B pathways and increase COX-2 expression, providing the molecular trigger for a mucosal immunoinflammatory reaction. These findings help understand the molecular mechanisms underlying genital mucosal inflammation and may be used to assist in the rational selection of safe microbicide candidates.

# POSTER SESSIONS

75

## Optimising Endocervical Mucosal Cell Sampling for the Study of HIV Mucosal Target Cells in Microbicide and Mucosal Vaccine Research

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**BACKGROUND:** Microbicides and vaccines interact with vaginal mucosal immune responses. Measuring cellular composition of cervical mononuclear cells (CMC) is gaining importance in research settings and clinical trials. A fine brush, designed for cytology, collects cells effectively but yield for flow cytometry assays is often low and red blood cell (RBC) contamination high. We hypothesised that an L-shaped flocked swab could be more mucosa-friendly and possibly better at absorbing and releasing cells especially in the case of ectopy, than a brush.

**METHODS:** A comparison is made for the yield of live leucocytes (LL) between the swab (Copan) and brush (Cellpath® 9 mm Ø) in a randomised cross over design over 2 cycles with samples taken on day 9 and day 23. The samples are kept in PBS on ice and cells are counted within 1 hour. LL are counted in a Neubauer chamber after trypan-blue staining. The supernatant is tested for free haemoglobin, RBC and leucocyte esterase (LE) with a urine dipstick (Servotest®5+NL).

**RESULTS:** The median value for LL was  $0.34 \times 10^6$  (mean of  $1.6 \times 10^6$ ) and no significant difference was seen for sampler used. Similarly, no difference in yield for sampler was seen over the ectopy groups separately. Absence of blood, presence of ectopy (visual inspection) and a positive LE test were significantly related to yield. Both swab ( $P=0.005$ ) and brush ( $P=0.000$ ) had a higher yield when ectopy was present (Table 75.2). No difference ( $P=0.3$ ) for presence of blood was seen between samplers.

TABLE 75.1 Yield of LL in Relation to Characteristics

27 Participants		N (%)	Median <sup>1</sup>	IQ range <sup>1</sup>	Range <sup>1</sup>	P Value*
		92 samples	0.31	0.12–1.55	0–14.2	
<b>Sampler</b>	<b>Swab</b>	46 (50)	0.41	0.21–1.99	0–14.2	–
	<b>Brush</b>	46 (50)	0.33	0.08–1.34	0–11.4	0.121
<b>Blood</b>	<b>Negative</b>	43 (47)	0.65	0.21–2.34	0–12.3	–
	<b>Positive</b>	49 (53)	0.22	0.08–0.7	0–14.24	0.036
<b>Ectopy</b>	<b>Absent (11)</b>	38 (41)	0.14	0.06–0.35	0–13.75	–
	<b>Present (16)</b>	54 (59)	0.82	0.27–1.99	0.06–14.24	0.000
<b>LE</b>	<b>Negative</b>	45 (49)	0.21	0.06–0.35	0–2.2	–
	<b>Positive</b>	47 (51)	1.34	0.32–2.85	0.06–14.24	0.000

TABLE 75.2 Yield of LL for Ectopy by Sampler

Participants (N)	Swab		Brush		P value*
	N samples	Median <sup>1</sup>	N samples	Median <sup>1</sup>	
<b>Ectopy</b>	No (11)	19	0.21	19	0.07
	Yes (16)	27	0.75	27	0.88

\*Wilcoxon rank – sum (Mann Whitney)

<sup>1</sup> LL  $\times 10^6$

**CONCLUSION:** Live leucocyte cell yield in this nulliparous healthy population is low and no difference is seen between the sampling devices tested in this interim descriptive analysis. Cell yield was significantly higher if ectopy was present.

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## Interlaboratory Variability in Detection of Cytokines by Bioluminex: Implications for Microbicide Safety Studies

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**BACKGROUND:** Measurement of cytokines in genital tract secretions is being promoted as a potential biomarker of microbicide safety. However, the interlab variability in measurements is not known.

**METHODS:** To address this gap, cervicovaginal lavage (CVL) samples that had been collected as part of ongoing Phase I clinical safety trials were evaluated in a blinded fashion in three independent laboratories to determine the interlaboratory variability in measurements. Thirty-six samples from 12 subjects (Day 0, Day 7 and Day 14 of a Phase I microbicide gel study) were aliquoted and distributed to each laboratory and analyzed using the identical multiplex kit for INF- $\alpha$ 2; INF- $\alpha$ 2, IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , IL-10 and IL-17. Data were analyzed on log transformed values by Friedman's (paired non-parametric) and then pairwise (Wilcoxon) tests.

**RESULTS:** There were significant differences between labs for INF- $\alpha$ 2 and IL-8 on all days and for IL-10 on Day 0, IL-1 $\beta$  on Day 14 and IL-6 on Day 7 with Lab C > Lab B ~ Lab A. However, there were no differences in the quartiles for the labs for any of the cytokines at Day 7 and Day 14 ( $p>0.3$ ); the ordering of the samples is similar between the labs in all cases.

TABLE 68.1

	Lab A Day 0	Lab B Day 0	Lab C Day 0	Lab A Day 7	Lab B Day 7	Lab C Day 7	Lab A Day 14	Lab B Day 14	Lab C Day 14
INF- $\alpha$	0.66	0.91	1.28	0.63	0.77	0.96	0.63	0.77	0.89
INF- $\gamma\gamma$	0.57	0.51	0.57	.52	.50	.52	.58	.77	.57
IL-1 $\beta$	1.24	1.12	1.31	.94	.92	1.05	1.37	1.32	1.46
IL-6	1.45	1.34	1.53	1.29	1.29	1.51	1.51	1.55	.1.77
IL-8	2.71	2.51	2.54	1.38	1.67	2.25	1.67	1.99	2.51
TNF $\alpha$	.51	.51	.51	.51	.51	.51	.62	.60	.58
IL-10	.55	.52	.64	.58	.55	.57	.60	.57	.58
IL-17	.51	.53	.56	.51	.51	.51	.51	.51	.53

**CONCLUSIONS:** Thus, although the absolute values may differ across laboratories and should not be directly compared, actual changes in local immune response to microbicides should be reliably detected across different laboratories.

## SESSION 14

## Oral Abstracts (OA7): Rectal Microbicides: Preclinical Studies

**Moderators:** Ross Cranston, Carolina Herrera

Sunday, May 23, 4:30pm–6:00pm

Ballroom

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**Expression of Membrane Drug Transporters in Recto-Sigmoid Colon from HIV Infected Men: Potential Role in Microbicide Permeability**M.F. De Rosa<sup>\*1</sup>, T. Hoque<sup>1</sup>, C.J. Kim<sup>2</sup>, G. Kandel<sup>3</sup>, C. Kovacs<sup>4</sup>, R. Kaul<sup>2,5</sup>, R. Bendayan<sup>1</sup><sup>1</sup>*Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Canada;* <sup>2</sup>*Faculty of Medicine, University of Toronto, Toronto, Canada;* <sup>3</sup>*St. Michael's Hospital, Toronto, Canada;* <sup>4</sup>*Maple Leaf Medical Clinic, Toronto, Canada;* <sup>5</sup>*University Health Network, Toronto, Canada*

**BACKGROUND:** Microbicides have gained significant global attention as potential strategy to prevent HIV transmission. We propose that the mucosal permeability of microbicides, and their efficacy and/or toxicity may be reduced by interaction with the drug efflux membrane transporter P-glycoprotein (P-gp), Multidrug Resistance-Associated Proteins (MRPs) and/or Breast Cancer Resistance Protein (BCRP). Specifically, we hypothesize that the activity of these transporters may reduce levels of these agents in the genital tract or rectum; in addition, interaction with drug influx membrane transporters such as organic anion transporting polypeptide (OATP), organic anion transporter (OAT) and/or organic cation transporter (OCT), may increase levels at these same sites. It has been reported that the uptake of tenofovir into renal proximal tubules is mediated by hOAT1 and hOAT3, and its transport in the kidney is mediated by MRP4; and that maraviroc has affinity for P-gp. At present, there are very few studies documenting the functional expression of these transporters in recto-sigmoid colon, a mucosal site that is highly HIV susceptible. The objective of this study was to investigate the expression of drug influx/efflux transporters in recto-sigmoid colon biopsies from HIV (+) treated and naïve men, and HIV (-) individuals.

**METHODS:** Recto-sigmoid colon biopsies were obtained from: i) HIV-infected, therapy naïve men, ii) HIV infected, HAART-treated men with an HIV viral load <50 copies/ml for at least 4 years (LT-HAART), and iii) HIV negative individuals. The mRNA expression of drug influx/efflux transporters in recto-sigmoid colon tissues obtained from the three groups was measured by RT-PCR. P-gp, MRP1 and MRP4 protein expression was determined by immunoblotting in the same set of recto-sigmoid colon samples.

**RESULTS:** The following tables summarize the results obtained:

TABLE 43.1 mRNA Expression of Drug Transporters in Recto-Sigmoid Colon Samples of HIV(+) Men

Group	Efflux Transporters (MDR1, MRPs, BCRP)	Influx Transporters (OATPs, OATs, OCTs)	Nucleosides Transporters (ENTs, CNTs)
Chronic naive (N=1)	(+) (MDR1, MRP3,4, BCRP)	(+) (OATP B, OAT4, OCT1,3)	(+) (CNT2 , CNT3)
LT-HAART (N=8)	(+) (MDR1, MRP1,3,4, BCRP)	(+) (OATP B, OCT1)	(+) (CNT2 , CNT3)
HIV Neg (N=3)	(+) (MDR1, MRP1,3, BCRP)	(+) (OATP B)	(-)

TABLE 43.2 Protein Expression of Drug Transporters in Recto-Sigmoid Colon Samples of HIV (+) Men

Group	Efflux Transporters		
	P-glycoprotein	MRP1	MRP4
LT-HAART (N=3)	(+)	(+)	(+)

**CONCLUSIONS:** Expression of MRP4 in the rectum of HIV infected men could alter the permeability and bioavailability of tenofovir, an anti-retroviral agent known to be effluxed by this transporter that is currently in clinical trials. Expression of P-gp in the sigmoid colon could alter the permeability of maraviroc, another potential microbicide that is known to be a substrate of this transporter. These results help to elucidate factors influencing the mucosal bioavailability and efficacy of microbicides.

# ORAL SESSIONS

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### **Effect of Storage Conditions on GALT T Cell Phenotype—Implications for Phase 1 Microbicide Studies**

I. McGowan<sup>\*1,4</sup>, S. Longinotti<sup>1</sup>, K. Duffill<sup>1</sup>, E. Sutton<sup>4</sup>, S. Abo<sup>4</sup>, R. Cranston<sup>4</sup>

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**BACKGROUND:** Assessment of mucosal immune function in Phase 1 rectal microbicide studies often includes characterizing gut associated lymphoid tissue (GALT) T cell phenotype using flow cytometry. To generate comparable data it is important that flow cytometric characterization of GALT is conducted on one machine or between sites that have cross validated their equipment. Cross validation is technically demanding and so currently Phase 1 studies routinely require that samples are shipped overnight to a central laboratory. It is not clear whether samples should be shipped on ice or frozen and whether GALT T cells should be isolated before shipping. Also there are limited data on what the immunological consequences of shipping would be. In this study we evaluated whether a variety of approaches to storage and shipping resulted in altered cell viability or phenotype.

**METHODS:** Endoscopic intestinal biopsies were collected from HIV negative participants (N=39). GALT T cells were isolated using collagenase enzymatic digestion. There were four groups; (1) Fresh: processed within 1 hour of collection, (2) Overnight: biopsies were stored in culture medium overnight at 4°C and then processed, (3) Frozen bx: biopsies were transferred to a cryopreservation container with a cooling rate of -10C/minute and stored for a minimum of 2 weeks at -80°C, and (4) Frozen cells: mucosal mononuclear cells (MMC) were isolated from the biopsies, pelleted in freezing medium and stored as in (3). Cells were subsequently stained with monoclonal antibodies (CD45, CD3, CD4, CD8, CCR5, CXCR4, CD69, and a viability stain (Aqua, Invitrogen). Flow cytometry was undertaken using an LSR-II (BD Biosciences). Non-viable cells were excluded from analysis. Data was compared for all markers between all four groups.

**RESULTS:** Overnight storage at 4°C did not result in any loss of viability whereas significant loss in viability was seen in both freezing techniques. Variable changes in phenotype were seen by group. In the overnight storage group the mean percentage of CD8+ T cells rose from 26.0% to 33.1 %.

	Viability (% ± SD)	T cell Phenotype with Significant Change
<b>Fresh (N=16)</b>	80.1 ± 9.6	
<b>Overnight (N=16)</b>	87.2 ± 4.8	CD8 (p < 0.01)
<b>Frozen bx (N=10)</b>	58.9 ± 11.5	CD4/CCR5 (p < 0.01), CD4/CCR5 (p < 0.05)
<b>Frozen MMC (N=13)</b>	59.6 ± 12.4	CD3 (p < 0.05)

**CONCLUSIONS:** Overnight storage of intestinal biopsies was not associated with a reduction in cell viability and only modest changes in CD8 phenotype. Techniques involving cryopreservation resulted in significant loss in cell viability. We conclude that overnight shipping can be used in multicenter studies requiring characterization of GALT T cell phenotype.

## 45

### **Assessing Colonic Epithelial Injury and HIV Penetration Following Simulated Ejaculation of Autologous Seminal Fluid in Men Who Have Sex with Men**

E. Fuchs\*, A. Koteish, R. Bakshi, N. Louissaint, K. King, M. Torbenson, E. Purdy, C. Hendrix

*Johns Hopkins University School of Medicine Baltimore, MD, USA*

**BACKGROUND:** In comparison to vaginal intercourse, epidemiologic and in-vitro evidence of increased risk of HIV transmission via receptive anal intercourse (RAI) have suggested that seminal fluid may be toxic to colonic mucosa. To determine if simulated RAI and rectal exposure to seminal fluid induces changes in the colonic mucosal barrier, we explored the effect of coitus and rectally-administered seminal fluid on colonic histology, mucosal permeability, and the ex-vivo susceptibility of colonic explant tissue to HIV infection.

**METHODS:** Ten subjects served as their own controls for each of two interventions: simulated coitus followed by rectal administration of 3mL of isotonic salt-balanced Normosol (NMSL) or 3ml frozen/thawed autologous seminal fluid (SF), each containing 500 microuncies of 99mTc-DTPA. Plasma was collected at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.33, 2.67, 3, 3.5 and 4 hours after dosing. Approximately 1 hour after dosing, flexible sigmoidoscopy was performed and pinch biopsies were obtained from the distal rectosigmoid. Immediately following collection, biopsy samples were exposed to HIV challenge. Explant supernatants were harvested every 3 days and analyzed for quantitative HIV-1 p24 antigen (Ag). Plasma aliquots were measured for radioactivity with results expressed as the fraction of administered isotope ( $\times 10^{-6}$ ). Blinded biopsies were graded for degree of epithelial surface disruption. All data were analyzed by non-parametric paired comparisons.

**RESULTS:** Low levels of plasma isotope were detectable in both interventions within fifteen minutes of sampling, with a small detectable increase noted following endoscopic biopsy. No statistically significant difference (NS) in median maximum concentration (Cmax) or median Area Under the Curve (AUC) for plasma isotope was noted for the two interventions (Cmax NMSL = 0.66, IQR 0.54-1.37; SF = 0.79, IQR 0.26-1.04; each  $\times 10^{-6}$  of dose administered, p=NS; AUC NMSL = 2.82, IQR 1.48-6.57; SF = 1.49, IQR 0.75-3.00; p=NS). Similarly no difference was noted when comparing for median grade of epithelial disruption (NMSL = 1.17, IQR = 1.04-1.46; SF = 1.00, IQR = 0.92-1.17; p= NS) or cumulative HIV p24 Ag 15 days following explant exposure (NMSL = 5,901 pg/mL, IQR 878-14,095; SF = 8,049 pg/mL, IQR 2,803-17,660; p= NS).

**CONCLUSIONS:** We found no evidence that frozen/thawed autologous seminal fluid alters the colonic mucosal barrier, or facilitates HIV infection as measured in explant supernatants.

# ORAL SESSIONS

SESSION 14/15  
Sunday, May 23, 2010

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## Testing Rectal Safety of Tenofovir in the Macaque Model

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**BACKGROUND:** A 1% tenofovir gel and HEC placebo gel were provided by CONRAD for enrollment in the pigtail macaque model for evaluating safety of rectally applied topical microbicide products.

**METHODS:** Six Macaca nemestrina were randomly selected for a three-arm crossover study in which 1% tenofovir gel, HEC placebo gel and no product (negative control) were compared. Each experiment ran for five days, during which rectal pH, microbiology and lavage were assessed daily. Each day that gel was applied (days 1 through 4), repeat assessments were collected 30-minutes after application. Rectal lavage samples were assessed under a dissecting microscope (7X) for evidence of fecal matter, cellular debris, epithelial desquamation (epithelial sheets ≥3mm in any dimension) and/or blood.

**RESULTS:** There was very little difference in rectal pH between the study arms. The presence/absence of each constituent of the rectal microflora remained quite stable throughout the experiment. However, semi-quantitative populations of certain constituents of rectal microflora, notably H<sub>2</sub>O<sub>2</sub> producing lactobacilli, tended to decline over time in the 1% tenofovir study arm. Individual organism growth rates fluctuated in all study arms, but by semi-quantitative assessment, the populations of H<sub>2</sub>O<sub>2</sub> positive lactobacilli decreased by two or more steps in the majority of animals using the test gel. Rectal lavage findings of epithelial sheets measuring at least 3mm in one dimension occurred more frequently in lavage samples collected thirty minutes after exposure to the 1% tenofovir gel, compared to lavage samples in the same arm collected just prior to product use. This pattern was not noted in the HEC placebo arm or in the no product arm.

**CONCLUSIONS:** Daily rectal use of the 1% tenofovir gel resulted in a higher rate of epithelial sloughing noted by rectal lavage assessment, and may have led to decreased populations of H<sub>2</sub>O<sub>2</sub> producing lactobacilli in the rectal environment, as compared to HEC placebo and no product use. This study was conducted with a small group of 6 animals, controlling for themselves to minimize individual animal variation between study arms, making it difficult to infer the significance of these potentially deleterious findings. Additional experiments are warranted to increase the total study population in order to determine whether the trends toward undesired safety concerns continue.

## SESSION 15

### Oral Abstracts (OA8): The Legacy of Study Participation: Social Harms, Seroconverters and Site Capacity

**Moderators:** Kim Eva Dickson, Shayhana Ganesh

Sunday, May 23, 4:30 pm–6:00 pm

Rooms 301–304

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## Social Harms in HPTN 035 in Lusaka, Zambia

V.L. Nhandu<sup>\*1</sup>, M. Njuma<sup>1</sup>, M.B. Guffey<sup>1,2</sup>

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**BACKGROUND:** It is the responsibility of investigators to prevent and minimize social harms (non-medical adverse consequences of study participation) in clinical research participants. However, data on social harms occurring during microbicide trials in sub-Saharan Africa are lacking.

**METHODS:** During HPTN 035, screening for social harms occurred quarterly and as needed during an average of 16 months follow-up. Women who experienced problems with others as a result of study participation were asked to describe the problem including whether it resulted in emotional, physical, or financial harm to her or her children. We reviewed study-related problems reported by HPTN 035 participants in Lusaka, and characterized their type, severity, instigator, and impact.

**RESULTS:** 57 of 320 participants (18%) reported a total of 70 problems related to study participation. Instigators of the problems were friends (43%); neighbors (14%); spouses or partners (13%); family members (8%); workmates (4%); and other members of the community (18%). Reported problems were accusations of Satanism, selling blood or children, or "being bought," (n=53) and from stigmatization resulting from suspicion in research (n=10). Women also reported problems with partners over belief that study gels would cause impotence or infertility (n=3) and arguments over family planning or condom use (n=4). Twelve problems resulted in social harms in 9 participants. One participant experienced emotional and physical harm (grade 1 adverse event) from neighbors following accusations of "going to Satanists to get rich." Eight participants experienced 10 distinct emotional harms and reported feeling "bad," "embarrassed," or "stigmatized." Study staff intervened directly to bring about conflict resolution when permitted and provided multi-disciplinary support to all participants reporting any problem or harm. All problems were followed until participant-reported resolution, although 1 woman withdrew from study due to of emotional harm associated with parental accusations of Satanism.

**CONCLUSIONS:** A minority (18%) of Lusaka HPTN 035 participants experienced social problems associated with their study participation, yet few (3%) incurred social harms. These problems and harms emerged from participants' relationships with friends, family, and others within the community. Although social harms were uncommon, routine screening and follow-up of reported problems is warranted to minimize risks to study participants.

# ORAL SESSIONS

**48**

## Uptake of Referrals to HIV Care and Treatment Services in Mwanza, Tanzania During the MDP 301 Phase III Clinical Trial of PRO 2000 Microbicide Gel

S. Lees<sup>\*1,3</sup>, G. Mtolela<sup>2</sup>, V. Selestine<sup>3</sup>, C. Barberousse<sup>1,3</sup>, J. Masanja<sup>2</sup>, A. Vallely<sup>4</sup>, C. Moffat<sup>1,2</sup>, S. Harvey<sup>1,2</sup>, R. Hayes<sup>1</sup>, and the MDP trial team

<sup>1</sup>London School of Hygiene and Tropical Medicine, London, UK; <sup>2</sup>African Medical and Research Foundation, Mwanza, Tanzania; <sup>3</sup>Mwanza Intervention Trials Unit, National Institute for Medical Research, Mwanza, Tanzania; <sup>4</sup>University of Queensland, Herston, Qld 4006, Australia

**BACKGROUND:** During the Microbicides Development Programme (MDP) clinical trial to assess the efficacy and safety of PRO 2000/5 gel participants who were diagnosed HIV positive at their screening visit and those who seroconverted during the clinical trial were referred to the government HIV Care and Treatment services (CTC). This study investigated the uptake of the CTC services by trial participants and using qualitative research methods, explored the enablers and barriers to uptake of these services.

**METHODS:** Staff at the CTC clinics were asked to collect MDP referral slips at the first visit. Trial clinicians visited the clinics regularly to collect slips. Qualitative research involved in-depth interviews (IDIs) with HIV positive women (2 screening/CTC attenders, 4 seroconverters/CTC attenders, 3 seroconverters/CTC non-attenders), focus groups (FGDs) with HIV negative trial participants (2) and participatory learning activities with participant representatives.

**RESULTS:** From September 2006 to February 2008, all women diagnosed HIV positive during screening (317) and all women who were positive at baseline or seroconverted during the trial (19 out of 1146 women enrolled) were referred to HIV CTC services. Women who seroconverted were given intensive support to attend the CTC centre. Of these, 42 (13%) women positive at screening attended CTC services and 7 (37%) seroconverters attended CTC services ( $p=0.005$ ). IDIs and FGDs revealed a good understanding of the cause and treatment of AIDS by HIV positive and negative women. Barriers to attending services were identified as disbelief of diagnosis, stigma, fear of disclosure to partners, disbelief in treatment effectiveness, or belief that God would provide a cure. Enablers to attending were good knowledge, supporting partner or family, or positive perceptions about the CTC clinic.

**CONCLUSION:** Referral was more effective with seroconverters due to the intensive support provided by the team, including follow up if they did not attend. Poor uptake of CTC services should be addressed during HIV prevention trials by offering support for HIV positive participants to attend, such as escorting the participants to the clinic or offering follow up counselling. Further investigation into community barriers to the uptake of CTC in Tanzania is also needed.

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## SPARTAC (Short Pulse AntiRetroviral Therapy at HIV Seroconversion)

N.P. Makeka\*, S.Gappoo, Prof. G. Ramjee  
HIV Prevention Research Unit, Medical Research Council

**BACKGROUND:** The HIV prevalence and incidence rates in South Africa continue to rise despite increased awareness and education and access to free condoms. HIV positive individuals still delay the initiation of Antiretroviral Treatment (ART) when needed. Reported 1000 South Africans die daily due to AIDS related illnesses. Reasons for this delayed uptake are numerous but mainly relate to fear of stigma, and discrimination. The following abstract examines some of the fears of acute seroconvertors enrolled in an ART trial in Durban, South Africa.

**METHODS:** HIV seroconvertors from ongoing Microbicide trials were enrolled in HIV treatment trials of acute infection. The SPARTAC trial was an open labeled, randomized controlled trial designed to assess use of ART at primary HIV infection. Participants were randomized into one of three arms: Long course combination ART for 48 weeks; Short course combination ART for 12 weeks and No Therapy.

**RESULTS:** Between November 2005 and May 2007, 87 women were enrolled into the trial and expected to attend regular clinic visit for 3.5 to 5 years depending on the date of enrollment. All women received intense adherence and supportive counseling in addition to clinical and laboratory monitoring throughout the trial. Below are some of the concerns and fears raised by women during enrollment.

### Fears:

- Taking pills everyday might lead to involuntary disclosure.
- Misconceptions that ARV's predisposed people to greater illness or death
- ARV might change sexual enjoyment (lower/higher libido).
- Potentially life threatening side effects
- ART may change body shape

### Concerns:

- Future resistance to ARV drugs.
- If I fall pregnant whilst on ARV, will ARV cause any harm to the fetus?
- Food restriction (to take before or after meal)
- Can I detoxify, or take traditional medication if I am on ARV's

Many of these fears and concerns were addressed by supporting the women through the initiation of treatment and disclosure to family and friends. Women were accompanied to DoH clinics to facilitate the process and allowed to contact clinic staff outside of their clinic hours for any additional concerns

**CONCLUSION:** Increased education and awareness is still required at a community level to address rumors and misconceptions that delay the initiation of ARTs. The trial was able to address this need for trial participants but this is required at a much larger scale for entire communities to benefit. This as well will be the implication on VOICE trial

# ORAL SESSIONS

SESSION 15  
Sunday, May 23, 2010

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## Long-term Follow up of HIV Seroconverters in Microbicide Trials—MTN 015

S. Riddler<sup>\*1</sup>, L. Wang<sup>2</sup>, S. Ganesh<sup>3</sup>, B. Makanani<sup>4</sup>, F. Martinson<sup>5</sup>, M. Kasaro<sup>6</sup>, F. Mhlanga<sup>7</sup>, S.S.Ou<sup>2</sup>, L. Levy<sup>8</sup>, K. Eskay<sup>1</sup>, U. Parikh<sup>1</sup>

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<sup>2</sup>Statistical Center for HIV/AIDS Research and Prevention, Seattle, WA, USA; <sup>3</sup>Medical Research Council, Durban, South Africa; <sup>4</sup>College of Medicine-Johns Hopkins University Research Project, Queen Elizabeth Central Hospital, Blantyre, Malawi; <sup>5</sup>UNC Project—Tidzive Centre, Kamuzu Central Hospital, Lilongwe, Malawi; <sup>6</sup>Centre for Infectious Disease Research in Zambia, Lusaka, Zambia; <sup>7</sup>UZ-UCSF Collaborative Research Programme, Harare, Zimbabwe; <sup>8</sup>Family Health International, Research Triangle Park, NC, USA

**BACKGROUND:** The effect of topical microbicide and oral pre-exposure prophylaxis (PrEP) use on the natural history of HIV-1 infection in participants who seroconvert is unknown. MTN 015 was established to monitor long term virologic, immunological, and clinical outcomes in women who become HIV-infected during prevention trials.

**METHODS:** MTN-015 study visits occur at enrollment and at 1, 3, 6 and every 6 months after seroconversion; additional visits occur after initiation of antiretroviral therapy (ART) (defined as any antiretroviral except single dose NVP). CD4+ T cell count, HIV-1 RNA, clinical history, and risk reduction counseling are completed at each visit. Behavioral questionnaires, CBC, chemistries, and STI tests are completed at most visits. Test results are provided to participants for clinical care at local ART programs. Genotypic resistance testing (ViroSeq) is performed at entry and when clinically indicated. Plasma, PBMC, cervicovaginal lavage and vaginal swabs are archived for future testing.

**RESULTS:** To date, 99/139 (71%) eligible women from HPTN 035 have enrolled at 5 African sites. At enrollment, the median age was 27 years; median time from seroconversion was 18 months; median follow up is 5.5 months to date. One participant died with presumptive TB 22 months after seroconversion. At enrollment, median CD4+ and HIV-1 RNA were 407 cells/mm<sup>3</sup> and 3.9 log<sub>10</sub> copies/mL for non-ART women and 560 cells/mm<sup>3</sup> and 2.6 log<sub>10</sub> copies/mL for those on ART. Thirty-six women have received ART (18 initiated prior to and 18 after enrollment). ART was initiated a median of 13.3 months after seroconversion. The most common initial ART regimen was NVP/3TC/d4T (58%). Twelve women stopped all ART during study follow up for side effects or other reasons. Resistance testing was completed on 68 participants (31 no results: 14 insufficient HIV RNA, 2 sequencing unsuccessful, 15 samples unavailable). A single PI mutation was observed (M46I or L) in 2 samples. One sample contained a two amino acid insertion in protease (M36I+NQ). Clade C virus was identified in 67/68.

**CONCLUSIONS:** Long term follow up of HIV-positive women in MTN-015 is feasible and acceptable to the participants. One-third of women have received some ART but discontinuation of ART was common. Transmission of HIV with significant drug resistance mutations was not observed. Ongoing enrollment into MTN-015 continues from current MTN trials including the VOICE study.

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## What Happens to Sites After a Trial; A Case Study From Nigeria

N. Onyejepu<sup>\*1</sup>, O.K. Obunge<sup>2</sup>, Umo-Otong<sup>3</sup>, T. Awopeju<sup>1</sup>

<sup>1</sup>University of Rome; <sup>2</sup>University of Port Harcourt,  
<sup>3</sup>Diacore

**BACKGROUND:** Development of a trial site requires considerable investment. Trial sponsors need to invest in local infrastructure including equipment, buildings, laboratories, transport and human resource development (training). The question of post-trial sustainability of sites is a key issue. This is more so when trials are halted due to questions related to low incidence of HIV as demonstrated in sites in West Africa. We present a case study looking at a site in West Africa two years after the study was halted.

**METHODS:** We developed a tool to assess the state of the trial sites two years after the study. The components of the tool included ability of sites to sustain infrastructures put in place using available resources, ability to integrate upgraded laboratory into institutional activities, application of skills acquired by personnel to enhance quality of service and involvement in research related activities attributable to the site upgrade.

**RESULTS:** The facility imbibed the need to sustain quality performance. The department of Medical Microbiology which was the main site of the study runs a revolving fund system and therefore was able to focus on maintaining infrastructures. The molecular based technology was not fully functional; the cost of reagents surpassed the funding presently attainable for research in the field. Integration into hospital services was limited since patients could not afford to pay the rate required to sustain specialized laboratory service. The clinical researchers have focused on other areas that readily attract funding.

**CONCLUSIONS:** The hospital gained significantly from the resources following the trial. However researchers will need to focus on partnership development that will enhance their ability to source for funds independently. Registration with in-country trial networks will allow for resource sharing and development of centers of excellence

# ORAL SESSIONS

## SESSION 16

### Oral Abstracts (OA9): Adherence in Microbicide Trials

**Moderators:** Elizabeth Tolley, Juliane Etima

Sunday, May 23, 4:30 pm–6:00 pm

Rooms 319–325

**52**

#### Development of a “Smart Applicator” for Vaginal Delivery of Microbicide Gels Can Help In Monitoring Adherence to Clinical Trial Protocol

D. Fairhurst<sup>1</sup>, S. Race<sup>\*1</sup>, S. Wilder<sup>2</sup>

<sup>1</sup>XiGo Nanotools, PA, USA; <sup>2</sup>International Partnership for Microbicides, MD, USA

**BACKGROUND:** While the need for an efficacious and acceptable vaginal microbicide gel is recognized, serious challenges remain regarding adherence with clinical protocols required to prove efficacy during large scale drug trials. The use of electronic devices and sensors to monitor adherence in microbicide trials is novel and, as yet, untested. The aim of this preclinical evaluation study was to develop a Smart Applicator (SA) system to accurately and precisely measure key vaginal use parameters. Such a system could be useful in verifying participant adherence to clinical trial protocol in future microbicide or other vaginal/reproductive health product trials.

**METHODS:** This Smart Applicator system comprises three elements: a single-use vaginal applicator containing a temperature sensor and an electronic module to regulate acquisition of biometric data during vaginal dosing, a Reader with USB interface (to receive data wirelessly from the SA after return to a clinic), and ComplianceChek Software. For test purposes the SA was filled with KY Jelly. Accuracy and precision of time, date and volume of gel delivery were verified under controlled laboratory conditions. ICH guidelines were followed for storage at elevated temperature (40°C; 65RH).

**RESULTS:** Extensive preclinical data for first a generation Smart Applicator design has been obtained, including power consumption during storage and use under developing world conditions, manufacturing process verification, temperature and humidity, durability, impact resistance, seal integrity, electrostatic discharge susceptibility, wireless communication robustness in high RF environments, time of deployment accuracy, temperature response, accuracy and repeatability. Results confirm that the device works as designed and suggest that it is robust enough to function under the environmental conditions in sub-Saharan Africa.

**CONCLUSIONS:** Preliminary results demonstrate the potential utility of the SA system to monitor adherence to clinical trial protocol. Work is in progress to develop a Gen II version that addresses some of the operational limitations found in the present study, to further optimize the design (smaller, re-usable), to implement the latest RFID Read/Write technology and to move to clinic-based feasibility.

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#### Predictors of Adherence in the Carraguard Phase 3 Trial in South Africa

B. Friedland<sup>\*1</sup>, D. Gawarecki<sup>1</sup>, M. Gehret<sup>1</sup>, S. Skoler<sup>1</sup>, L. Altini<sup>2</sup>, K. Ahmed<sup>3</sup>, G. Ramjee<sup>4</sup>, S. Patel<sup>2</sup>, C. Monedi<sup>3</sup>, N. Morar<sup>4</sup>, S. Govender<sup>1</sup>, R. Maguire<sup>1</sup>, P. Lahteenmaki<sup>1</sup>

<sup>1</sup>Population Council, New York, NY, USA; <sup>2</sup>University of Cape Town, Cape Town, South Africa; <sup>3</sup>University of Limpopo, Medunsa Campus, Soshanguve, South Africa; <sup>4</sup>Medical Research Council, Durban, South Africa

**BACKGROUND:** Determining adherence to product use in microbicide trials is critical for evaluating safety and efficacy. In an effort to understand reasons for low adherence in the Carraguard Phase 3 trial (gel used in 42.1% of sex acts), and to improve adherence in the future, an analysis was undertaken to identify factors that were predictive of adherence.

**METHODS:** A randomized, double-blind, placebo-controlled trial of Carraguard was conducted among 6,202 women in South Africa to assess its efficacy in preventing HIV. Product use was assessed with a dye test that indicated if opened applicators had been inserted vaginally. The proportion of sex acts with gel was estimated by dividing the aggregate number of inserted applicators by the aggregate number of sex acts. Logistic regression was used to assess factors predictive of adherence (ratio of inserted applicators to sex acts ≥ 80%) in the efficacy population (n=6005).

**RESULTS:** The strongest predictors of adherence were baseline coital frequency and study site; women who reported having sex 1–2 times per week were half as likely to be adherent as those who reported sex less than once a week (AOR 0.48, 95% CI 0.40–0.58). Women from Isipingo (Durban) and Soshanguve (Pretoria) were four times more likely to be adherent than those from Gugulethu (Cape Town) (AOR 4.16, 95% CI 3.32–5.21; AOR 4.07, 95% CI 3.27–5.07). Age was also highly predictive of adherence; compared to 16–21 year olds, women 39 and older were more likely to be adherent (AOR: 1.41, 95% CI: 1.12–1.78), although 22 to 29-year-olds were less likely to be adherent (AOR: 0.84, 95% CI: 0.68–1.04). Baseline reports of sex in exchange for money or abuse by steady partner and longer study participation were significant predictors of lower odds of adherence. Treatment group (Carraguard versus placebo), marital status, baseline condom use, post-enrollment HIV status, and number of partners were not significant predictors of adherence in the final model.

**CONCLUSIONS:** While further exploration is necessary to explain the large differences in odds of adherence at the three study sites, the results of this analysis suggest that the same factors associated with risk of HIV acquisition—being aged 22–29, having sex regularly, having an abusive partner, or having sex in exchange for money—may also be associated with difficulty using a coitally-dependent microbicide. Continued research is critical for developing products that women are able to use consistently.

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### The Performance of the Population Council Applicator Test in a Simulated Microbicide Trial

S. Littlefield<sup>1</sup>, J. Rankin<sup>1</sup>, B. Friedland<sup>1</sup>, B. Mensch<sup>1</sup>, P.C. Hewett<sup>2</sup>, S. Abbott<sup>1</sup>, K. Ahmed<sup>3</sup>, S. Patel<sup>4</sup>, G. Ramjee<sup>5</sup>, T. Palanee<sup>6</sup>

<sup>1</sup>The Population Council, New York City, New York USA; <sup>2</sup>The Population Council, Lusaka, Zambia;

<sup>3</sup>University of Limpopo/Medunsa Campus, Setshaba Research Centre, Soshanguve, South Africa;

<sup>4</sup>University of Cape Town, Emplisweni Centre for Wellness Studies, Gugulethu, South Africa; <sup>5</sup>Medical Research Council, Isipingo Clinic, Isipingo, South Africa

**BACKGROUND:** The discrepancy between high rates of self-reported gel use and the low proportion of covered sex acts based on the Population Council's applicator test in the Phase 3 Carraguard trial highlights the importance of understanding and improving measures of adherence. This 3-month simulated microbicide trial, conducted in the same three sites as the Phase 3 trial, improved upon the analysis of adherence; in this study adherence data were collected and analyzed by month, enabling a more accurate assessment of adherence, as well as the ability to examine changes in adherence over time.

**METHODS:** 849 women were enrolled and instructed to use applicators filled with placebo gel for three months. Participants were randomly assigned to complete monthly interviews via ACASI or FTFI, with responses validated by the applicator test, a dye test which indicated whether applicators had been inserted in the vagina. The percentage of sex acts covered by gel was calculated by dividing the number of opened and inserted applicators, based on the applicator test, by rounds of sex reported for the prior month. Self-reported gel use was calculated by dividing the total reported rounds of sex covered by gel by rounds of sex reported for the prior month. To compare the percentage of sex acts covered by gel by month, F tests were used.

**RESULTS:** Based on the applicator test, the aggregate percentage of sex acts covered by gel was estimated to be 47.2%, similar to the 42.1% found in the Phase 3 trial; estimates for months 1-3 were 53.2%, 47.4%, and 39.9%, respectively, a significant decline over time. This decline was consistent at all three sites over the course of the trial. As was the case in the Carraguard trial, self-reports of gel use suggested a considerably higher level of coverage, with an overall estimate of 86.3%; 85.2% in month 1, 87.8% in month 2, and 86.3% in month 3.

**CONCLUSIONS:** The magnitude of the discrepancy between the aggregate estimate of sex acts covered by gel as indicated by the applicator test and self-reported gel use in this simulated trial is similar to that observed in the Carraguard Phase 3 trial. These results suggest that the applicator test may be a useful tool both to objectively estimate adherence in microbicide trials and to understand how adherence changes over the course of a trial.

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### Feasibility and Utility of Returning Used and Unused Applicators in Microbicide Trials: Experiences in CAPRISA 004

A. Naidoo\*, M. Upfold, T. Gengiah, T.G. Masinga, C. Ngcobo, Z. Mkhize, L. Zondi, T. Nzimande, K. Naidoo, B. Moodley, B. Maharaj and L. Chelini on behalf of the CAPRISA 004 study team

*Centre for the AIDS Programme of Research in South Africa (CAPRISA), University of KwaZulu-Natal, Durban, South Africa*

**BACKGROUND:** In antiretroviral microbicide trials, study product accountability and monitoring is essential. Further, complete accountability for every applicator is useful to monitor adherence to assigned gel and to identify any problems that the participant may have with the applicator usage and mechanics. We report here on our experiences in collecting used and unused study applicators and elaborate further on the feasibility and utility of this procedure.

**METHODS:** Participants in CAPRISA 004 received uniquely identifiable new product at each visit and were required to return all used and unused applicators as part of monthly study visit procedures. Study product dispensed in the previous month was reconciled with the used and unused returns at the next visit.

**RESULTS:** A total of 172320 applicators were dispensed over 25 months, between 22 Oct 07 and 22 Nov 09, with an average of 6628 applicators issued per month (range: 430–9570). During this period, sustained high return rates were observed; only 1.7% of applicators could not be accounted for at the end of the study. 54.2% of the dispensed applicators were returned as used and 44.1% were returned as unused. On two separate occasions applicators belonging to another microbicide trial were identified through this procedure, leading to the identification of participants co-enrolled in other microbicide trials. Twenty four instances of product sharing were identified during gel reconciliation; the majority appear to be inadvertent due to participant's sharing storage spaces, especially when there is more than one study participant in a household. Each used applicator returned is visually assessed, thereby making it possible to ascertain when participants may be having difficulty with gel mechanics due to partially engaged plungers or partial expulsion of gel or visible excess gel loss into the strip bag provided for used returns. These instances were used as an opportunity to provide additional counselling and support to study participants on correct applicator use.

**CONCLUSIONS:** While the process of collecting and visually assessing applicator returns is time-consuming, this can be facilitated by creating special packaging for used returns and storing unused returns. In CAPRISA 004, full monthly reconciliation of all applicators was found to be feasible and an important source of data that was used not only to validate participant self-report of product use but to also to identify and prevent inadvertent or deliberate product sharing. It also provides the study team with an objective estimate of actual product requirement based on use, ensures adequate numbers of gel applicators are dispensed.

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### Assessing the Reporting of Adherence and Sexual Activity in a Simulated Microbicide Trial

B. Mensch<sup>1</sup>, P.C. Hewett<sup>2</sup>, S. Abbott<sup>1</sup>, J. Rankin<sup>1</sup>, S. Littlefield<sup>1</sup>, K. Ahmed<sup>3</sup>, N. Cassim<sup>4</sup>, S. Patel<sup>4</sup>, G. Ramjee<sup>5</sup>, T. Palanee<sup>6</sup>, S. Mierzwa<sup>1</sup>, S. Skoler-Karpoff<sup>6</sup>

<sup>1</sup>Population Council, New York; <sup>2</sup>Population Council, Lusaka, Zambia; <sup>3</sup>University of Limpopo/Medunsa Campus, Setshaba Research Centre, Soshanguve, South Africa; <sup>4</sup>University of Cape Town, Emplisweni Centre for Wellness Studies, Gugulethu, South Africa; <sup>5</sup>Medical Research Council, Isipingo Clinic, Isipingo, South Africa; <sup>6</sup>Memorial Sloan Kettering Cancer Center, New York, NY, USA

**BACKGROUND:** Misreporting of adherence and sexual behavior undermines detection of an association between product use and HIV infection in microbicide trials. Obtaining accurate self-reports minimizes the possibility that a candidate microbicide would be deemed ineffective when in fact low adherence is the underlying issue. This study investigates whether, in a placebo trial, audio computer-assisted self-interviewing (ACASI) produces more accurate reporting of adherence and higher reporting of sexual behavior than a standard face-to-face interview (FTFI).

**METHODS:** At the three South African clinics that participated in the Phase 3 Carraguard trial, 849 women were enrolled and instructed to use applicators filled with a placebo gel for three months. Participants were randomly assigned to FTFI or ACASI for behavioral interviews. Validation of self-reports was assessed through two biomarkers: a dye test, which indicates whether the applicator has been inserted in the vagina, and Rapid Stain Identification of Human Semen, which detects the presence of semenogelin in the vagina and is a biomarker of sex without a condom in the prior 48 hours. To compare reporting by interview mode, t tests and z tests were conducted. For the combined analyses across all follow-up visits, generalized estimating equation models were used to account for the within participant correlation.

**RESULTS:** For the most part ACASI generated significantly higher reporting of sexual behavior and vaginal hygiene practices, although the effect of the computer on reporting diminished somewhat over time. Women were about two times more likely to report sex without gel with ACASI. However, comparisons of reported and tested applicators did not indicate greater honesty about gel insertion with the computer. Comparisons of self reports of unprotected sex with the validated marker of recent semen exposure revealed more agreement with ACASI but differences were not large or consistent over time.

**CONCLUSIONS:** While it appears that ACASI encourages higher reporting of sensitive behaviors in HIV prevention trials, the results from this study suggest that even self-interviewing with a computer does not lead to full disclosure regarding adherence.

# ORAL SESSIONS

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## The "Wisebag:" An Innovative Strategy for Enhancing Measurement of Microbicide Gel Use in Clinical Trials

T. Gengiah<sup>\*</sup>1, L. Mansoor<sup>1</sup>, A. Naidoo<sup>1</sup>, M. Upfold<sup>1</sup>, K. Naidoo<sup>1</sup>, B. Maharaj<sup>1</sup>, B. Moodley<sup>1</sup>, Q. Abdool Karim<sup>1</sup>, S. Abdool Karim<sup>1,2,3</sup>, on behalf of the CAPRISA 004 Wisebag study team

<sup>1</sup>Centre for the AIDS Programme of Research in South Africa (CAPRISA); <sup>2</sup>University of KwaZulu-Natal, Durban, South Africa; <sup>3</sup>Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, USA

**BACKGROUND:** Direct adherence assessment methods in microbicide trials rely mainly on participant self-report of product use and are therefore prone to bias from inaccurate participant recall and social desirability in responses. The *Wisebag* (which records opening events and can provide automated reminders) was developed to provide an indirect, objective measure of adherence and support participant's adherence with cell phone reminders. This pilot study aimed to assess reliability of self-report by comparing it with the *Wisebag* generated data.

**METHODS:** Ten participants, from the 24 screened, were enrolled after providing informed consent and were followed up for a maximum of 4 months. A short questionnaire was administered at enrollment, months 1, 3 and at exit to assess acceptability of the *Wisebag* and challenges experienced during use. Additionally, each time the participant opened the *Wisebag*, a reminder text message was sent to the participant. *Wisebag* opening dates and times were recorded via an electronic unit that sensed when the bag was opened by means of a reed-switch in the lid and the unit then transmitted a signal to a central server. These data were then correlated with self-reported gel use and the number of used applicators returned to the study pharmacy.

**RESULTS:** Thirty three monthly study visits were completed on 10 participants. During these 33 months, there were 161 recorded opening events, self-reported use of 170 applicators and 183 returned used applicators. A total of 129 of the 161 opening events involved removal of a single applicator. There were 26 *Wisebag* opening events which involved removal of more than one applicator, with one incident of 4 applicators retrieved at once. Six openings of the *Wisebag* involved no applicators being removed and used. Only 14 of the 33 monthly reports had the same number of return used applicators as *Wisebag* openings; principally due to participants retrieving more than one applicator in 16.1% of the *Wisebag* openings. Only 63% (21/33) of participant reports of the date of last gel insertion corresponded accurately with the *Wisebag*-recorded opening events. One visit had no used applicators and no reported sex but one recorded opening event. In the remaining 11 visits, the variation in recall compared to *Wisebag* recordings was on average 9.5 days with a range from -20 to +7 days (ie. participants reported gel insertion up to 20 days before the last recorded opening event or up to 7 days after the last recorded opening event). For comparative purposes, there was 91% (30/33) concurrence between self report and returned used applicators. In terms of acceptability, 9 of the 10 participants preferred the *Wisebag* compared to the CAPRISA 004 gel storage bag, due to its compact size, exclusivity and content anonymity. No difficulties were reported regarding *Wisebag* storage in the home. The sms reminder on correct gel use was considered very helpful by 8 of the 10 participants.

**CONCLUSIONS:** The *Wisebag* was found to be acceptable and its cell phone reminders useful. The *Wisebag*'s opening event data served as a useful measure of adherence, providing a more accurate measure of date of last use. However, it underestimates overall applicator use due to occasional multiple applicator removals from a single opening. Openings without applicator removal also occur but are less common. These results suggest that the *Wisebag* could be a valuable tool to measure adherence, especially to assess accuracy of recall of the last applicator use. We recommend the *Wisebag* for larger scale testing before it can be considered for implementation in microbicide trials.

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## Daily Monitored Adherence as a Possible Adherence Enabler in Microbicide Trials

A. Nel, A. Lambert, M. Isaacs\*, M. Albertse, L. Coetzee

International Partnership for Microbicides, Paarl, Western Cape, South Africa

**BACKGROUND:** Participant adherence to daily product use is a significant challenge in microbicide efficacy trials. To reduce the influence of adherence on interpretation of efficacy results, IPM is piloting Daily Monitored Adherence (DMA) based on the Daily Observed Therapy method used in tuberculosis treatment. The trial is assessing the logistics of DMA implementation and the feasibility to scale-up into a large Phase III efficacy trial.

**METHODS:** Six research centers enrolled participants in a 6-week trial of a microbicide gel using the DMA method. Gel application was not directly observed, but monitored through daily contact on weekdays with trial participants and collection of used applicators. Home visits, drop-off centers or a combination of these methods were employed. Designated staff monitored adherence through the daily collection of used applicators Monday to Friday. If participants forgot to apply the gel, they were asked to do so in privacy and immediately return the used applicator to the study staff member. Participants do not have contact with study staff over weekends or on public holidays.

During adherence contacts, a short questionnaire is completed documenting reasons for non-adherence, missed visits, as well as any other relevant information. Adherence counseling is provided as and when indicated.

**RESULTS:** Preliminary results indicate that more than half of participants prefer home visits by a member of staff. The first home visit on average lasts 15 minutes and thereafter approximately 8 minutes. Drop-off center visits are slightly shorter. Most participants prefer to see the DMA staff in the morning whereas gel is inserted either in the morning or evening. Less than 5% of participants reported to have used the gel outside of time and/or date decided upon. All applicators were returned and less than 5% outside of the time and/or date decided upon. A smaller percentage of participants withdrawn due to partner concern or community disclosure reasons.

**CONCLUSION:** Preliminary results indicate that participants do comply with DMA requirements. DMA provides an ongoing opportunity for counseling and AE reporting and is in general well managed by Outreach Workers. To date, implementation of the DMA process has had no apparent impact on participant retention in the trial.

## SESSION 17

### Mini-Symposia (MS3): Product Selection Strategies

**Moderators:** Roberta Black, Gustavo Doncel

Sunday, May 23, 4:30pm–6:00pm

Rooms 403–405

# 59

### Product Selection— An Industry Perspective

Louise Pedneault

Pfizer Vaccine Clinical Research, USA

# 60

### Presentation of Product Profiles

Randy Tressler

Independent Consultancy, USA

The HIV microbicide field has been moving from development of polyanionic microbicides to the development of antiretroviral (ARV) based prevention strategies. These strategies include topical, subcutaneous and oral routes of administration. With nearly thirty unique oral ARV agents approved by regulatory agencies, and with more ARVs in development there are a large number of possible individual as well as combination ARVs that can be considered for prevention approaches. Candidates for development can be selected based on careful evaluation of product characteristics such as: mechanism of action; physicochemical characteristics; pharmacokinetics including concentration in vaginal secretions; safety; resistance (probability of selecting or inducing resistance) as well as the impact of resistance on subsequent therapy; acceptability of administration/adherence; and cost. The product profile of the top combinations will be presented and discussed by a panel of experts in microbicide formulation and clinical development.

# 61

### Round Table Discussion of Products and Criteria for Advancing Products to Later Stage Development

Zeda Rosenberg

International Partnership for Microbicides, USA

A decision paradigm is critical to being able to rank and progress development of microbicides. Some criteria such as mechanism of action, potency, and redundancy in the field are important early in lead selection while others such as ease of manufacture, acceptability, cost, and the intellectual property landscape are important in later stages. All however should be weighed prior to reducing efforts to a single lead with appropriate backups.

Consideration must also be given to potential dosage forms, each of which has its pros and cons. The physical and chemical properties of the microbicide candidate must be consistent with the dosage form. High, medium, and low priority products can be established based on key criteria with concomitant prioritization of resources for their development.

Justification for product advancement occurs at each stage in the development process: CMC, safety, and efficacy. The product must be proven safe in *in vitro* genotoxicity and cytotoxicity studies as well as preclinical animal studies. The product must also be proven effective in cell culture and *ex vivo* tissue models, and have an appropriate PK profile in women.

Finally, potential risks are assessed and monitored throughout the drug development process to increase the likelihood of bringing an effective microbicide product to market.

# ORAL SESSIONS

**62****Round Table**Henry Gabelnick  
CONRAD, USA

An algorithm has been developed to evaluate potential candidates for clinical evaluation. Initial testing includes *in vitro* and *ex vivo* screening for activity and safety parameters. A modified rabbit vaginal irritation test for toxicity including evaluation of biomarkers also is employed. At the time a formulated active pharmaceutical ingredient (API) is first evaluated for safety, initial assessments are made of acceptability, particularly ease of insertion and messiness, if a gel product. Important considerations in the selection process include stability of the API and the product, ease of manufacture and cost to manufacture. Finally comparative advantages compared to other APIs in the same class and other classes must be considered.

**63****Round Table**Yasmin Halima  
*Global Campaign for Microbicides, USA*

The vulnerability of women and high-risk groups posed by the biological and social determinants of HIV, drive the development pathway for microbicides and pre-exposure prophylaxis (PrEP). However, the decision to invest in the development of an effective prevention agent is ultimately determined by a combination of complex factors.

Product selection must begin with an understanding of the social and cultural environment in which a woman might use a microbicide or PrEP, as well as its scientific rationale. Some of the questions from an advocate perspective that would influence the decision to recommend an agent for clinical development include:

- is the product effective (level of efficacy)
- is it safe (safety profile)
- does it fit her life style (dosing strategy)
- is it accessible and affordable
- can it be used without her partners knowledge if she fears violent consequences (used without detection; application of nanotechnology)
- does it enhance sexual pleasure (viscosity, smell, taste)
- will it protect against pregnancy (contraceptive)
- enable her to practice safer sex while maintaining the ability to become pregnant
- if it contains an antiretroviral, might it compromise future treatment options or lead to transmission of resistant virus (treatment and public health impact)

**64****Round Table**Stephen Becker  
*Bill & Melinda Gates Foundation, USA*

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**Characterization of Seminal Enhancer of Viral Infectivity (SEVI) on HIV-1 Penetration in the Female Genital Tract**

S. Allen\*, M. Anderson, Z. Okocha, T.J. Hope

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**BACKGROUND:** One mode of human immunodeficiency virus type 1 (HIV-1) transmission occurs via vaginal exposure to the semen of HIV-1 infected men. For a productive infection to occur, HIV-1 within semen must gain access to target cells by penetrating natural mucosal barriers including the cervical epithelium. Seminal enhancer of viral infectivity (SEVI) amyloid fibrils enhance HIV infectivity by sequestering the virus and promoting viral/host cell interactions as shown by Munch et al., 2001. However, these *in vitro* studies were conducted using multiple cell-lines and immune cells isolated from whole blood and may not be optimal for studying the role of SEVI in the context of HIV transmission.

**METHODS:** To characterize the effects of SEVI on HIV-1 penetration of the cervical epithelium, studies were conducted using a more biologically relevant approach: human cervical explants. For *ex vivo* studies, fresh human cervical specimens were dissected into 1cm cubes of endo- and ectocervix and incubated with photo-activatable GFP-Vpr HIV in the presence or absence of SEVI for four hours. Tissues were then removed from inoculum, sectioned, stained and imaged.

**RESULTS:** Results show that in the human ectocervix, SEVI/HIV-1 complexes aggregate at the outermost surface of the ectocervical epithelium. Moreover, the number of penetrating virions was decreased three-fold in SEVI treated ecto-cervical tissues although the degree of penetration was similar. The introduction of SEVI to endocervical tissues seemed to modify filamentous SEVI into a spherical conformation. Analysis of penetration in the endocervix illustrates that in both treated and untreated conditions, the number of penetrating virions and the extent of penetration were similar.

**CONCLUSIONS:** Taken together, these results indicate the likelihood of two distinct roles for SEVI. In the ectocervix, SEVI may act to inhibit penetration of the epithelium while in the endocervix, SEVI may shuttle HIV through the epithelium to enhance penetration.

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**Comparison of *Ex Vivo* HIV Infection of Polarized and Non-polarized Cervical Explant Cultures as Surrogates for HIV Efficacy**J.D. Lynam<sup>\*1</sup>, N. Billitto<sup>1</sup>, M. Cost<sup>1</sup>, R.P.K. Na Ayudhya<sup>1</sup>, L.C. Rohan<sup>1,2</sup>, C.S. Dezzutti<sup>1,2</sup><sup>1</sup>Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, PA, USA; <sup>2</sup>University of Pittsburgh, Pittsburgh, PA, USA

**BACKGROUND:** Microbicides are a promising approach for HIV prevention. One of the greatest challenges facing microbicide research is the development of a model that accurately represents real life situations and that can successfully predict clinical trial outcomes. To address this challenge, we compared the ability to infect polarized and non-polarized cervical biopsies with HIV *ex vivo*.

**METHODS:** A Tischler biopsy punch was used to excise 5mm × 3mm biopsies from each tissue. Non-polarized cervical biopsies were placed in a 48 well plate. Polarized biopsies were positioned on a 3mm hole punched into the membrane of a transwell. Matrigel was used to seal the biopsies, leaving only the epithelium of the biopsy apically exposed. Biopsies were placed in 1 ml of complete cervical medium + 1% IL-2 (c-medium). HIV-1<sub>BaL</sub> was added to the apical surface of polarized biopsies and added directly to non-polarized biopsies. They were incubated for 4 hours and washed with 1 ml of HBSS in triplicate. C-medium was added to each biopsy and cultured for 21 days. Media was collected from each biopsy every 3 to 4 days and replenished with c-medium. Collections were stored at -80°C until needed. Upon completion of the 21 day culture, biopsies were washed, fixed in 10% formalin, and submitted for immunohistochemistry (IHC) analysis with p24 specific antibodies. HIV-1<sub>BaL</sub> infection was assessed using HIV-1 p24 ELISA on the stored culture supernatant.

**RESULTS:** Non-polarized biopsies demonstrated higher levels of p24 in culture. Median p24 log<sub>10</sub> on day 14 of culture was 2.4 pg/mL for polarized and 3.6 pg/mL for non-polarized biopsies, respectively. This 1.2 log<sub>10</sub> difference was statistically significant ( $p < .001$ ) (Unpaired t test with Welch correction). IHC confirmed the p24 ELISA data with 71% of polarized biopsies and 88% of non-polarized biopsies showing p24 antigen positive cells.

**CONCLUSIONS:** These data suggest that non-polarized biopsies are more susceptible to infection with HIV-1<sub>BaL</sub> than polarized biopsies. Higher rates of infection are important for reproducibility and elimination of false negatives. Using non-polarized cervical biopsies for clinical studies would be the most efficient method for testing the *ex vivo* efficacy of a microbicide (topical or oral) because they are more readily infected.

# SUNDAY POSTERS

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## Interlaboratory Variability in Detection of Cytokines by Bioluminex: Implications for Microbicide Safety Studies

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**BACKGROUND:** Measurement of cytokines in genital tract secretions is being promoted as a potential biomarker of microbicide safety. However, the interlab variability in measurements is not known.

**METHODS:** To address this gap, cervicovaginal lavage (CVL) samples that had been collected as part of ongoing Phase I clinical safety trials were evaluated in a blinded fashion in three independent laboratories to determine the interlaboratory variability in measurements. Thirty- six samples from 12 subjects (Day 0, Day 7 and Day 14 of a Phase I microbicide gel study) were aliquoted and distributed to each laboratory and analyzed using the identical multiplex kit for INF- $\alpha$ 2; INF- $\alpha$ 2, IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , IL-10 and IL-17. Data were analyzed on log transformed values by Friedman's (paired non-parametric) and then pairwise (Wilcoxon) tests.

**RESULTS:** There were significant differences between labs for IFN- $\alpha$ 2 and IL-8 on all days and for IL-10 on Day 0, IL-1 $\beta$  on Day 14 and IL-6 on Day 7 with Lab C > Lab B ~ Lab A. However, there were no differences in the quartiles for the labs for any of the cytokines at Day 7 and Day 14 ( $p > 0.3$ ); the ordering of the samples is similar between the labs in all cases.

TABLE 68.1

	Lab A Day 0	Lab B Day 0	Lab C Day 0	Lab A Day 7	Lab B Day 7	Lab C Day 7	Lab A Day 14	Lab B Day 14	Lab C Day 14
INF- $\alpha$	0.66	0.91	1.28	0.63	0.77	0.96	0.63	0.77	0.89
INF- $\gamma\gamma$	0.57	0.51	0.57	.52	.50	.52	.58	.77	.57
IL-1 $\beta$	1.24	1.12	1.31	.94	.92	1.05	1.37	1.32	1.46
IL-6	1.45	1.34	1.53	1.29	1.29	1.51	1.51	1.55	.1.77
IL-8	2.71	2.51	2.54	1.38	1.67	2.25	1.67	1.99	2.51
TNF $\alpha$	.51	.51	.51	.51	.51	.51	.62	.60	.58
IL-10	.55	.52	.64	.58	.55	.57	.60	.57	.58
IL-17	.51	.53	.56	.51	.51	.51	.51	.51	.53

**CONCLUSIONS:** Thus, although the absolute values may differ across laboratories and should not be directly compared, actual changes in local immune response to microbicides should be reliably detected across different laboratories.

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## Early Colorectal Responses to HIV-1 and Modulation by Microbicides

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**BACKGROUND:** The extreme vulnerability of the colorectal tract to HIV-1 infection is likely due to histological and immunological characteristics of the intestinal mucosae. The aims of this study were to determine the genomic responses to HIV-1 exposure in the colorectum and if these responses are dependent upon infection and/or modulated by microbicides.

**METHODS:** Colorectal explants were incubated with or without drugs for one hour before addition of virus. Viral penetration within colorectal lamina propria was assessed with fluorescent viral particles (BaLPAGFPGag). Early responses to viral exposure were analyzed by Luminex and RNA microarray, at two set time points: 6 h (representing responses induced by viral attachment, entry and early reverse transcription) and 24 h (corresponding to viral integration and productive infection).

**RESULTS:** Viral exposure studies using fluorescent BaLPAGFPGag demonstrated virus penetration of up to 39 m into the lamina propria within 6 h. HIV exposure to tissue for 6 h induced an increase in the levels of IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, MCP-1 and GCSF secretion. Further secretion of these pro-inflammatory cytokines was detected after 24 h of incubation independently of viral tropism. The X4-isolate NL4.3 induced higher levels of RANTES, MIP-1 $\alpha$  and MIP-1 $\beta$  secretion than R5-HIV-1 YU.2. Potential drug candidates for colorectal microbicides, including entry, fusion or reverse transcriptase inhibitors demonstrated differential capacity to modulate these responses.

**CONCLUSIONS:** Gene expression is differentially affected by viral tropism and time. Early responses of colorectal tissue after exposure to HIV-1 can be modulated by RTI and entry inhibitors.

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## Effects of Cellulose Sulfate on the Structural and Functional Integrity of Human Cervicovaginal Epithelium

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**BACKGROUND:** Cellulose sulfate (CS) has been reported to exhibit in vitro and in vivo antiviral activity against HIV. However, a phase III clinical trial of a vaginal gel formulation of CS showed lack of effectiveness. Furthermore, subgroup data analysis suggested the possibility that CS might increase the risk of HIV acquisition. Among plausible biological causes for this finding are disruption of epithelial function and induction of a proinflammatory state in the cervicovaginal mucosa. We investigated these hypotheses using cell and tissue-based models.

**METHODS:** Human vaginal epithelial (VK2/E6E7) and endocervical (END-1/E6E7) cell lines and human cervicovaginal (CV) reconstructed tissues (EpiVaginal) were incubated with multiple concentrations of CS and hydroxyethyl cellulose (HEC, placebo) for different times. Main study endpoints for cell-based experiments were: cytotoxicity (MTS, LDH), gene expression (microarray, qPCR), and immune mediator release (multiplex, ELISA). In addition, transepithelial electrical resistance (TEER), fluorescein (NaFl) permeability, tight junction protein expression and HIV infection were evaluated in tissues treated in vitro with the clinical gel formulations.

**RESULTS:** Neither CS nor HEC were cytotoxic to VK2 and END cells or to reconstructed CV epithelium at the highest concentrations tested. Treatment of these cells or tissues with CS did not induce proinflammatory mediators when compared to HEC. Except for an early, transient upregulation of CYP450, CS gene expression profile was similar to that of HEC. CV tissues treated with CS gel did not show a decrease in viability or TEER or an increase in NaFl permeability. Epithelial expression of tight junction proteins, E-cadherin and desmoglein-3, was not different in CS and HEC treated tissues either after single or multiple 6hr daily doses. Pre-treatment of tissue with CS blocked HIV infection.

**CONCLUSIONS:** CS does not appear to show cytotoxic or proinflammatory effects on vaginal or cervical epithelial cells and tissues. Furthermore, the epithelial barrier function of reconstructed CV tissues is not compromised after a single or multiple doses of 6% CS gel.

**71****Comparison of Ex Vivo Colorectal Explant Infection Using Fresh Versus Freeze Thawed Tissue**

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**BACKGROUND:** Recently Phase 1 microbicide studies have included ex vivo/in vitro explant challenge studies to generate pharmacodynamic data. The ability to freeze intestinal explant tissue and conduct standardized infection studies in one center would likely improve the precision of explant experiments. We conducted freeze/thaw experiments to address this question.

**METHODS:** 20 biopsies were acquired from each of 14 healthy seronegative controls via flexible sigmoidoscopy at 30cm from the anal verge. 10 biopsies were used for immediate explant infection, MTT assay and histology; 10 were frozen and thawed for later infection using combinations of 3 freeze protocols (FP) and 2 thaw protocols (TP). FP#1: snap frozen in liquid nitrogen; FP#2: collection on ice prior to the addition of freeze medium (7%DMSO/FBS); FP#3: collection in cold culture medium (RPMI/10%FBS) prior to the addition of freeze medium; TP#1: rapid thaw at 37°; TP#2: thaw by swirling at 37° with gradual exposure to incremental volumes of culture medium. Explant infections, in triplicate, used 2 titers ( $10^4$  and  $10^2$  TCID<sub>50</sub>) of HIV-1BaL virus with endpoint being the mean cumulative p-24 at day 14. MTT and histology were assessed at time of infection.

**RESULTS:** There was no difference in tissue viability or histology between the fresh or frozen tissue. Using fresh tissues, 100% infection was achieved with TCID<sub>50</sub>  $10^4$  (mean p-24 7332 pg/ml  $\pm$  5874); 93% infection occurred using TCID<sub>50</sub>  $10^2$  (mean p-24 3258 pg/ml  $\pm$  3102). For FP#1/TP#1 infection at day 14 was <1% of control at TCID<sub>50</sub>  $10^4$  and  $10^2$ ; FP#2/TP#2 was 16.5% $\pm$ 18% of control at TCID<sub>50</sub>  $10^4$  and 1%  $\pm$  2% at TCID<sub>50</sub>  $10^2$ ; FP#3/TP#2 was 37% $\pm$  44% of control at TCID<sub>50</sub>  $10^4$  (n=9) and 33%  $\pm$ 44 of control at TCID<sub>50</sub>  $10^2$ .

**CONCLUSIONS:** Fresh intestinal explants were infectible ex vivo but no FP/TP combination produced equally productive infections. Interestingly, tissue viability by MTT assay did not predict the inability to establish productive ex-vivo HIV-infection. FP/TP for colorectal tissues, are challenging and additional efforts are needed if this approach is to be utilized.

**72****Broad Spectrum Endogenous Antimicrobials in Secretions from Female Reproductive Tract Epithelial Cells in Culture**

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**BACKGROUND:** Relatively little is known about the mechanisms through which the female reproductive tract (FRT) is protected against the diverse sexually transmitted infections (STI) that threaten the lives and reproductive health of women. In addition to providing a physical barrier to infectious microorganisms, FRT epithelial cells, which are the sentinel cells to first see potential STI pathogens, actively secrete a spectrum of constitutive and induced factors that inhibit infection by some STI pathogens. To understand the scope of these secreted factors, we hypothesized that epithelial cells secrete innate antimicrobial factors that inhibit a range of potential pathogens of the FRT.

**METHODS:** Epithelial cells were isolated from FRT tissues (Fallopian tube, uterus, endocervix and ectocervix) following hysterectomy and cultured in cell inserts until confluence for lower tract cells or polarization for upper tract cells. Apical secretions from primary cultures from epithelial cells were incubated for one hour with HIV-1, *Neisseria gonorrhoeae*, or *Candida albicans* prior to being tested for their ability to grow and/or infect target cells. Secretions from FRT epithelial cells incubated with *Lactobacillus crispatus* was also tested to determine whether they were inhibitory to commensals found in the lower FRT. Several factors in uterine epithelial secretions were identified by ELISA or Luminex analysis.

**RESULTS:** Epithelial cell secretions from throughout the FRT inhibit, *N. gonorrhoeae* and *C. albicans* infection of target cells. When HIV-1 R5 strains were analyzed, secretions from the uterus, cervix, and ectocervix had HIV neutralizing activity. In contrast, HIV-1 X4 has less activity in secretions from epithelial cells from the upper and lower FRT. Interestingly the commensal *L. crispatus* was not affected by any of the secretions tested. Thus, epithelial cells from throughout the FRT secrete factors with anti-viral, anti-bacterial and anti-fungal activity. Antimicrobial, cytokine and chemokine analysis of uterine secretions revealed several candidate molecules that could account for pathogen inhibition.

**CONCLUSIONS:** These findings provide definitive evidence for the critical role of epithelial cells in protecting the FRT from infections, without comprising the beneficial presence of commensals.

**73****Mechanisms of Cellulose Sulfate Interaction with the Human Bacteria-Colonized Cervicovaginal Epithelium—Use of a New *In Vitro* Model to Assess Microbicide Safety**

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**BACKGROUND:** Several anti-HIV microbicides including cellulose sulfate (CS) have passed conventional preclinical evaluation and safety trials, yet ultimately failed to succeed in Phase III trials despite their anti-HIV activity in surrogate models of efficacy. Concerns have been raised that current preclinical algorithms are deficient in addressing the complexity of the human vaginal environment. A limitation of traditional vaginal in-vitro models is that they have lacked the physiologic host-microflora interactions and thus ignored the role of the normal microbial biofilm as a modifier of the epithelial immune function. We applied a novel microflora-colonized cervicovaginal epithelial model to investigate potential interferences of CS with the vaginal barrier function.

**METHODS:** Immortalized and primary cervical and vaginal epithelial cells were colonized with normal and bacterial vaginosis associated microflora (BV) and exposed to a panel of microbicide compounds including CS for up to 24h. Epithelial colonization was assessed by colony forming units of bacteria stably associated with the vaginal epithelial cells in the presence of microbicides and pathogenic bacterial and viral determinants. NF- $\kappa$ B activation was assessed by a luciferase reporter assay. Extracellular and intracellular inflammatory, immunoregulatory, antimicrobial and apoptotic markers were measured. Dose responses were determined and differences between treatments were assessed by ANOVA

**RESULTS:** At low concentrations (<100 g/mL), CS enhanced NF- $\kappa$ B activation but not cytokine production. In bacteria colonized vaginal cells, low dose CS even decreased cytokine production. At higher doses, CS increased cytokine production by BV-exposed endocervical cells but reduced TLR-mediated cytokine production against viral and bacterial determinants by bacteria-colonized vaginal and endocervical cells. Epithelial responses to viral determinants were suppressed by BV, and CS did not modify this effect. Hydroxyethyl cellulose did not induce any of these changes.

**CONCLUSIONS:** These results suggest that microbicides can differentially modulate vaginal-microflora interactions and suggest that CS may have variable pro- and anti-inflammatory effects depending on drug concentration and variations in the vaginal microflora. Further studies are needed to assess the importance of these interactions regarding the safety of microbicide candidates.

# SUNDAY POSTERS

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### Predictors of Unprotected Sex among Female Sex Workers in Madagascar: Comparing Semen Biomarkers and Self-reported Data

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**BACKGROUND:** Research on the determinants of condom use and condom non-use generally has relied on self-reported data with questionable validity. The detection of prostate-specific antigen (PSA) in vaginal fluid is a biological marker of exposure to semen within the previous 48 hours. We identified predictors of recent, unprotected sex among female sex workers (FSWs) using two outcome measures: self-reports of unprotected sex within the past 48 hours and detection of PSA.

**METHODS:** The study was conducted among 331 FSWs in two sites in Madagascar participating in an 18-month, randomized controlled trial on condom promotion. Participants were recruited at their last study visit for this additional research, which consisted of a short questionnaire on recent coitus and condom use and the collection of vaginal swabs to test for PSA. We simultaneously fit models for the two outcome measures using bivariate logistic regression; we chose this approach because assessing whether two models differ requires a direct comparison of the models. We used manual backwards elimination to reduce the full model; variables were only removed if they were non-significant at the 0.05 level for both outcomes.

**RESULTS:** Multivariable logistic regression revealed that self-reported unprotected sex was associated with three factors. Younger women had a greater odds of self-reported unprotected sex compared to women ≥35 years of age (adjusted OR [aOR], 2.1; 95% CI, 1.1–4.0). Women reporting current hormonal contraception use were less likely to report unprotected sex (aOR, 0.4; 95% CI, 0.2–0.9). Finally, women who reported having ≥1 sipa (emotional partner) in the previous seven days had an odds of reporting unprotected sex 4.8 times (95% CI, 2.9–8.1) that of women without a sipa. The sole factor related to having PSA detected was prevalent chlamydial infection (aOR, 4.5; 95% CI, 2.0–10.1).

**CONCLUSIONS:** Differences in predictors identified suggest that determinants of unprotected sex, based on self-reported behaviors, might not correlate well with risk of semen exposure. The variables significantly associated with self-reported unprotected sex might be more appropriately interpreted as correlates of the reporting behavior, rather than the behavior of interest (i.e., unprotected sex). Caution must be taken when interpreting self-reported sexual behavior measures or when adjusting for them in analyses evaluating interventions for the prevention of HIV/STIs.

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### Optimising Endocervical Mucosal Cell Sampling for the Study of HIV Mucosal Target Cells in Microbicide and Mucosal Vaccine Research

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**BACKGROUND:** Microbicides and vaccines interact with vaginal mucosal immune responses. Measuring cellular composition of cervical mononuclear cells (CMC) is gaining importance in research settings and clinical trials. A fine brush, designed for cytology, collects cells effectively but yield for flow cytometry assays is often low and red blood cell (RBC) contamination high. We hypothesised that an L-shaped flocked swab could be more mucosa-friendly and possibly better at absorbing and releasing cells especially in the case of ectopy, than a brush.

**METHODS:** A comparison is made for the yield of live leucocytes (LL) between the swab (Copan) and brush (Cellpath® 9 mm Ø) in a randomised cross over design over 2 cycles with samples taken on day 9 and day 23. The samples are kept in PBS on ice and cells are counted within 1 hour. LL are counted in a Neubauer chamber after trypan-blue staining. The supernatant is tested for free haemoglobin, RBC and leucocyte esterase (LE) with a urine dipstick (Servotest®@5+NL).

**RESULTS:** The median value for LL was  $0.34 \times 10^6$  (mean of  $1.6 \times 10^6$ ) and no significant difference was seen for sampler used. Similarly, no difference in yield for sampler was seen over the ectopy groups separately. Absence of blood, presence of ectopy (visual inspection) and a positive LE test were significantly related to yield. Both swab ( $p=0.005$ ) and brush ( $P=0.000$ ) had a higher yield when ectopy was present (Table 75.2). No difference ( $P=0.3$ ) for presence of blood was seen between samplers.

TABLE 75.1 Yield of LL in Relation to Characteristics

27 Participants	N (%)	Median <sup>1</sup>	IQ range <sup>1</sup>	Range <sup>1</sup>	P Value*
	92 samples	0.31	0.12–1.55	0–14.2	
<b>Sampler</b>	<b>Swab</b>	46 (50)	0.41	0.21–1.99	0–14.2
	<b>Brush</b>	46 (50)	0.33	0.08–1.34	0–11.4
<b>Blood</b>	<b>Negative</b>	43 (47)	0.65	0.21–2.34	0–12.3
	<b>Positive</b>	49 (53)	0.22	0.08–0.7	0–14.24
<b>Ectopy</b>	<b>Absent (11)</b>	38 (41)	0.14	0.06–0.35	0–13.75
	<b>Present (16)</b>	54 (59)	0.82	0.27–1.99	0.06–14.24
<b>LE</b>	<b>Negative</b>	45 (49)	0.21	0.06–0.35	0–2.2
	<b>Positive</b>	47 (51)	1.34	0.32–2.85	0.06–14.24

TABLE 75.2 Yield of LL for Ectopy by Sampler

Participants (N)	Swab		Brush		P value*
	N samples	Median <sup>1</sup>	N samples	Median <sup>1</sup>	
<b>Ectopy</b>	No (11)	19	0.21	19	0.07
	Yes (16)	27	0.75	27	0.88

\*Wilcoxon rank – sum (Mann Whitney)

<sup>1</sup> LL ×10<sup>6</sup>

**CONCLUSIONS:** Live leucocyte cell yield in this nulliparous healthy population is low and no difference is seen between the sampling devices tested in this interim descriptive analysis. Cell yield was significantly higher if ectopy was present.

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### HIVgp120 Binding to a CD4 Independent Protein of 160kDa on Human Spermatozoa

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**BACKGROUND:** HIV infection of target cells requires binding of HIV gp120 to the host cell CD4 receptor. However, HIV has also been reported to bind and enter into cells devoid of the CD4 receptor. Our laboratory has identified a protein of 160kDa on human spermatozoa (CD4 -ve) that binds to HIV. The present study aimed at identifying, characterizing and understanding the mode of action of this receptor on human spermatozoa.

**METHODS:** The N-terminal amino acid sequence of the 160kDa protein and its peptide obtained by tryptic digestion were determined. Polymerase chain reaction amplification of human testicular cDNA was performed using degenerate primers corresponding to peptide sequences of the 160kDa protein. Localization of 160kDa protein on sperm was performed using fluorescently labeled gp120, followed by inhibition experiments using antagonists to determine the specificity. An HIV-gp120-tracking experiment was performed, wherein spermatozoa were examined for surface HIV-gp120 and internalization over a time course of 24 hours.

**RESULTS:** The partial cDNA sequence of the 160kDa protein demonstrated 99% identity with human macrophage mannose receptor. Sequence of testicular mannose receptor was obtained and exhibited 99% identity with that of macrophage mannose receptor. Furthermore, mannose receptor protein from sperm extract was found to have a molecular weight of 160kDa, congruent with that of 160kDa HIV-binding protein. HIVgp120 binding and mannose receptor expression were localized to the equatorial segment in 10% of ejaculated sperm, which increased after capacitation. Direct involvement of hMR in HIV gp120 binding was further supported by displacement of HIVgp120-FITC using excess of cold unlabeled gp120 and in competitive binding experiments, mannan at molar excess concentrations completely inhibited gp120 binding to sperm. HIV gp120 appears not to internalize but remained bound to mannose receptor at the surface over a time course of 24 hrs.

**CONCLUSIONS:** Spermatozoa may thus serve as carriers of HIV from the male to the female, through the binding of HIV gp120 to sperm mannose receptor. The current study presents hMR as a potential CD4-independent HIV-binding protein on human spermatozoa.

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### Carrageenan Gel, an HIV-1 Entry Inhibitor, Retains HIV-1 Anti-infective Activity in Female Genital Samples After Vaginal Application During a Phase I Safety Trial

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**BACKGROUND:** Carrageenan (CG) is the active ingredient in a vaginal microbicide gel (30 mg/ml carrageenan) that was tested in a phase III clinical trial which failed to reduce HIV-1 sexual transmission. To investigate whether CG retained anti HIV activity over time in the genital tract, we conducted ex vivo analysis of cervico-vaginal lavages (CVLs) from 16 HIV-1 infected Thai women in a crossover safety trial (CG, placebo and no gel arms).

**METHODS:** We measured CG concentrations and the HIV anti-infective activity of CVLs taken before ( $T_0$ ) and 15 min ( $T_{15\text{min}}$ ) after vaginal application of gel (4ml), and 8-24 hrs ( $T_{\text{week}}$ ) after a week of gel use. CG levels were measured with a methylene blue colorimetric assay (sensitivity = 25ug/ml) and the anti-infective activity of each CVL supernatant was assayed using a microtiter-based HIV-1 infectious titer reduction assay with TZMbl cells and a CCR5-using CRF01\_AE virus stock. Infectious HIV-1 titer reductions for a woman's CVLs were reported as the difference between the  $\log_{10}$  TCID<sub>50</sub> at  $T_0$  compared to  $T_{15\text{min}}$  and  $T_{\text{week}}$ . The Rank Sum Test was used to evaluate CG levels, TCID<sub>50</sub> values, and titer reductions in CVLs between groups.

**RESULTS:** All 16 women at  $T_{15\text{min}}$  and 12 women at  $T_{\text{week}}$  had measurable CG levels in their CVLs (ranges: 103–3154 and 32–282 ug/ml, respectively). CG levels were higher at  $T_{15\text{min}}$  versus  $T_{\text{week}}$  ( $p<0.001$ ; medians of 304 versus 98 ug/ml, respectively). There was significant anti-infective activity in  $T_{15\text{min}}$  and  $T_{\text{week}}$  CVLs with measurable CG levels compared to those at  $T_0$  ( $p<0.001$  and  $p<0.001$ , respectively); median  $\log_{10}$  TCID<sub>50</sub> values for CVLs at  $T_0$ ,  $T_{15\text{min}}$  and  $T_{\text{week}}$  were 4.9, 2.8, and 2.9, respectively. There was a corresponding increase in anti-infective activity with increasing CG levels (ug/ml): <30, 30 to 150, >150 to <300, and >300 had 0.6, 1.9, 2.1, and 2.3  $\log_{10}$  median infectious titer reductions, respectively ( $p<0.001$ , ANOVA on Ranks). In addition, there was no difference in titer reductions between  $T_{15\text{min}}$  and  $T_{\text{week}}$  CVLs which had similar concentrations of CG ( $p=0.5$ ). CG levels between  $T_{15\text{min}}$  and  $T_{\text{week}}$  decreased <10 fold in 10 and >10 fold in 6 women; however, there were significant titer reductions at  $T_{\text{week}}$  for both groups ( $p<0.001$  and  $p=0.015$ , respectively). Anti-infective activity was not detected in the placebo or no gel arms.

**CONCLUSIONS:** Our results suggest that CG gel retains its ability to act as an HIV-1 anti-infective agent when measurable at 8 to 24 hours after vaginal application.

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### Comparison of HIV Infection Rates in the Intestinal Explant Model Using Endoscopic or Surgically Derived Tissue Samples

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**BACKGROUND:** The evaluation of potential microbicidal agents benefits from clinically relevant and practical models of ex vivo HIV-1 infection, such as explants. Given the trade-off between amount of tissue (surgical) and time-proximity to acquisition (endoscopic), we compared the readout of explant infectability using surgical and endoscopic large intestine (LI) samples.

**METHODS:** LI endoscopic biopsies from healthy subjects were obtained via flexible sigmoidoscopy at 30cm from the anal verge (n=5). LI surgical samples were obtained from colonic resection margins (n=3). Histology of all samples showed viability without evidence of ischemia. Explants of equal size were submerged in culture medium with HIV-1<sub>Bal</sub> at TCID<sub>50</sub> 10<sup>3</sup> to 10<sup>1</sup>, in duplicate or triplicate, washed and established on gel-foam rafts as previously described. Culture supernatants were collected at 3–4 day intervals for 14 days. Each donor's mean, cumulative p-24 at day 14 was reported. Infectability was defined as the detection of p-24>100pg/ml at any time point.

**RESULTS:** At TCID<sub>50</sub> of 10<sup>1</sup>, 50% of surgical explants (n=2) and 80% (4/5) of endoscopic explants were infected. At TCID<sub>50</sub> of 10<sup>2</sup> and 10<sup>3</sup>, 100% of both surgical and endoscopic derived LI explants were infected. There were no significant differences in the cumulative HIV-1 p-24 related to viral inoculum or source of explant tissue.

	Mean Cumulative Day 14 HIV-1 p-24 (pg/ml) ± SD		
TCID <sub>50</sub>	10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>
Surgical LI (N=3)	2308± 1870	6917± 4129	8362 ± 8248
Endoscopic LI (N=5)	1604 ±2269	4228 ± 1939	5708 ± 5260

**CONCLUSIONS:** Surgically and endoscopically acquired LI explants exhibit similar virological HIV-1 infectability profiles using the submersion protocol, supporting the use of either for screening rectal microbicidal agents

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## Potential HIV-1 Microbicide Agent: Green Tea Catechin, Epigallocatechin Gallate

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**BACKGROUND:** The first lines of defense against viral infection are the mucosal dendritic cells; Langerhans cells (LC) and monocyte-derived dendritic cells (MDDC). They are initial targets for mucosal HIV-1 infection, expressing both CD4 and HIV-1 coreceptors. HIV-1 infection results in impaired immune function by binding of gp120 to the CD4 receptor in complex with a chemokine receptor. Previously, we presented evidence that the green tea catechin, epigallocatechin gallate (EGCG) binds with high affinity ( $K_d=10\text{nM}$ ) to the CD4 D1 domain at the gp120 attachment site (Phe43, Arg59, Trp62) on T cells and now present evidence of EGCG as an inhibitor of the mucosal route of HIV-1 transmission.

**METHODS:** CD34+ hematopoietic stem cells differentiated to LC (CD1a+/HLA-DR+/CD80+/CD11c+/CD207+/birbeck granule-+/CD34-, expressing 70% CD4+/CCR5+) or CD14+ cells differentiated to MDDC (CD1a+/HLA-DR+/CD80+/CD209+/CD206+/CD14-, expressing 60% CD4/CCR5+) were isolated from the peripheral blood of HIV-1 seronegative donors. Assessment of HIV-1-gp120 inhibition of binding was made by flow cytometry. HIV-1 infectivity was assessed by an HIV-1 p24 ELISA. Cytotoxicity was determined by ViCell and MTT assays. Dendritic cell function of antigen uptake and presentation and phagocytosis was assessed by flow cytometric analysis and radioisotope assays. Statistical significance was determined by Student's *t* test.

**RESULTS:** HIV-1 p24 antigen production was significantly inhibited by EGCG in both LC and MDDC HIV-1 infectivity cultures in a dose-dependent manner with the greatest responses at 100% (50uM;  $p<0.001$ ), 67% (25uM;  $p<0.01$ ), 45% (10uM;  $p<0.05$ ). EGCG significantly inhibited HIV-1-gp120 binding to CD4 in a dose-dependent manner on LC by 95% (50uM;  $p<0.001$ ), 70% (25uM;  $p<0.01$ ) and 27% (10uM;  $p<0.05$ ) with similar results on MDDC. Control catechin, (-)-catechin, did not alter HIV-1-gp120 binding nor infectivity. There were no significant cytotoxic effects by EGCG at these concentrations. Dendritic cell function, as measured by phagocytic efficiency and capacity and antigen uptake and presentation was not significantly altered in the presence of EGCG.

**CONCLUSIONS:** The inhibitory effect on HIV-1-gp120 binding on mucosal cells and the inhibition of HIV-1 infectivity by EGCG may prove to be a therapeutic benefit relevant in the development of an effective microbicide against HIV-1.

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## CD4 Independent Mechanism of Sexual Transmission of HIV

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**BACKGROUND:** HIV1 is known to be primarily transmitted through sexual route. Besides free virus present in urogenital secretions, sperm and vaginal epithelial cells associated HIV is also responsible for sexual transmission of HIV. However the spermatozoa as well as vaginal epithelial cells are known to be devoid of conventional CD4 receptor, therefore suggesting the presence of alternate protein responsible for HIV binding and entry into these cells.

**METHODS:** CD4 independent HIV binding protein was identified by Western blot analysis of human sperm extract using cell free HIV and gp120 *env* glycoprotein. Partial N-terminal amino acid sequence of HIV binding protein and its peptide was determined. Human testicular cDNA was PCR amplified using the degenerated primers based on the partial amino acid sequence of HIV binding protein and sequenced. Presence of human Mannose Receptor (hMR) was also investigated by immunofluorescent localization using FITC labeled antibody to hMR in the vaginal epithelial cells of normal and HIV negative serodiscordant females. Binding displacement of FITC labeled gp120 to sperm and vaginal epithelial cells was checked in the presence of Mannan by immunohistochemical localization.

**RESULTS:** Cell free HIV as well as gp120 binds specifically binds only to 160kDa sperm protein. Partial amino acid sequence of 160kDa protein showed homology with hMR. PCR amplified testicular cDNA showed 99% sequence identity with hMR. FITC labeled antibody to hMR binds specifically to vaginal epithelial cells. Presence of hMR was observed in less than 10% of the vaginal epithelial cells of HIV negative Serodiscordant females while 85 to 95% of the vaginal cells of the normal females from general population showed presence of hMR. Binding of FITC labeled gp120 was displaced in the presence of Mannan.

**CONCLUSIONS:** Study demonstrated presence hMR on sperm as well as vaginal epithelial cells responsible for sexual transmission for the first time. The differential expression of hMR determines risk of sexual transmission of HIV. Further studies also demonstrated that Mannan prevents the binding of HIV to these cells. This suggests the role of hMR in sexual transmission of HIV and also the need for revised strategies for development of microbicide which will prevent HIV entry into both CD4 independent (sperm and vaginal epithelial cells) and CD4 dependent cells (endo and ecto cervical cells).

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## A New Model for Studies on Cervicovaginal Inflammation and Barrier Disruption

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**INTRODUCTION:** Several microbicide candidates have failed in clinical trials because they induce inflammation and epithelial barrier disruption in the female reproductive tract. Unfortunately, many of the cervicovaginal epithelial models currently used to test microbicides are limited due to poor availability, reproducibility and cost.

**OBJECTIVE:** The purpose of this study was to establish a reliable and cost-effective in vitro ectocervical/vaginal inflammation model, and to further determine the molecular basis for inflammation-induced changes in barrier permeability.

**METHODS:** EpiVaginal™ cultures (MatTek Corp., Ashland, MA) were treated with increasing doses of the proinflammatory cytokine TNF- $\alpha$  for 24hours. The culture medium of treated tissues was assessed for IL-6 and IL-8 production by Bioplex Cytokine Immunoassay. Epithelial permeability was determined by measuring transepithelial electrical resistance (TEER). The integrity of the epithelium is regulated by junctional proteins, which were characterized by immunohistochemistry.

**RESULTS:** Treatment with 2ug/ml of TNF- $\alpha$  significantly increased secretion of IL-6 and IL-8 into the culture medium, and increased epithelial permeability by 25%. Expression of junctional proteins JAM-A, JAM-C, E-cadherin and ZO-1 in the untreated Epivaginal model resembled that of normal vaginal tissue, and was reduced following TNF- $\alpha$  treatment.

**CONCLUSIONS:** We have developed a new model to characterize molecular events underlying increased permeability induced by proinflammatory cytokines in vaginal epithelial tissue. These findings could provide important insight into how vaginal permeability is regulated in order to guide future microbicide development. Further, the identification of biomarkers to detect barrier dysfunction could advance the efforts to formulate safe and effective microbicides to prevent the transmission of sexually transmitted pathogens.

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### Polyfunctional HIV-1 CD4 Responses in HIV-Resistant Versus Infected Sex Workers Due to HIV-1 Specific Epitopes in the Majengo Cohort (Nairobi, Kenya)

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**BACKGROUND:** A longitudinal study of low social economic status commercial sex workers has demonstrated variable susceptibility to HIV-1 infection, where some of the highly exposed subjects remain persistently seronegative signifying resistance to HIV-1. There has been intense interest in understanding mechanisms responsible for this phenomenon. However, since both HIV infected and resistant individuals have HIV specific T helper responses, and then there is a certain uniqueness that protects the latter group from getting infected. Combinations of MHC alleles like HLA -B18, HLA -A2 and TAP gene variants have been associated with this resistance. This study evaluated the protective immunity in the light of specific selective peptide recognition.

**METHODS:** The peptide pools were designed from the entire HIV-1 Clade -A proteome giving 778 overlapping peptides between 11 & 16 amino acids in length, grouped into 20 pools. Each pool had 40 peptides except pool 20 which had 18 peptides. The immune was determined using 10 colour LSR II flow cytometry to measure INF $\gamma$ , TNF - $\alpha$ , IL- 2 production at day 3 and proliferation (using cells loaded with CFSE) at day 6 from a total of 70 individuals of whom, 4 were low risk controls, 33 were resistant, 20 negatives and 18 infected.

**RESULTS:** The resistant group shows more INF $\gamma$ , TNF - $\alpha$ , IL-2 production and proliferation, especially in response to peptide pools 1, 12, 13 and 14 (Env, P24, P31 and P2P7P1, P6 P7, Protease and REV peptide pools). The difference was significant at with a p value of 0.05 using non parametric tests.

**CONCLUSIONS:** There was no correlation between cytokine production and proliferation indicating that polyfunctionality is important which can be used to determine efficacy of vaccines. Future directions include breaking down the specific peptide pools with unique responses and making use of Epitope locator finder to characterize the magnitude of HIV specific reactivity.

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### Host Genetic Polymorphisms in Couples Discordant for Human Immunodeficiency Type 1 Infection

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**BACKGROUND:** Some individuals remain HIV-1 seronegative despite multiple sexual exposures to the virus. The mechanisms underlying this resistance remain unclear and several host genetic factors have been implicated, among which are the homozygosity for mutations in CCR5 gene (CCR5 Delta 32) and CCR2 gene (CCR2 64I).

This study sought to determine the possible role of CCR5 Delta 32 and CCR2 64I polymorphisms in the lack of HIV-1 transmission in Ghanaian HIV-1 highly exposed serologically discordant couples.

**METHODS:** HIV-1 antibody testing was done using Abbott HIV-1/2 Determine assay and confirmed with Innolia HIV-1/2 assay on 32 couples, comprising of 12 HIV-1 serologically discordant (SDC) and 20 serologically concordant couples (SCC) visiting the Korle-Bu Teaching Hospital (KBTH) of Ghana in 2007. HIV-1 negative serostatus of discordant partners was confirmed by polymerase chain reaction (PCR) using primers for the long terminal repeat region (LTR) of HIV-1. CCR5 and CCR2 genotypes were identified by PCR and PCR-RFLP (Restriction Fragment Length Polymorphisms) respectively using DNA extracted from peripheral blood.

**RESULTS:** HIV antibody testing with PCR confirmation revealed 8 SDCs and 24 SCCs. CCR5 genotyping for SDCs and SCCs showed mainly wild type alleles, with one female seronegative individual having heterozygous CCR5 allele. None of the individuals had CCR5 Delta 32 homozygous genotype.

The allele frequency of CCR2 64I was 37.5% and 12.5% in seronegative and seropositive partners of SDCs respectively. None had CCR2 64I homozygous allele. SCCs had an allele frequency of 31.8% with 4.5% having homozygous alleles. The allele frequency of CCR2 64I in the entire study population was 30%.

**CONCLUSIONS:** The infrequent occurrence of CCR5 Delta 32 allele was unlikely to explain discordance in this cohort but the frequent occurrence of the CCR2 64I allele could explain discordance but may not be implicated in natural resistance to HIV-1 infection. Counselling should be intensified in this at risk group.

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### Glycosidase Activity and Effects in Cervical Vaginal Lavages

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**BACKGROUND:** Mucins are highly glycosylated proteins that are a major component of cervical mucus and function as a protective barrier against pathogens in the upper female genital tract. The effects of glycosidases on the degradation of mucins have been reported. We examined cervical vaginal lavages (CVLs) from ten women and measured CVLs for total protein, glycosidase activity, lectin-carbohydrate binding specificity, and mucin content. Our hypothesis is that mucin degrading enzymes will be directly related to carbohydrate composition and be reflected in lectin and mucin ELISA assays.

**METHODS:** CVLs were collected (with informed consent) in saline and stored at -80°C until use. ELISA assays for lectins and mucins were developed and optimized in our laboratory. Methylumbelliferyl derivatives were used in fluorometric assay to measure 5 different enzyme activities in the CVLs including: sialidase,  $\alpha$ -fucosidase, galactosidase,  $\alpha$ -glucosidase and  $\beta$ -glucuronidase. Protein content was determined using the method of Lowry et al.

**RESULTS:** All ten CVLs showed unique enzyme activity and protein patterns. All data are available for two of the CVLs. These CVLs did not show sialidase activity and had similar  $\alpha$ -fucosidase activity. Also, both CVLs had similar Wheat Germ Agglutinin (WGA) binding activity. Enzyme activity assays showed a major difference in  $\alpha$ -glucosidase activity between CVL 1 and CVL 2 (58.9 units and 0.2 units, respectively). CVL 1 had more  $\alpha$ -galactosidase activity than CVL 2. Other enzyme activities were similar between the two CVLs. CVL 2 had greater lectin-carbohydrate binding with Helix pomatia which binds terminal N-acetyl- $\alpha$ -D-galactosamine.

**CONCLUSIONS:** Although oligosaccharide side chains vary among glycoproteins such as mucins, there are also common features. Terminal sugars, usually sialic acid or fucose prevents attack by some glycosidases. Galactosides are the most common sugars. Our data support our hypothesis: there was a lack of sialidase in the samples and bound WGA was the same. Both samples had  $\alpha$ -fucosidase but only CVL 1 had  $\alpha$ -galactosidase, thus the mucins in both CVLs lost their protective fucose but the  $\alpha$ -galactosidase in CVL 1 removed more of the galactosides and therefore these samples no longer contained as much galactoside and bound less Helix pomatia lectin. We observed unique enzyme patterns in all the CVLs, but conclusive statements must await the completion of these complex studies.

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## Characterization of HIV Variants in Blood and Semen and Its Association with Pathogenesis of HIV

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**BACKGROUND:** Genetic variability of human immunodeficiency virus type - 1 (HIV-1) is a potential threat for diagnosis and treatment of HIV/AIDS, as well as the development of effective vaccines. Presence distinct viral variants have been detected in PBMCs, lymph node, spleen, brain and lung in the same individual. Sexual transmission though an inefficient but is the most common route of HIV infection. The viral isolates present in the genital secretions and cells including vaginal epithelial cells, seminal leukocytes and sperm represent the initial virus responsible for sexual transmission of HIV

**METHODS:** Genotypic and phenotypic characterization of viral variants in blood and compare that with seminal plasma, leukocytes and sperm of the same HIV infected individuals. The DNA/RNA isolated and Variants will be analyzed by Heteroduplex mobility assay (HMA) phenotypic characteristics *in-vivo* will be evaluated by estimation of viral load and CD4 counts.

**RESULTS:** Analysis of HIV proviral DNA from spermatozoa and PBMCs of the same individual by HMA showed the presence of distinct variants. Sequence analysis of C2/V3 region of HIV1 C env gp 120 region showed 97% homology from spermatozoa while that with the PBMCs of the same individual showed 91% homology. Further, in-vitro studies in few cases demonstrated that the viral variants present in the spermatozoa of same individuals were found to be infectious.

**CONCLUSIONS:** The study demonstrated the presence of distinct viral variants in spermatozoa which determine the risk of sexual transmission of HIV. Characterization of the HIV variants present in the spermatozoa may help in designing the strategies for the prevention of sexual transmission as well as therapeutics of HIV/AIDS.

### ABSTRACT WITHDRAWN BY AUTHORS

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## Estimating Population Attributable Risk for HIV Seroconversion Among Women Who Were Recruited for Microbicide Site Preparation Studies in Durban, South Africa: Implications for Prevention Policy and Planning

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**BACKGROUND:** Currently, the province of KwaZulu-Natal is at the most advanced stage of the HIV/AIDS epidemic. Identifying what causes HIV to explode in this region is likely to be complicated. However, it is now known that the severity of the epidemic varies by geographic location in this region. Therefore, it is important to determine the "hot" spots in this region.

**METHODS:** The aim of this study was to characterize the geographical variation of HIV infection in 158 sub-censuses in Durban, South Africa. Geographical data from a cohort of women who were recruited for various microbicide site preparation and feasibility studies were used to determine the areas with high HIV prevalence and incidence by using geostatistical methodologies.

**RESULTS:** This study identified three significant clusters (one primary and two secondary) of high HIV prevalence in small rural communities of Durban. Among women who tested HIV positive at screening, 458 were geographically clustered. Of these 146 (32%) were determined to be centered within a 4.5 km radius and exclusively from west of Durban. This study also identified areas of high HIV incidence which were broadly consistent with high prevalence areas.

**CONCLUSIONS:** Geographic excesses of HIV infections were detected in rural communities of Durban where the women were exclusively targeted for microbicide studies. Results reinforce the inference that risks for HIV infection are associated with definable geographical areas. The regions identified are critical in controlling the HIV epidemic and may provide important direction for future interventions.

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## HIV Risk Among Young Single Non-cohabiting Women Participating in HIV Prevention Studies in KwaZulu-Natal, South Africa

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**BACKGROUND:** South Africa carries the burden of the highest number of persons infected with HIV in the world; the most affected province is KwaZulu-Natal, where 26% of those in the 15-49 year age group are infected. Among non-pregnant women in KwaZulu-Natal, the population most vulnerable to HIV acquisition are those under 24 years of age, who do not identify themselves as married, and who do not cohabit with a male partner. We analysed the factors influencing the risk of HIV acquisition in this sub-population using combined data from two microbicide trial feasibility studies (HPTN 055 and the MDP 301 feasibility study), and one phase III trial of the diaphragm for HIV prevention (MIRA) conducted in greater Durban, and in a rural district north of the city.

**METHODS:** A total of 2523 women meeting the various study criteria were enrolled, 38% of whom were 24 years of age or younger, single and non-cohabiting. HIV incidence, sexual behaviour and socio-demographic data were collected as per the protocols of the respective studies. The association between demographic and behavioral risk factors and HIV seroconversion was investigated using Kaplan-Meier curves and log-rank tests.

**RESULTS:** 57% of the women who seroconverted were 24 years old or younger, single and non-cohabiting (high risk group). The majority of the high risk group ( $n=959$ ) lived in urban areas (71%), and 16% had an STI at screening ( $p=0.0004$ ). The HIV incidence rate in the high risk group was 10/100 py (95% CI 8, 12), while that in the rest of the population was 4.3/100 py (95% CI 3.5, 5.4). The HIV incidence rate was 13/100 py (95% CI 9, 19) among high risk group women who had an STI at baseline vs. 9/100 py (95% CI 7.6, 11) in those who did not.

**CONCLUSIONS:** The results suggest that targeted interventions, including microbicides, PrEP and STI prevention and treatment among young unmarried women and their sexual partners could have a significant influence on the acquisition of HIV in this high risk group.

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## High, Sustained HIV Incidence Among Sexually Active Women in Madibeng, South Africa

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**BACKGROUND:** Phase 3 safety and efficacy clinical trials of candidate microbicides require recruitment and retention of populations at high risk of HIV infection. Pre-trial epidemiologic studies allow researchers to measure HIV incidence rates (IR) in candidate populations for planning trial sample size and power, and to strengthen site research capacity. An observational study in preparation for a Phase 3 efficacy trial in Madibeng, South Africa, was conducted during 2007-2009.

**METHODS:** Sexually active women ( $N=798$ ) age 18 to 35 years were screened for eligibility for a prospective HIV seroconversion study. Women were tested for HIV and pregnancy, interviewed for sexual and other risk behaviors, and screened for sexually transmitted infection (STI) symptoms. HIV-negative, non-pregnant women ( $N=299$ ) were enrolled in the cohort and had quarterly visits for one year. Participants were required to use condoms plus another contraceptive method, not to be breastfeeding, and to refrain from anal intercourse during follow-up.

**RESULTS:** HIV and pregnancy prevalence at screening were 24% (95% CI 21.1, 27.1), and 2.5% (95% CI 1.4, 3.6), respectively. Median age was 24 years, 91% of women had started or completed high school, and 73% were single. Nearly all women (86%) reported one male sex partner in the prior 3 months. Forty-one percent of women felt they were at high risk for HIV infection, primarily because of inconsistent condom use or partner's possible infidelity. One-third of women reported an STI symptom at baseline, usually lower abdominal pain or vaginal discharge. Nearly all (99%) women said they would be willing to participate in a microbicide trial. The retention rate was 89%. Fifteen HIV seroconversions occurred for an overall HIV IR of 6.0 (95% CI 3.0, 9.0) per 100 person-years. The HIV IR remained relatively stable during follow-up, with 1st and 4th quarter IR of 5.9 (95% CI 0.1, 11.7) and 6.9 (95% CI 0.1, 13.8). Reported condom use at last sex act increased during follow-up (73% vs. 45% at screening). Despite providing contraceptive counseling at nearly all study visits, and actual methods at half of visits, 12 pregnancies occurred during follow-up for a pregnancy IR of 4.8 (95% CI 2.2, 7.5).

**CONCLUSIONS:** This population appears to be well-suited for microbicide trials, with high and sustained HIV IR over time, good retention, and interest among participants and the community. However, the high pregnancy IR suggests contraceptive services should be strengthened.

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## HIV Incidence Among Women at Higher Risk in Bloemfontein, South Africa

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**BACKGROUND:** Phase 3 safety and efficacy clinical trials of candidate microbicides require recruitment and retention of populations at high risk of HIV infection. Pre-trial epidemiologic studies allow researchers to measure HIV incidence rates (IR) in candidate populations for planning trial sample size and power, and to strengthen site research capacity. An observational study in preparation for a Phase 3 efficacy trial in Madibeng, South Africa, was conducted during 2007-2009.

**METHODS:** Sexually active women ( $N=798$ ) age 18 to 35 years were screened for eligibility for a prospective HIV seroconversion study. Women were tested for HIV and pregnancy, interviewed for sexual and other risk behaviors, and screened for sexually transmitted infection (STI) symptoms. HIV-negative, non-pregnant women ( $N=299$ ) were enrolled in the cohort and had quarterly visits for one year. Participants were required to use condoms plus another contraceptive method, not to be breastfeeding, and to refrain from anal intercourse during follow-up.

**RESULTS:** HIV and pregnancy prevalence at screening were 24% (95% CI 21.1, 27.1), and 2.5% (95% CI 1.4, 3.6), respectively. Median age was 24 years, 91% of women had started or completed high school, and 73% were single. Nearly all women (86%) reported one male sex partner in the prior 3 months. Forty-one percent of women felt they were at high risk for HIV infection, primarily because of inconsistent condom use or partner's possible infidelity. One-third of women reported an STI symptom at baseline, usually lower abdominal pain or vaginal discharge. Nearly all (99%) women said they would be willing to participate in a microbicide trial. The retention rate was 89%. Fifteen HIV seroconversions occurred for an overall HIV IR of 6.0 (95% CI 3.0, 9.0) per 100 person-years. The HIV IR remained relatively stable during follow-up, with 1st and 4th quarter IR of 5.9 (95% CI 0.1, 11.7) and 6.9 (95% CI 0.1, 13.8). Reported condom use at last sex act increased during follow-up (73% vs. 45% at screening). Despite providing contraceptive counseling at nearly all study visits, and actual methods at half of visits, 12 pregnancies occurred during follow-up for a pregnancy IR of 4.8 (95% CI 2.2, 7.5).

**CONCLUSIONS:** This population appears to be well-suited for microbicide trials, with high and sustained HIV IR over time, good retention, and interest among participants and the community. However, the high pregnancy IR suggests contraceptive services should be strengthened.

# SUNDAY POSTERS

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### HIV Incidence Among Women at Higher Risk in Rustenburg, South Africa

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**BACKGROUND:** The USAID-funded Site Identification and Development Initiative (SIDI) aims to increase capacity for HIV prevention research by supporting new research entities and ascertaining HIV incidence at their sites. As part of this initiative, Family Health International (FHI) collaborated with the Aurum Institute, a non-profit health research organization based in Johannesburg, to support a research clinic in Rustenburg, a small South African city in North West Province. We helped to obtain ministerial, scientific and ethical approvals for the study. We developed and delivered a core HIV training curriculum, and study-specific training. We created an infrastructure for recruitment, study visits and participant care, rapid and EIA testing for HIV, data entry/management, referrals, and financial management.

**METHODS:** The incidence study comprised cross-sectional screening and a subsequent prospective cohort study of HIV-seronegative, sexually active women aged 18–35 years. Women were considered at higher risk of HIV on the basis of self-reported behavioral factors and/or medical history features. They made up to 6 monthly visits for interviews, testing, and counseling. HIV serostatus was determined by 2 rapid tests (Determine and Unigold) and a third tie-breaker rapid test (SD Bioline), with additional confirmatory information provided by PCR and Western blot.

**RESULTS:** Initially slow recruitment was overcome as this new research entity established linkages with communities and stakeholders in the city. We enrolled and tested 546 women in screening and 223 women in the prospective cohort, with high retention. HIV seroprevalence was 23.0%. The HIV incidence rate was 3.2 per 100 women-years overall (95% CI 0.4, 11.5), and more than twice that in women 18–25.

**CONCLUSIONS:** The prospective HIV incidence rate was substantial, albeit imprecise, in these Rustenburg women. Vigorous prevention programming is needed in the municipality. Follow-up continues, and the site is well-prepared to conduct further HIV prevention research in the district.

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### High HIV Incidence in a Candidate Trial Population in Mbekweni, South Africa

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**BACKGROUND:** Feasibility studies allow microbicide researchers to measure HIV and pregnancy incidence rates (IR) in candidate populations, estimate retention, and strengthen site research capacity. A feasibility study in preparation for a Phase 3 safety and efficacy clinical trial in Mbekweni, South Africa was conducted.

**METHODS:** Healthy, sexually active women (N=800) age 18 to 35 years were screened for a prospective HIV seroconversion study. At screening, women were tested for HIV and pregnancy, and interviewed for sexual and other risk indicators. HIV-negative, non-pregnant women were eligible for the cohort if they agreed to use condoms plus another contraceptive method during follow-up, to refrain from anal sex, and were not breastfeeding. After enrollment, participants (N=299) had quarterly visits for 12 months, with HIV and pregnancy testing and interviewing at each visit.

**RESULTS:** HIV and pregnancy prevalence at screening were 21.8% (95% CI 18.9, 24.7), and 2.3% (95% CI 1.2, 3.3), respectively. Median age was 24 years, and median residence in the study area was 12 years (range: <1–35 years). Most women (65%) were single, and 26% had completed high school. Only 5.1% of women reported >1 male sex partner in the prior 3 months, but only 44% reported condom use at last sex act. About one-third (32%) reported having a sex partner they knew to be HIV infected. All but one woman said they would be willing to participate in a microbicide trial. The retention rate was 85%. Eleven women became HIV infected for an overall HIV IR of 4.5 (95% CI 1.8, 7.1) per 100 person-years. HIV incidence decreased during follow-up, with IR of 5.9 (95% CI 1.8, 9.9) and 2.7 (95% CI 0, 5.8) in the first and second six months, respectively. Reported condom use at last sex nearly doubled during follow-up (82% vs. 44% at screening). Pregnancy IR increased during follow-up, from 5.8 (95% CI 1.8, 9.9) to 8.4 (95% CI 2.9, 13.9) in the first and second six months. The proportion of women who reported STI symptoms decreased during follow-up (5% vs. 10% at screening).

**CONCLUSIONS:** In this sample of South African women, overall HIV incidence was high, although the IR decreased over time. High HIV risk combined with good retention make this population well-suited for microbicide or other HIV prevention trials. However, the high and increasing pregnancy IR over time suggests that family planning services, especially onsite provision of contraception, should be strengthened in future studies.

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### High-Risk Women for Contracting HIV in South Africa—Who Are They and Are They Ideal for Microbicide Trials?

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**BACKGROUND:** Phase 2/3 microbicide trials need to identify women at high risk of HIV. Epidemiologically, having a new sex partner elevates HIV risk but the characteristics of such women, including their suitability for microbicide trials, is unknown. We describe women reporting a recent, new sex partner among participants who screened for a cohort study in South Africa.

**METHODS:** Data derive from a cross-sectional study involving a structured interview. The following were evaluated for associations with the outcome: demographics, contraceptive use, pregnancy intentions, STI symptoms, sexual behavior, risk perceptions and knowledge of microbicides. Black African women (n=736) comprise the analytic sample. Women who, in the 3 months prior to interview, reported having sex with a partner whom they had never had sex with before, were defined as having a new partner. Variables significant at p<0.15 in cross tabulations were included in a final logistic regression model.

**RESULTS:** Women were predominantly Tswana (82%) with a mean age 23.2 years (18–35), 77% with a HS diploma, 20% cohabitating/married, 18% had recent STI symptoms, 52% reported condom use, 24% reported hormonal injection use, 25% had a new recent sex partner. Women with new sexual partner(s) were more likely to be single AOR (95%CI) 2.99 (2.03–4.40), report STI symptoms 1.55 (1.01–2.39), consume alcohol 1.66 (1.14–2.40), and were more informed about microbicide gels 2.87 (1.32–6.22). Women with new sexual partner(s) were less likely to report unprotected sex 0.45 (0.28–0.73) but more likely to express regret about not using condoms 2.26 (1.47–3.47). They were also less likely to use hormonal injection contraception 0.50(0.31–0.81), have unprotected sex and be long-term residents of the local area 0.44(0.29–0.65).

**CONCLUSIONS:** Women with new partners do appear to be a group at higher risk of HIV. Although more likely to report protected sex it was insufficient given their STI symptoms. This group may be more challenging to include in microbicide trials as they are less likely to use hormonal contraception; which is often needed for microbicide trials. They may also be more difficult to identify during recruitment as they cannot be readily distinguished by demographic profiling; may be difficult to retain due to greater transiency; but may require less education given their awareness about microbicide development. Paradoxically these women have ideal risk profiles for efficacy trials yet may be difficult to enroll.

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### Network of Infections Affecting Rural Communities in Northern Nigeria: Implications for Prevention Interventions

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**BACKGROUND:** There are many sexual activities that go on in Nigeria, its part of the sexual networks that highly contribute to HIV transmissions. Studies into the structure of sexual networks at a social level indicated that a thorough investigation of what drives HIV infections, and analyzed the many reasons for the uneven behavioral prevention strategies that are been put in place for men, women, young people and adolescents in this part of the country.

**METHODS:** Because of the rural setting of the Nigerian environment we used the ethnographic research in Numan, a rural communal residence in Northern Nigeria. Participant observation and unscripted interviews with older and younger residents of a village elicited data on sexual relationships and their structure. Post hoc analysis of field notes describes a sexual network with detailed socio-economic info-education of most community members.

**RESULTS:** A thorough researched case study of 52 individuals that included 22 men and 30 women, this was a mixed number of infected people, MSMs and other diversified groups within the ages of 18 to 49, the men and the women were almost of the same age except for the young people. The distinctive characteristics of the network are its inclusiveness, membership uniqueness, and geo-political spread across the six zones of the country: South east, South south, south west, North east, North Central and North west. Firm results indicated that men partnered with younger women across board and membership was not restricted in terms of individual vocation and livelihood status, socio-economic, religious and cultural-gender status, the network were connected to Ghana with multisectoral and worldwide links for growth and development. Some members of the network died from AIDS suspected cases; also members did not share a strong sense of being part of a broader network of HIV infection.

**CONCLUSIONS:** At broader look the network provided the members an open forum to expression and common dialogue for local and national issues affecting members at any level, created an atmosphere of sense of belonging commitment, purpose and potential opportunities for HIV prevention, restructured the behavioral attitudes of members in the six geo-political zones where they were in existent. More or less the relevance was attached to prevention with abstinence, condom use and faithfulness as approaches.

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### MicroCT for Noninvasive Evaluation of Microbicide Distribution and Retention in the Progesterone-treated Mouse Model

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**BACKGROUND:** The mouse is an important model for pre-clinical evaluation of candidate microbicides. After intravaginal or intrarectal treatment, various assays can be used to assess microbicide toxicity and protective efficacy. With many research groups now employing this model, we hypothesized that differences in intravaginal treatment technique could influence experimental outcome. To test this hypothesis we used microCT to evaluate the distribution and retention of a radiopaque microbicide-surrogate in both inactive and active mice.

**METHODS:** Animals were hormonally synchronized 1 week prior with injections of Methyprogesterone Acetate (2mg/0.1ml). Before imaging, 25 l of Omnipaque 300®, a viscous clinical contrast agent was delivered intravaginally to both active (n=5) and inactive (n=5) mice. Inactive mice remained under anesthesia throughout the experiment. Active mice were treated using hand-restraint, released, and anesthetized immediately prior to imaging. MicroCT imaging was performed using a Siemens Inveon scanner at 20 minutes post-treatment in both groups. Three-dimensional images were collected at 107 m/pixel isotropic resolution. Differences in contrast agent distribution and retention were quantified using Amira and Inveon Research Workplace post-image processing software. Volumes (Mean +/- SD) between treatment groups were compared using Student's t-test to determine statistical significance.

**RESULTS:** Inactive mice retained 4 times more contrast agent than did active mice in the mid (P=.002) and upper (P=.007) cervicovaginal regions. On average, the contrast agent volumetric distribution was 7 times higher (P=.004) for the inactive (35 +/- 15 l) vs. active (5 +/- 6 l) groups. The majority of contrast agent retained was pooled above the pubic bone in the mid-vaginal region. Inactive mice (3/5) showed higher tendency for retaining agent near the cervix compared to active mice (0/5).

**CONCLUSIONS:** Significant differences in the biodistribution of a microbicide-surrogate were demonstrated in vivo between active and inactive mice using microCT imaging. This suggests that similar differences in biodistribution may be seen with candidate microbicides that could ultimately lead to variability in treatment outcomes. MicroCT provides valuable, supplemental information that can enhance treatment consistency and lead to improved standardization for microbicide candidate screening experiments in this model.

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### Multi-Study Assessment of Vaginal Cytokines and Microflora in the Safety Evaluation of Intravaginal Rings in Pig-Tailed Macaques

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**BACKGROUND:** HIV microbicide trials have emphasized the need to evaluate the safety of topical microbicides and delivery platforms in an animal model prior to conducting clinical efficacy trials. An ideal delivery device should provide sustainable and sufficient concentrations of effective products to prevent HIV transmission while not increasing transmission risk by either local mucosal inflammation and/or disruption of the normal vaginal microflora.

**METHODS:** Safety analyses of macaque-sized elastomeric silicone and polyurethane intravaginal rings (IVRs) loaded with candidate antiretroviral (ARV) drugs were tested in four studies ranging in duration from 49 to 73 days with retention of the IVR being 28 days in each study. Macaques were assigned to 3 groups: blank IVR, ARV-loaded IVR, and naïve. In sequential studies, the same macaques were used but rotated into different groups. Mucosal and systemic levels of cytokines were measured from vaginal fluids and plasma, respectively, using multiplex technology. Changes in vaginal microflora were also monitored. Statistical analysis (Mann-Whitney test) was used to compare data between two groups of unpaired samples (with and without IVR, and IVR with and without ARV) for the groups collectively, and also for individual macaques.

**RESULTS:** There were few statistically significant differences in mucosal and systemic cytokine levels measured longitudinally when the ring was present or absent, with or without ARVs. Of the 8 proinflammatory cytokines assayed a significant increase ( $p = 0.015$ ) was only observed for IL8 in plasma with the blank and ARV loaded IVR (median of 9.2 vs. 5.7 pg/ml in the absence of IVR). There were no significant differences in the prevalence of H<sub>2</sub>O<sub>2</sub>-producing lactobacilli or viridans streptococci, or other microorganisms indicative of healthy vaginal microflora. However, there was an increase in the number of anaerobic gram negative rods in the presence of the IVR ( $p < 0.0001$ ).

**CONCLUSIONS:** IVRs with or without ARVs neither significantly induce the majority of potentially harmful proinflammatory cytokines locally or systemically, nor alter the lactobacillus or *G. vaginalis* levels. The increase in anaerobic gram negative rods alone suggests minimal disruption of normal vaginal microflora. The use of IVRs as a long-term sustained delivery device for ARVs is promising and preclinical studies to demonstrate the prevention of transmission in the HIV/SIV nonhuman primate model should continue.

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## Progress in the Development of a Transgenic Rabbit Model for the Testing of Anti-HIV Microbicides.

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**BACKGROUND:** An important scientific goal has been the development of small animal models of human immunodeficiency virus type 1 infection that can be used for testing and development of microbicides. Previous studies have demonstrated that rabbit cells transfected with CD4 and CCR5 are susceptible to SIV and HIV infection and that rabbits transgenic for CD4 show limited replication of HIV following IV challenge. This study aims to establish transgenic rabbits expressing human CD4 and CCR5 as a small animal model to evaluate microbicides.

**METHODS:** Expression constructs containing appropriate promoter, enhancer and silencer sequences were designed for expression in rabbit T cells and macrophages. Transgenic animals were generated by microinjection of embryos that were implanted in recipient female rabbits (by Renova Life Inc, MD, USA). Integration of the transgene was determined by PCR.

**RESULTS:** 474 microinjected embryos for the CD4 construct, yielded 51 animals, of which 9 animals were positive by PCR, with varying signal intensity suggesting some level of mosaicism. 339 embryos microinjected with CCR5 construct generated 32 animals 7 of which were positive by PCR, also with varying signal intensity. Backcrossing has generated pure heterozygous F1 transgenic animals (CD4+/- and CCR5+/- respectively), strongly positive for DNA by PCR. Furthermore, isolated rabbit lymphocytes were positive for transgene expression by rtPCR for mRNA. Ongoing work will require backcrossing to generate double transgenic F2 animals (CD4+/-, CCR5+/-) followed by crossing of F2 males and females to generate F3 homozygous dual positive (CD4+/, CCR5+/) animals.

**CONCLUSIONS:** Significant progress has been made in the generation of CD4+/CCR5+ transgenic rabbits as a model for testing microbicides.

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## A Chinese Rhesus Macaque Model for Testing "Live" Microbicides

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**BACKGROUND:** The mucosa of the cervix and vagina on which most HIV transmission occur is normally coated by a biofilm of commensal microorganisms including Lactobacillus. We have genetically engineered human vaginal *Lactobacillus jensenii* to secrete anti-HIV peptides and proteins that inhibit HIV viral fusion and entry into host cells to serve as a "live" microbicide. Testing the efficacy of this approach required development of a non-human primate model to examine colonization and expression of the live microbicide (*in situ*). We examined the macaque flora and cytokines in order to provide a better understanding of this model.

**METHODS:** We chose the Chinese rhesus macaque model, similar to the Indian rhesus used in most vaccine testing. Chinese rhesus macaques have a similar menstrual cycle to humans and support colonization with Lactobacilli. We determined the basal bacterial vaginal flora in 16 female macaques once a week for 4 weeks (during one menstrual cycle). We also measured cytokine/chemokines values each week on the same day as bacterial collection. Animals were then intentionally colonized with a recombinant *Lactobacilli jensenii* and again bacterial flora and cytokine/chemokine measurements were examined.

**RESULTS:** Cultured bacteria from the macaque were identified to the species level and a modified Nugent score was determined. *Lactobacillus johnsonii* was the dominant Lactobacillus found in a majority of the macaques in one or more time points. *Staphylococcus*, *Streptococcus*, anaerobes and small numbers of *Enterics* were also identified. Animals with Lactobacillus dominated flora showed a cytokine profile that was anti-inflammatory.

**CONCLUSIONS:** In the rhesus macaque model, Lactobacillus flora may induce an anti-inflammatory response. The response to recombinant *Lactobacilli jensenii* will be determined. A better understanding of the model will help to validate and improve its relevance to natural HIV infection.

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## In Vivo Detection of Microbicide-Induced Damage in Small Animal Rectal Epithelium Using Confocal Microendoscopy

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**BACKGROUND:** Microbicide safety has primarily been evaluated in the vagina but use of microbicides rectally necessitates effective preclinical methods to detect toxic effects of candidate agents on rectal epithelium. Real-time noninvasive imaging for assessment of epithelial disruption could provide unique information regarding microbicide effects. In this feasibility study, confocal microendoscopy was evaluated for *in vivo* visualization of the mouse rectal epithelium treated with either PBS or benzalkonium chloride (BZK) to determine if acute (20 minutes) injury could be detected.

**METHODS:** Ten mice were given 50 µl of 0.2% BZK or PBS rectally for 20 minutes. The rectal epithelium was then labeled with the nuclear staining fluorescent dye acriflavine orange (0.05%) for 5 minutes and rinsed. *In vivo* imaging was performed using the Cell-Vizion confocal microendoscope having a 1.5mm diameter imaging probe. Twelve images per mouse were obtained then mice were sacrificed and rectal tissue removed for histological processing. Images were evaluated for features associated with PBS or BZK treatment including surface topography, presence of surface debris, and presence and shape of crypts. Using criteria established in a five mouse training subset prior to this study, images from the mice treated with 0.2% BZK or PBS were classified (blinded assessment) and a determination of treatment made.

**RESULTS:** PBS controls had smooth surface topography with one of two appearances: 1) areas in which crypts appeared as dark circular regions with smooth edges and continuous transition between crypts and 2) highly uniform fields where crypts were not evident but the surface appeared smooth. The most prominent feature associated with BZK treatment was presence of crypts retaining a generally circular shape but with highly rugated edges and distinct gaps between crypts. Surface topography was nonuniform and textured. Surface debris was present in both groups. Histology revealed loss of surface columnar cells in BZK animals and areas of disrupted superficial crypt structure. Visual classification resulted in the correct identification of 4/5 BZK and 4/5 PBS cases.

**CONCLUSIONS:** These preliminary *in vivo* results are highly promising and indicate that confocal microendoscopy may provide a sensitive surface assessment of rectal epithelial integrity in small animal models for evaluating microbicide effects.

**100****Gamma Scintigraphic Evaluation of the Distribution and Retention of Multiparticulates after Intravaginal Administration to Sheep**

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**BACKGROUND:** Multiparticulates could be promising formulations for the delivery of microbicide drugs to the vagina, having good spreadability and effective drug release. We developed the starch based multiparticulates for vaginal drug delivery. The main objectives of this study were: (i) to evaluate the vaginal tolerance of the pellets, using the Slug Mucosal Irritation assay (SMIA) (ii) to evaluate the *in vivo* behavior of the pellets after intravaginal administration to an animal model (i.e. sheep), using gamma scintigraphy.

**METHODS:** To estimate the irritation and tissue damage potential of the formulation to vaginal mucosa the SMIA was carried out for 5 days. The slugs were in contact with 20 mg test substance for 30 min each day, and the mucus, enzymes and proteins produced were measured. Gamma scintigraphy in sheep was carried out to evaluate the distribution and retention time of pellets in the vagina by administering radiolabelled (<sup>111</sup>In) pellets and gel. Pellets were prepared by extrusion-spheronisation using high amylose starch as main excipient. The radiolabeled pellets were filled into a hard gelatin capsule and administered intravaginally to 6 anoestrous sheep. Scintigraphic scans were taken immediately after administration; 4h, 6h 8h 12h and 24h of post dose. The sheep vagina was outlined by administering <sup>111</sup>In labeled gel, followed by scans taken immediately after administration, 1h, 4 h, and 8h. The spread in gel scans were used as reference ROI. Based upon the region of interest (ROI), the % of activity retained in the vagina after each time point of pellet administration was calculated.

**RESULTS:** The SMIA indicated no irritation or tissue damage to the slugs, confirming the suitability of the formulation for vaginal administration. The low amount of progesterone (<0.6 ng/ml) confirmed the oestrous stage of all sheep. The capsule was found intact, after 4h of administration. Following slow intravaginal disintegration of capsule between 6 to 8 h, the pellets dispersed throughout the vaginal area. After 24 h the radioactivity was found still in the entire vaginal area confirming the presence of disintegrated pellets in the vagina indicating long retention time of this formulation.

**CONCLUSIONS:** The novel multiparticulate pellet system did not cause irritation or tissue damage (based on the SMIA). Gamma Scintigraphy scans proved that the pellets dispersed well throughout the vagina with an acceptable retention time. These data should now be confirmed by an imaging study in human volunteers.

**101*****In Vivo* Optical Coherence Tomography for Noninvasive Assessment of Microbicide Safety in a Small Animal Model**

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**OBJECTIVE:** Application of a non-invasive technique for the assessment of cervicovaginal tissue integrity following topical applications of microbicide agents could improve the evaluation of safety of microbicides in pre-clinical and clinical studies. *In vivo*, in-depth tissue imaging, such as Optical Coherence Tomography (OCT), may provide an effective tool for high-resolution visualization of morphological changes associated with topical microbicide application. We investigated the potential of OCT to assess the integrity of mucosal and submucosal tissue following a single application of topical microbicide.

**METHODS:** A single dose of BZK solution (.02, .2 & 2%), or PBS was intravaginally administered in mice. At 16 hours post-treatment, an FDA clinically approved OCT imaging device (Imalux Niris) with a 2.7 mm diameter imaging probe was used to acquire images from cervicovaginal tissue *in vivo*. Images were quantitatively assessed using a grading system designed to assess microbicide-induced changes in tissue morphology as well as thickness of mucosa and vaginal wall. Results from these assessments were compared to histology.

**RESULTS:** The mouse cervicovaginal tract can be imaged *in vivo* and non-invasively with OCT. In PBS treated animals, the vaginal wall was easily delineated showing a bi-layer structural morphology. The mucosa, vaginal wall (submucosa, muscularis, and serosa) and surrounding adipose and colorectal tissue are identifiable in real-time and measurable with post-image analysis software. Following application with 2% BZK, a significant degree ( $p=0.0001$ ) of changes occurred including epithelial denuding and vaginal wall thickening as compared to PBS controls. Changes were less pronounced with lower doses of BZK. These observations were compared and supported by histology. The grading system detected a significant, dose-dependant increase in OCT image abnormality related to increasing BZK concentration (0.2%:  $p=.04$ ; 2%:  $p<.0001$ ).

**CONCLUSIONS:** *In vivo* OCT can be performed non-invasively to delineate cervicovaginal tissue morphology and microstructure, including epithelial mucosa and vaginal wall structures. Tissues treated with a single application of BZK exhibited structural changes indicative of cellular toxicity and injury. This technique can provide a non-invasive approach for rapid and quantitative *in situ* assessment of microbicide-induced changes in tissue morphology and integrity.

**102****Repeated Agent Exposures Affect *In Vitro* and *In Vivo* Measures of Microbicide Safety**

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**BACKGROUND:** The disappointing clinical failures of vaginal microbicides have provided new insights into factors that impact microbicide effectiveness, including repeated product application and its association with increased HIV-1 infection. Clinical trials examining the nonoxynol-9 (N-9)-containing product COL1492 determined that daily applications increased the risk of HIV-1 infection. Therefore, despite being an effective spermicidal agent, N-9 was determined to be an ineffective microbicide. This increased risk of HIV-1 infection can be attributed to N-9-induced breaks in the epithelial barrier combined with local inflammation and immune cell recruitment. These results clearly indicate the need to study the impact of multiple applications on microbicide safety and efficacy.

**METHODS:** To investigate the effects of repeated use on microbicide safety, multiple exposure experiments used a mouse model of cervicovaginal toxicity and cervicovaginal cell lines. Toxicity studies using the Swiss Webster mouse model used H&E tissue staining to reveal changes in cervicovaginal epithelial integrity. In *in vitro* experiments, End 1 cells were exposed daily for 4 days to unformulated N-9 and cell viability was measured at 10 minutes, 2, 4, 8, and 24 h daily using the MTT cellular viability assay. For initial *in vitro* cytokine studies, real-time PCR was performed to measure changes in mRNA levels after multiple N-9 applications.

**RESULTS:** In the mouse experiments, the first application of 1% N-9 caused considerable damage to the cervical epithelium. Subsequent daily exposures were characterized by diminished cervical toxicity. Multiple daily exposures also increased the exposure duration required to elicit levels of epithelial damage similar to those seen after the first exposure. The vaginal epithelium was unaffected by single and multiple exposures. However, *in vitro* cytotoxicity experiments conversely demonstrated that End 1 cells became increasingly sensitive to N-9 after multiple exposures. Changes in cytokine expression *in vitro* were both time- and exposure-dependent.

**CONCLUSIONS:** Multiple N-9 exposures do not appear to result in cumulative cervical epithelial damage. In fact, multiple exposures cause changes in the cervical epithelium that increase its tolerance to N-9 exposure. Furthermore, comparisons of *in vitro* and *in vivo* results indicate that that *in vitro* assays designed to assess the effects of multiple exposures may not be predictive of *in vivo* outcomes.

# SUNDAY POSTERS

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### **Microbicide Excipients that Increase Susceptibility to Genital Herpes**

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**BACKGROUND:** Prior studies have reported that several microbicide active ingredients cause toxic effects that increase susceptibility to infection in mouse HSV-2 vaginal susceptibility models (Cone et al., BMC Inf Dis 2006;6:90; Galen et al., JID 2007;195:1332). Moreover, these results correlate well with instances of increased HIV transmission observed in microbicide clinical efficacy trials. These mouse models thus appear useful for detecting significant toxic effects prior to Phase III clinical trials. Excipients or "inactive ingredients" used in microbicide formulations might also have toxicities that increase susceptibility, but have not previously been tested in susceptibility models.

**METHODS:** Excipients commonly used in topical products were formulated in a well-studied non-toxic vehicle (the "HEC universal placebo"), or other formulations as specified. One of these excipients, the surfactant/emulsifier glycerol monolaurate (GML), is also being developed as an active microbicide, formulated in K-Y Warming Jelly (KYWJ). Twelve hr after delivering the test agent or PBS control, mice were challenged with 1.0 intravaginal infectious dose<sub>50</sub> of HSV-2, and 3 d later were assessed for infection by vaginal lavage culture. Agents were compared to PBS control by two-tail Fisher's exact test.

**RESULTS:** Significantly increased susceptibility to HSV-2 was observed after a single exposure to 5% GML in the extremely hyperosmotic vehicle (osmolality 10,300 mOsm/kg) KYWJ ( $P<0.0001$ ), to KYWJ alone ( $P<0.0001$ ), to each of the neat humectant/solvent components of KYWJ (propylene glycol ( $P<0.006$ ) and PEG-8 ( $P<0.02$ )), and to 5% GML as a colloidal suspension in PBS ( $P<0.006$ ). A trend toward increased HSV-2 transmission was seen after 0.1% disodium EDTA ( $P<0.095$ ), but not 0.0186% disodium EDTA. Increased susceptibility was not observed following exposure to the solvent/humectants 10% glycerin, or 10% propylene glycol, or with the preservatives 0.18% methylparaben plus 0.02% propylparaben, or 1% benzyl alcohol.

**CONCLUSIONS:** As reported with other surfactants, a single exposure to GML markedly increased susceptibility to HSV-2. Consistent with reports that extremely hyperosmotic formulations cause mucosal toxicity, the non-aqueous KYWJ and each of its undiluted humectant/solvent constituents also markedly increased susceptibility. EDTA at high levels caused a trend towards increased susceptibility.

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### **A Promising New Microbicide Gel: PC-707**

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**BACKGROUND:** Previously we have shown that PC-710 (1.5% zinc acetate in Carraguard) was effective in an HSV-2 mouse model for up to 18 hours after gel dosing. In the current study, we varied the zinc acetate concentration to strike a balance between efficacy in the HSV-2 mouse model and safety.

**METHODS:** Gels containing varying amounts of zinc acetate in Carraguard were prepared and evaluated in the HSV-2 mouse model and the XTT and TEER assays. The gels were characterized and placed on stability at 30°C and 40°C. CC<sub>50</sub> was measured using the XTT assay while the Biocoat HTS Caco-2 assay system was used for the TEER studies. For the HSV-2 mouse model, female Balb/C mice were vaginally infected at either high dose (10<sup>6</sup> pfu/mouse) or low dose (10<sup>4</sup> pfu/mouse). To assess the window of time that a formulation remains effective in protecting against HSV-2 exposure, an experiment looking at adding virus (10<sup>4</sup> pfu/mouse) from 1 h following gel application up to 8 h was run. The survival curves in the HSV-2 mouse model were analyzed by the Mantel-Cox test. A non-linear curve fit program was used to determine the CC<sub>50</sub> and EC<sub>50</sub> values.

**RESULTS:** Formulations of the PC-700 series containing 1.0%, 0.5%, 0.3%, 0.1%, and 0.03% zinc acetate were tested and no significant differences between 1.0%, 0.5%, and 0.3% formulations was observed in the HSV-2 survival curves. From these three formulations, 0.5% and 0.3% zinc acetate concentrations showed the best safety data according to XTT and TEER. Moving forward with PC-707, (0.3% zinc acetate) the data showed it was stable for 6 months at 30°C and 40°C, with pH, viscosity, CC<sub>50</sub> and EC<sub>50</sub> values within the acceptable range. In a high dose HSV-2 challenge experiment, PC-707 (85% uninfected) protected significantly better than Carraguard (15% uninfected). For the low dose challenge, both formulations afforded significant protection compared to both PBS and HEC in the 10 min and 1 h pre-exposure groups. However, PC-707 was significantly more effective at preventing the low dose HSV-2 vaginal infection compared to Carraguard in the 4 h post exposure treatment group.

**CONCLUSIONS:** PC-707 shows good protection against HSV-2 and is safe according to our XTT and TEER data.

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### **Saving Lives and Resources through Multipurpose Prevention Technologies**

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**BACKGROUND:** The various risks associated with unsafe sex are inextricably linked: most women at risk for HIV are also at risk for other STIs or an unintended pregnancy. Thus there is an urgent need to develop and facilitate access to safe, acceptable, and affordable multipurpose prevention technologies, especially for populations with limited access to health services. A multipurpose prevention technology is one that meets the multiple needs for protection against the various risks associated with sexual activity. Such technologies, including current and emerging interventions, would protect users against multiple risks even if they obtained the product for a single need, enabling them to purchase, understand, store, and use fewer products, and allow health care providers to store, supply, and advise women on a more compact range of products. Currently, male and female condoms are the only available methods known to provide simultaneous protection from pregnancy and STIs, including HIV, but they are so under-utilized that there is a strong case for intensifying efforts to develop alternative methods.

**METHODS:** 55 individuals with expertise in microbicides, barrier devices, and vaccines were sent an email survey on current and new multipurpose prevention technologies. The 26 responses were used to begin mapping the landscape of available and prospective technologies that could be defined or developed as *multipurpose*.

**RESULTS:** More than 30 devices, drugs, and vaccines with multipurpose prevention potential were identified as either in existence or in preclinical or clinical development. Some of these involve combinations of devices with drugs or drugs with vaccines; others are entirely new prevention approaches. These include: new physical barriers such as single-sized diaphragms in combination with a drug; vaginal rings adapted for time-release delivery of a drug; next-generation probiotics that could prevent and treat bacterial vaginosis, urinary tract infections, HIV, and other infections; and combination vaccines such as the HPV and HBV vaccine.

**CONCLUSIONS:** These promising multipurpose approaches constitute building blocks for a new generation and new vision of prevention. To accelerate their development, the global health community must pursue multiple, coordinated product development paths that will demand technical innovation, scientific persistence and collaborations, and possibly significant resources.

**106****Development of a Humanized-Cervicovaginal Murine Model for the Study of HIV-1 Transmission and Infection**

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**BACKGROUND:** The objective of this study was to demonstrate microbicide-induced inflammation and toxicity, the recruitment of human PBMC into the cervicovaginal epithelia, and the utility of this model for determining the relationship between cervicovaginal toxicity and susceptibility to infection.

**METHODS:** Human PBMC were successfully engrafted into NOD/SCID animals using an intraperitoneal injection. Animals were analyzed by fluorescence-activated cell sorter (FACS) analyses for evidence of specific immune cell populations in the blood circulation. Animals were then sacrificed at two-week intervals for 2 months post-injection and cervicovaginal tissues were harvested for immunohistochemical analyses of specific human immune cell populations.

**RESULTS:** Past studies demonstrated that NOD/SCID-hu mice surgically implanted with human vaginal xenografts and reconstituted with human PBMCs, detected human immune cell populations in the peripheral blood and infiltration of human CD45+ cells in the mouse spleens and vaginal xenografts for at least 2 months after reconstitution. In addition, experiments demonstrated this model was capable of being infected by HIV-1 BaL and IIIB. In the current model, immunohistochemical analyses demonstrated infiltration of human CD45+ cells in the mouse cervical and vaginal epithelium at levels similar to those observed in human cervicovaginal tissue.

**CONCLUSIONS:** Although the NOD/SCID human PBMC-xenograft model may provide a unique small animal system for the study of HIV-1 transmission, use of this model is limited by its complexity. This study demonstrated infiltration of human CD45+ immune cells into the cervicovaginal epithelium of NOD-SCID mice. Current studies will measure the establishment of HIV-1 infection in this model. Furthermore, this model is ideal as a large-scale screening tool for assessing safety/toxicity of pre-clinical microbicides, as well as for determining the efficacy of candidate compounds.

**107****Safety Studies of a Recently Developed Microboidal Contraceptive Gel (UniPron) in Female Baboons (*Papio anubis*)**

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**BACKGROUND:** To identify any toxicity on the vaginal epithelium, liver and kidney following UniPron administration. With the number of unwanted/unplanned pregnancies on the rise, there is a need for safe, effective and reliable contraceptives now more than ever before. Overpopulation, particularly in developing countries, is complicated by the pandemic of sexually transmitted infections (STI) including human immunodeficiency virus (HIV). As the HIV/AIDS epidemic continues to grow, people must find a way to protect themselves against HIV and other sexually transmitted infections (STIs).

**METHODS:** Ten healthy female olive baboons (*Papio anubis*) of reproductive age and of proven fertility were used. Five baboons were each treated with 15g of UniPron intravaginally twice a week for 20 weeks, venous blood collected before and after each treatment. Venous blood was collected from five control animals as in the experimental females, but these control animals were not given any treatment. The endpoints that were evaluated included clinical chemistry profiles on kidney and liver functions and vaginal histopathology.

**RESULTS:** Female baboons treated with 15g of UniPron intravaginally showed no detectable adverse effects on clinical chemistry profiles investigated and vaginal histopathology. Repeated intravaginal exposure of female baboons to UniPron did not induce detectable vaginal irritation and there were no detectable histological changes. Histopathological results of biopsies obtained from UniPron treated and control baboon vaginal tissues clearly showed that UniPron is non-toxic and did not induce any detectable vaginal irritation. There were no detectable histological changes in any of the ten biopsies examined. All the animals had normal histology comprising of the non-keratinized stratified squamous epithelia, well-vascularized lamina propria, muscularis mucosae and adventitia for each group of baboons.

**CONCLUSIONS:** Repeated administration of UniPron into baboon vagina did not cause any detectable toxicity.

**108****Protection of Macaques from Vaginal SHIV Transmission by Topical Maraviroc, a Potent Inhibitor of HIV-1 Entry via the CCR5 Co-receptor**R.S. Veazey\*, T.J. Ketas<sup>2</sup>, J. Dufour<sup>1</sup>, L. Green<sup>1</sup>, P.J. Klasse<sup>2</sup>, J.P. Moore<sup>2</sup><sup>1</sup>Tulane National Primate Research Centre, Covington, LA, USA; <sup>2</sup>Weill Medical College of New York, Cornell University, NY, USA

**BACKGROUND:** An effective vaginal microbicide could reduce human immunodeficiency virus type 1 (HIV-1) transmission to women. The detergent- or polyanion-based microbicide candidates have all failed to demonstrate efficacy in clinical trials, so the emphasis has finally switched to anti-retroviral drugs. Among microbicide candidates now in clinical development is Maraviroc (MVC), an FDA-approved small molecule drug that binds the CCR5 co-receptor and impedes HIV-1 entry into cells. Delivered systemically, MVC reduces viral load in HIV-1-infected people, but its ability to prevent transmission is untested.

**METHODS:** Groups of adult female rhesus macaques were treated with Depo-provera and 30 days later intravaginally treated with ranging doses of MVC or placebo gel and at strategic intervals, intravaginally challenged with SHIV162P3 (an R5 virus) or SHIV-KU1 (an X4 virus) and subsequently monitored for infection.

**RESULTS:** Gel-formulated MVC derived from prescription-grade tablets provided dose-dependent protection of macaques from vaginal challenge with a high-dose of a CCR5-using virus, SHIV-162P3. Protection was half-maximal at 0.5 mM (0.25 mg/ml) and fully protective at 6 mM (3 mg/ml). The duration of protection was transient; the longer the delay between MVC application and virus challenge, the less protection ( $T_{1/2} \sim 4$  h). As expected, MVC did not protect against vaginal challenge with a CXCR4-using virus, SHIV-KU1, but its presence also did not exacerbate post-infection viremia in animals infected with this virus.

**CONCLUSIONS:** These findings validate MVC development as a vaginal microbicide for women, and should guide clinical programs. Of note is that a single 300 mg tablet of prescription-grade MVC, contains enough active drug to fully protect about 25 macaques, and by implication, a similar number of women, if applied vaginally in a gel.

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### Elafin/Trappin-2, a Novel Innate Immune Factor with Anti-HIV-1 Activity in Cell Cultures

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**BACKGROUND:** Studies of natural models of individuals able to resist infection, such as HIV resistant commercial sex workers from Kenyan have found antimicrobial proteins present at higher levels in cervical secretions than control groups. Purification and tandem mass spectrometry resulted in the identification of a novel innate immune factor named elafin/trappin-2 which is highly associated with HIV resistance. We hypothesized that elafin/trappin-2 interferes with HIV infection and/or replication in genital tract conferring protection against HIV.

**METHODS:** The anti-HIV activity of elafin/trappin-2 was evaluated using tissue culture assays and HIV-1 laboratory strain IIIB. Mechanism of action was investigated by using different cell models, flow cytometry analyses and a cell-to-cell fusion assay.

**RESULTS:** The current study shows that elafin prevented HIV-1 replication in infected SupT1 cells with a similar activity, at a concentration of 1 µg/mL, as 10 µM AZT. We found that anti-HIV activity of elafin is not related to an interference with receptor CD4 and co-receptor CXCR4 expression on the surface of target cells. We also demonstrated that elafin does not bind to CD4+ target cells or to viral glycoproteins gp120 and gp41. Cell-to-cell fusion assays showed that syncytium formation is not blocked by elafin. Our preliminary data suggest that elafin might act at an earlier step than binding and entry into target cells such as virion coating.

**CONCLUSIONS:** Elafin/trappin-2 inhibited HIV-1 replication *in vitro*. More experiments are needed to identify the mechanism of action underlying the anti-HIV activity of elafin but this study allowed to determine that elafin does not bind to CD4+ T cells nor to viral gp120 and gp41. Elafin/trappin-2 is a new anti-HIV small molecule that represents potential for the development of a topical microbicide.

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### Oral Administration of a Potential Living Rectal Microbicide

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**BACKGROUND:** Numerous proteins have been identified with potent anti-HIV-1 activity yet their clinical utility is questionable given the intrinsic instability of proteins upon mucosal instillation and the exorbitant costs associated with their manufacture. Using cyanovirin-N (CV-N) as a prototype, we demonstrate that lactic acid bacteria (LAB) can be engineered to secrete CV-N and that engineered bacteria can be formulated as oral yogurt preparations.

**METHODS:** LAB species were engineered to secrete CV-N after electroporation of LAB-specific CV-N gene cassettes. Bacterial supernatants were tested for antiviral activity against a broad array of lab derived and primary patient isolates. Fermentation procedures were optimized to achieve milk-flavored products harboring the engineered bacteria. Six pigtailed macaques were trained to ingest 50ml of fermented CV-N product daily. Pre- and post-ingestion rectal swabs and lavages were performed for downstream identification of CV-N.

**RESULTS:** Western blot of lavage samples and primary culture of swabs revealed that CV-N was produced in the rectal vault as early as 24 hours after feeding had begun. CV-N expression could be documented as long as product was ingested. After stopping, variable amounts of time lapsed before the end of CV-N expression ranging from 2-4 days. Colonization with engineered bacteria was not observed in any of the pigtailed macaques.

**CONCLUSIONS:** Monkeys who ingested bioengineered LAB produce CV-N product in the rectal vault. LAB serve as potential delivery vehicles of protein microbicides. Given their accepted safety profile as Generally Regarded as Safe, LAB pose little risk after oral ingestion. This delivery system may serve as a cost effective and acceptable manner to forward the prevention of HIV-1 mucosal transmission.

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### Phytochemical Analysis of Plants with Anti-Herpes Simplex Virus Activity

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**BACKGROUND:** Persons having sexually transmitted infections are at a greater risk of acquiring HIV/AIDS if exposed to the deadly virus. These infections have been managed traditionally using various herbal preparations. Several plants have been recorded as having potential for treating STIs. Genital Herpes viral infections are increasingly becoming a common problem especially to the immunocompromised individuals. It is therefore necessary to establish the chemistry of those plants that have been shown to have anti-herpes viral properties with an aim of identifying a potential compound that can be developed as microbicide to prevent transmission of genital herpes infections.

**METHODS:** Five plants *Azadirachta indica*, *Warburgia ugandensis*, *Acacia nilotica*, *Acacia mellifera* and *Centella asiatica* whose extracts are reported in the literature as having anti-Herpes simplex activity were collected in their natural habitats in Kenya. Water extracts were made from various parts of the plants and freeze-dried. The dried extracts were then analysed for various phytochemicals using thin layer chromatographic plates.

**RESULTS:** *A. indica* (leaves), *Warburgia ugandensis* (stem bark) and *Centella asiatica* (leaves) were shown to contain terpenoids with little phenolic compounds. *Acacia nilotica* and *Acacia mellifera* root barks were shown to have phenolic compounds.

**CONCLUSIONS:** The phytochemical agents found in these plants can be used as markers for development as a microbicide from plants for use against transmission of genital herpes infections

**112****Clinical Failures of Select Polyanionic Microbicide Candidates May Be Attributed to In Vitro Enhancement of HIV-1 Infection**

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**BACKGROUND:** Increasing efforts are being directed toward the development of microbicides, which will be used to reduce or eliminate the risk of human immunodeficiency virus type 1 (HIV-1) sexual transmission. Polyanionic compounds, which interact non-specifically with HIV-1 gp120 to block infection, were among the first agents evaluated clinically for their potential as microbicide agents. Unfortunately, Phase III clinical trials involving Carraguard, Ushercell, and PRO 2000 demonstrated that these products were ineffective and may have, in some instances, increased the risk of HIV-1 infection. These findings precipitated reassessments of the in vitro activities of these agents to determine if variables that can affect agent safety and efficacy had been overlooked during pre-clinical testing. One such variable is product retention and loss following topical application in the female reproductive tract.

**METHODS:** *In vitro* washout experiments were performed using P4-R5 MAGI indicator cells or primary human peripheral blood mononuclear cells (PBMCs). Cells were incubated with IC50 or IC90 concentrations of compound for 1 h. Cells were then challenged with HIV-1 Bal (R5) or IIIB (X4) for 1 h either concurrent with compound incubation or up to 7 h after compound removal and incubation in new media without compound. Following infection, cells were washed, incubated for 48 h (P4-R5 cells) or 6 days (PBMCs), and assayed for HIV-1 infection.

**RESULTS:** By mimicking product loss *in vitro*, we showed that several polyanionic compounds, including carrageenan and cellulose sulfate, caused enhancement of HIV-1 infection following compound removal, despite potent antiviral activity when introduced simultaneously with the viral challenge. The presence and magnitude of this effect was compound-specific, dependent on the interval between compound removal and virus challenge, and dependent on HIV-1 co-receptor usage. Compounds that enhanced HIV-1 infection in this assay increased levels of HIV-1 infection up to 10-fold.

**CONCLUSIONS:** HIV-1 infection is significantly enhanced *in vitro* following polyanionic compound washout despite demonstrated effectiveness in assays where compound and virus are introduced concurrently. More detailed studies are now underway to determine the mechanism responsible for this enhancement effect, and to determine the contributions of this effect to the clinical failures of these agents.

**113****A Novel High-Throughput Approach for Screening Inhibitory Compounds Against *Chlamydia trachomatis***

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**BACKGROUND:** A primary challenge in microbicide and excipient development is to assess the safety and efficacy of novel compounds that can prevent both HIV and non-HIV sexually transmitted infections. *Chlamydia trachomatis* are sexually transmitted bacteria that contribute significantly to an increased rate of HIV transmission. *C. trachomatis* are obligate intracellular bacteria for which accurate detection is dependent on immuno-fluorescent staining of infected tissue culture cells. Currently, compounds with potential anti-chlamydial activity are analyzed using fluorescence microscopy and manual enumeration. In this study, a novel high-throughput screening method for rapidly and accurately assessing anti-chlamydial activity of excipients and microbicides is described.

**METHODS:** To develop a high-throughput screening method, two compounds that have previously been demonstrated to exhibit anti-chlamydial activity were employed: the microbicide Polymyxin B and a hydroxyethylcellulose (0.09 mM) excipient. Infectious chlamydial forms (EBs) were incubated with the compounds prior to addition to mouse fibroblast cells (L929). Dose and time dependent analyses were performed in triplicate within a 96-well-format. Cells were stained with MicroTrak (Trinity Biotech, NY) and DAPI. Images for 16-20 fields per well were acquired using automated fluorescent microscopy. Following image acquisition, data were analyzed using CellProfiler: an open-source software package designed for high-throughput cell image analysis. A customized pipeline was created within CellProfiler to quantify *C. trachomatis* infection rates.

**RESULTS:** Approximately 2000 cells per well were analyzed and intracellular chlamydial forms accurately identified. Automated processing of the collected images demonstrated dose and time dependent inhibition of *C. trachomatis* confirming observations in previous studies. This technique streamlined the collection and processing of thousands of images to rapidly assess anti-chlamydial activity.

**CONCLUSIONS:** This method provides a facile and accurate process to screen anti-chlamydial compounds in a high-throughput format. This approach decreases subjectivity associated with manual counting and yields robust data sets. This is a tool that can be applied by the microbicide community to efficiently screen numerous compounds and concentrations for inhibitory activity against *Chlamydia* or for similar processes that are reliant on immunofluorescent microscopy for enumeration.

**114****Screening of Indigenous Plant Products as Potential Candidate Microbicides**

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**BACKGROUND:** Secondary metabolites of plants have been found to inhibit HIV and thus biologically active natural products may serve as good source for microbicide development. We present here *in vitro* anti-HIV and anti-STI screening of extracts from wild plants, selected on the basis of their ethno-pharmacological and chemo-taxonomical profile.

**METHODS:** Based on the preset stringent criteria including HPLC and HPTLC profile, 13 wild plants belonging to eight families Convolvulaceae (3), Polygonaceae (2), Anacardiaceae (1), Combretaceae (2), Lauraceae (1), Asteraceae (2), Zingiberaceae (1) and Rhamnaceae (1) were selected. The methanol extracts of the plant parts were assessed for toxicity and subsequently for inhibition of X4 (HIV-1IIIB, UG070) and R5 (HIV-1 Ada5, VB59) tropic lab adapted strains and primary isolates (cell free and cell associated), using TZM-bl cells. Extracts with anti-HIV activity were further broadly separated into less, medium and highly polar sub fractions using column chromatography or solvent separation/partitioning and tested for anti-HIV activity. Eight extracts and three sub fractions with anti-HIV activity were additionally tested for inhibition of *C. albicans* and *N. gonorrhoea* by disc diffusion method.

**RESULTS:** Seven of thirteen primary extracts were found to inhibit cell free and cell associated X4 and R5 HIV-1 (CC80:15-117ug/ml, IC80:3-156ug/ml) and one extract inhibited only cell free HIV-1 (CC80:15ug/ml, IC80:4ug/ml). Fifteen sub fractions showed inhibition of cell free and cell associated X4 and R5 HIV-1 (CC80:3-117ug/ml, IC80: 1-312ug/ml). Additionally, four sub fractions were found to inhibit either cell free or cell associated X4 and R5 HIV-1 (CC80:35-78ug/ml, IC80:10-156ug/ml). These results will be confirmed in T lymphocytes, cervical explants and epithelial transwell model systems. Interestingly, one primary extract and its sub fraction showed less toxicity, good anti-HIV activity at low concentrations (IC80:3-16ug/ml), as well as anti-Candida and anti-Neisseria activity. Furthermore, one extract was found to exhibit only anti-Candida activity and another two showed anti-Neisseria activity.

**CONCLUSIONS:** The results demonstrate that ethno-pharmacological and chemo-taxonomical based plant selection is a good system of identifying newer safer leads that has potential of inhibiting HIV and other sexually transmitted pathogens, which could be moved into microbicide development, an effort towards HIV prevention.

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### Evaluation of Saquinavir as a Candidate Microbicide Compound in Cellular and Mucosal Tissue Models of HIV-1 Infection

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**BACKGROUND:** The maturation of newly formed HIV-1 virions is a critical step in establishing productive infection. The viral protease enzyme is heavily involved in the maturation of HIV and during post-translational processing cleaves the viral precursor proteins p55 and p160 to their mature forms. Saquinavir (SQV) is an antiretroviral drug used in combination HAART, and is a potent inhibitor of the protease enzyme, blocking the maturation of newly budded virus from host cells. We have investigated the efficacy and safety of SQV in cellular and tissue-based models of HIV-1 infection in order to evaluate it as a potential microbicide candidate.

**METHODS:** SQV was assessed for its anti-HIV activity in human cell lines and primary cell cultures, as well as cervical, penile and rectal tissue explants. Cellular and tissue viability was assessed by MTT assay. Tissue explants were exposed to SQV and HIV-1<sub>BaL</sub> for a 2-hour pulse. Twenty-four hours post-infection, tissue explants were either transferred to new culture plates and migrating cells were co-cultured with PM-1 T cells, or the explants were directly co-cultured with PM-1 T cells. Similarly, peripheral blood mononuclear cells (PBMCs), primary macrophages and immature dendritic cells (iDCs) were only exposed to SQV and HIV-1<sub>BaL</sub> for 2h. Viral replication was assessed by measurement of p24 antigen.

**RESULTS:** Cellular studies demonstrated a dose response to SQV (2h pulse). SQV inhibited infection of PM-1 T cells and Monomac-1 cells with IC<sub>50</sub> values of 0.65μM and 0.4μM respectively. In assays using PBMCs, primary macrophages and iDCs, the IC<sub>50</sub> values of SQV against HIV-1 infection were 0.06μM, 10.9μM and 2μM respectively. SQV inhibited production of infectious virus in cervical, penile and colorectal explants co-cultured with T cells at an IC<sub>50</sub> value of 2μM for both tissue types. Moreover, SQV inhibited the trans-infection of T cells by penile and cervical tissue migratory cells with an IC<sub>50</sub> of 1μM and 10μM respectively. Finally, SQV had no effect on cellular and tissue viability.

**CONCLUSIONS:** SQV demonstrates a potent ability to inhibit HIV infection of cell lines and primary cells. In addition, it inhibits production of infectious HIV-1 in tissue explants, while exhibiting a high inhibitory effect against trans-infection of T cells by tissue emigrating cells. These data suggest that SQV could be utilised as a microbicide to block the viral maturation and transmission of HIV-1 at mucosal surfaces.

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### Multiple Development Pathways of Pyrimidinediones as Topical Microbicides to Prevent the Transmission of HIV

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**BACKGROUND:** The pyrimidinediones (PYDs) are small molecules which act as highly potent nonnucleoside RT inhibitors at subnanomolar concentrations and inhibit virus entry at nanomolar concentrations. The suppression of two critical early occurring (pre-integration) steps in HIV transmission and infection renders these agents valuable as potential topical microbicides. Two PYDs, IQP-0528 and IQP-0532, have been defined as possessing the potency, lack of toxicity, stability and pre-formulation characteristics required of an effective microbicide product.

**METHODS AND RESULTS:** Significant progress has been made with the development of the PYDs along multiple development pathways. Efficacy and toxicity data in fresh human PBMCs infected with clinical strains of virus demonstrates that IQP-0528 and IQP-0532 act as highly potent and safe microbicides and interact in an additive to synergistic manner with other microbicides in development, providing a rationale for a combination microbicide product. Activity of the PYDs in cervical explant challenge models provided additional rationale for continued development of the compounds. The preformulation characteristics of the various PYDs have been evaluated and suggest multiple pathways for development of a PYD containing product formulated as a gel, intravaginal ring, or film alone or in combination. IND-directed safety and toxicology studies has been completed with our lead PYDs and demonstrate a significant safety margin, suggesting that a long-acting microbicide product containing a PYD will possess attractive pharmacokinetics and pharmacodynamics for prolonged microbicide product use. Like other RTIs, the PYDs also lend themselves for use as pre-exposure prophylaxis agents; data obtained with the congener IQP-0410 demonstrates that the oral bioavailability and pharmacokinetics of the PYDs will yield effective blood and tissue concentrations of the compounds.

**CONCLUSIONS:** The PYDs represent novel microbicide candidates based on their mechanism of action, high potency, lack of toxicity, compatible formulation characteristics, and toxicology profile. As a microbicide, the PYDs could be effectively combined with other microbicide products. Three PYDs, IQP-0410, IQP-0528, and IQP-0532 are currently undergoing multiple pathway development in gel, ring, film and PrEP formats.

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### In Vitro Evaluation of the Safety Profile of Pharmaceutical Excipients for the Use in Microbicide Formulations

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**BACKGROUND:** The results of the nonoxynol-9 and cellulose sulfate clinical trials have shown an increased risk of HIV infection and an absence of efficiency, respectively. These failures suggest that more extensive *in vitro* strategies must be implemented to anticipate the *in vivo* efficiency/toxicity rate of future candidate microbicide formulations. Not only the safety of the active pharmaceutical ingredient used in the formulation, but also the safety of pharmaceutical excipients is of great importance.

**METHODS:** The safety of excipients belonging to various classes, i.e. preservatives (PRES), co-solvents (CS), surfactants (SF) and cyclodextrins (CD), was evaluated on HEC-1A epithelial layers, grown in a dual chamber setup and on cervical explants cultured in a non-polarized fashion. Upon 24h exposure of the explants and the HEC-1A epithelial layers to pharmaceutically relevant concentrations of the excipients, viability was determined (WST-1 assay). Passage of fluorescent beads (100 nm, similar to an HIV virion) across cell layers was used as a measure of epithelial layer integrity. IL-8 production was assessed as a marker for induction of inflammation by excipients.

**RESULTS:** All tested SF exerted toxic effects on HEC-1A cells and cervical explants at concentrations typically used in formulations (sodium lauryl sulfate > TPGS1000 succinate >Polysorbate 80 >Cremophor® EL). Cell viability and epithelial layer integrity were also compromised by the PRES benzalkoniumchloride and benzylalcohol, but less by sorbic acid, methyl- and propylparaben. Among CD, the following rank order for toxicity was observed: dimethyl-β-CD >α-CD, β-CD >γ-CD >hydroxypropyl-β-CD >sulfobutylether-β-CD >hydroxypropyl-γ-CD (no toxic effects). The CS ethanol and glycerin, propylene glycol and polyethylene glycol 400 and 1000 exerted considerably lower toxic effects. Finally, IL-8 production was differentially affected by the various excipients.

**CONCLUSIONS:** The present study revealed a reduced viability of epithelial cells and cervical explants and impaired epithelial layer integrity upon apical exposure to various pharmaceutical excipients. Identification of these safety issues in early development is of great assistance to formulation scientists. Careful selection of excipients for microbicide formulation seems warranted.

**118****Development of Flavonoids for Use in a Combination Microbicide**C.E. Isaacs<sup>\*1</sup>, M.A. Parniak<sup>2</sup>, L. Rohan<sup>2,3</sup>, S. Hillier<sup>2,3</sup>, G. Merz<sup>4</sup>, W. Xu<sup>1</sup>, G. Wen<sup>4</sup>

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**BACKGROUND:** Developing a combination microbicide that inactivates multiple sexual pathogens is desirable since genital infections are often polymicrobial and synergistic. The development of a combination microbicide directed against HIV and HSV could reduce HIV transmission both by directly inactivating HIV and by disrupting the biological synergy link between HSV infection and HIV transmission. Flavonoids derived from green (catechins) and black (theaflavins) tea, are on the Food and Drug Administration's GRAS (generally recognized as safe) list of compounds approved for human consumption. The objective of this study was to measure their virucidal activity against HSV and HIV.

**METHODS:** The antiviral activity of catechins from green tea and theaflavins from black tea was measured against panels of HIV-1 and HSV-2 and HIV. Electron microscopy and confocal microscopy were used to determine the effects of flavonoids on HSV and Vero cell morphology. Aggregation of HSV-1 glycoproteins was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

**RESULTS:** Oxidative dimerization of the tea flavonoid (-)-epigallocatechin gallate (EGCG) produces compounds with increased antiviral activity against HSV-1 and HSV-2 at acidic, neutral and alkaline pH when compared to monomeric EGCG, which is only antiviral at neutral and alkaline pH. EGCG inactivated HSV-1 and HSV-2 at pH 8.0 by 1,000–10,000 fold but was ineffective at pH 5.7. However, the EGCG digallate dimers theasinensin A, P2 and theaflavin-3,3'-digallate inactivated both viruses by 1,000–10,000 fold at pH 5.7 and as much as 100,000-fold at pH 8.0. EGCG did not inactivate vesicular stomatitis virus (VSV) in the pH range of 5.7–7.4 and by ≤ 100-fold at pH 8.0 while the digallate dimers theasinensin A and P2 reduced VSV titers by ≥ 100,000-fold from pH 5.7–8.0. Examination of purified HSV-1 virions and Vero cells utilizing electron and confocal microscopy showed that dimerized EGCG (100 μM) destroyed HSV-1 virions but did not damage Vero cells. EGCG dimers inactivated cell surface HSV-1 virions as effectively as cell free virus and produced aggregation of HSV-1 glycoprotein B but not glycoprotein D within 1 hour. EGCG dimers decreased the titer of HIV virions to the same or greater degree as the monomer at neutral pH.

**CONCLUSIONS:** EGCG dimers have equivalent or greater anti-HSV activity at pH 7.4–8.0 than the EGCG monomer but have much greater antiviral activity at acidic pHs which is relevant for the vaginal environment. EGCG dimers, both relative to the degree of HSV inactivation and the broad range of effective pH, appear to have excellent potential to be utilized in a topical microbicide in conjunction with an NNRTI such as UC781 in the pH range found vaginally.

**119****Potent Low-Cost Neutralizing Antibodies against HIV-1 from Hyperimmune Bovine Colostrum**M. Kramski<sup>\*1</sup>, R.J. Center<sup>1</sup>, C. Siebentritt<sup>1</sup>, A.K. Wheatley<sup>1</sup>, G. Rawlinz<sup>2</sup>, D.F.J. Purcell<sup>1</sup>

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**BACKGROUND:** Bovine colostrum, also known as first milk, contains antiviral proteins and highly concentrated maternal immunoglobulin drawn from serum during pregnancy. Our aim was to establish a vaccination strategy in cows using oligomeric HIV-1 Env gp140 to obtain large quantities of colostrum-derived HIV-1 neutralizing antibodies (Ab) with high intrinsic safety at relatively low cost. Vaccination of each pregnant cow can yield up to 1kg of polyclonal Ab and, if broadly potent, may be incorporated into a cost effective global-scale microbicide.

**METHODS:** Soluble clade A, B and C Env gp140 oligomers were purified from the supernatant of stably transfected HeLa and 293T cell-lines. In a pilot study, two pairs of pregnant (P) and non-pregnant (NP) cows were vaccinated 3 times intramuscularly with 100μg of a clade B or with equal amounts of clade A, B and C Env (trimix) gp140 oligomers in adjuvant. Upon becoming pregnant, NP-cows received an additional vaccination 4 weeks before calving. Colostrum was collected postpartum and analyzed for presence and breadth of HIV-1 neutralizing antibodies.

**RESULTS:** All animals seroconverted within 9 weeks of the first vaccination with reciprocal endpoint serum IgG titers of up to  $1 \times 10^5$  for NP-cows but only  $1.5 \times 10^2$  for P-cows. Within colostrum samples, Abs specific to HIV-1 Env gp140 were concentrated up to reciprocal endpoint IgG titres between  $3.5 \times 10^3$  and  $1 \times 10^6$ . While non-immune colostrum showed some intrinsic neutralizing activity, hyperimmune colostrum from the P-trimix cow and the NP-clade B cow demonstrated highest HIV-1 neutralizing activity against HIV-1 clade A, B and C Env-pseudotyped reporter viruses. Neutralizing activity ranged between 30%–90% for 27 Env-pseudotyped reporter viruses, including Env clones of the clade B and C NIH reference panels (ARRP #11227, #11326), and was mediated primarily by bovine IgG

**CONCLUSIONS:** Vaccination with native structured HIV-1 Env gp140 oligomers yields a high concentration of colostrum-derived polyclonal bovine Ab with potent cross-clade neutralizing activity against HIV-1. With the safety profile of food they can be easily produced in large-scale to a fraction of the cost of monoclonal neutralizing Abs. Colostrum-derived anti-HIV-1 Abs warrant further development for topical, mucosal application ahead of safety and efficacy evaluation.

**120****Evaluation of Retrocyclin and Lactobacillus-derived Cyanovirin for Antiviral Activity in Cell-Free and Cell-Associated HIV-1 Transmission Assays**C. Lackman-Smith<sup>\*1</sup>, B.A. Snyder<sup>1</sup>, M.C. Osterling<sup>1</sup>, K.M. Marotte<sup>1</sup>, A.M. Cole<sup>2</sup>, B.Ramaratnam<sup>3</sup>, L. Rohan<sup>4</sup>, D. Patton<sup>5</sup>, J.E. Cummins, Jr<sup>6</sup>, P. Gupta<sup>4</sup>

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**BACKGROUND:** Retrocyclin (RC-101) and Lactobacillus-derived cyanovirin (LB-CVN) have been proposed for development as topical microbicides to prevent sexually transmitted HIV-1 infection. In conjunction with a collaborative program evaluating these compounds for formulation characteristics, safety in non-human primates, and post-administration tissue distribution, these compounds were evaluated using *in vitro* assays in the presence and absence of human seminal plasma or at pH transition from acidity to neutrality similar to what may occur in the vagina before and during coitus.

**METHODS:** Unformulated compounds were tested in established *in vitro* assays to determine inhibition of cell-free (CF) HIV-1 transmission in MAGI-R5 cells infected with HIV-1 Ba-L +/- 12.5% human seminal plasma (SP) and also with pH transition from 4.0 to 7.4 (pH). Compounds were also evaluated in cell-associated (CA) transmission assays using 1) H9/HIV-1SK-1 and ME180 or GHOST(3)X4/R5 target cells and 2) MOLT-4/R5/JRCSF and GHOST(3) X4/R5 target cells +/- 25% SP.

**RESULTS:** LB-CVN was active in the ME180, CXCR4- and CCR5-tropic CA, CCR5-tropic CF and CCR5-topic fusion assays with mean IC<sub>50</sub> values ranging from 0.05 to 0.15μg/mL. The IC<sub>50</sub> for SP and pH assays ranged from 0.06 to 0.09μg/mL. The LB-CVN inhibited of *Lactobacillus crispatus* and *L. jensenii* (MIC<sub>50</sub> 0.45μg/mL (+/-0.03) and 0.15 (+/-0.03) μg/mL, respectively). RC-101 was active in the CCR5-tropic CA with mean IC<sub>50</sub> of 0.25 (+/-0.09), and 2.70 (+/-0.34) μg/mL respectively for the standard and SP assay, and similarly in the CCR5-tropic CF assays the mean IC<sub>50</sub> values were 0.74 (+/-0.34), 3.08 (+/-0.92), and 7.26 (+/-0.68) μg/mL respectively in the standard, SP, and pH assays. RC-101 was active in the ME180 and X4-CA with IC<sub>50</sub> of 4.07 (+/-2.06) and 2.34 (+/-0.34) μg/mL, respectively. RC-101 was not inhibitory to *Lactobacillus* sp. at the highest concentration tested (100μg/mL).

**CONCLUSIONS:** LB-CVN demonstrated activity in all of the HIV assays with and without SP or pH transition, but was inhibitory to viability of Lactobacillus species. RC-101 was active in all assays yet demonstrated reduced activity in the presence of SP, with no inhibition of viability of Lactobacillus species. Given the reported stability of RC-101, its non-toxic and non-inflammatory nature, and its ability to inhibit HIV-1 infection in cervical organ culture, our studies further suggest the continued development of this peptide as a topical intra-vaginal microbicide.

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### Safety Evaluation of the Aflavin Derivatives Gel as Microbicide for Preventing HIV Sexual Transmission

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**BACKGROUND:** Theaflavin derivatives (TF), including theaflavin (TF<sub>1</sub>), theaflavin-3-gallate (TF<sub>2A</sub>), theaflavin-3'-gallate (TF<sub>2B</sub>), and theaflavin-3,3'-digallate (TF<sub>3</sub>), are the principal polyphenols in black tea. The TF, in which 90% (W/W) are the above four compounds, showed anti-HIV activities through inhibiting HIV entry in our earlier study. The purpose of the present study is to develop TF gel (containing 2% theaflavin derivatives) as a novel microbicide and evaluate its vaginal mucosal toxicity.

**METHODS:** The toxicity study of TF gel was carried out *in vitro* on women reproductive tract epithelial cells and in New Zealand rabbit vaginal irritation model. Sera level of pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, IL-8 and TNF- $\alpha$ ) and immuno-regulatory cytokines (IL-10 and GM-CSF) were measured by ELISA kits. PCNA immunostain was performed to evaluate the vaginal tissues for inflammation. The systemic absorption of TF gel was also investigated for evaluating its bioavailability.

**RESULTS:** According to its physical and chemical profiles, TF gel was prepared by mixing 2% theaflavin derivatives and several pharmaceutical excipients, like Carbopol<sup>®</sup> 974P, glycerin and propylene glycol. Ethanol was added to improve the solubility of TF. TF showed low cytotoxicity on women reproductive tract epithelial cells. No apparent cervicovaginal toxicity was observed at any time point evaluated following the intravaginal administration of TF gel to rabbits. In contrast, application of N-9 resulted in damage on vaginal epithelium. TF gel did not trigger pro-inflammatory and immuno-regulatory cytokines productions. Only low expression of PCNA was observed in vaginal tissues of TF gel treated animals. Further studies indicated that systemic absorption of TF was not detected in plasma 2 hours after single vaginal administration of TF gel. In contrast, the amount of TF was still detected in the cervicovaginal lavages (CVLs) drawn 6h after treatment. These results suggested TF could retain in vaginal cavity and maintain biological activity for a relatively long time.

**CONCLUSIONS:** Combining the profiles of marked stability at acidic condition, low mucosal toxicity and lacking of systemic absorption, theaflavin derivatives gel is a potential safe and inexpensive microbicide candidate for preventing HIV sexual transmission.

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### Anti-HSV Agent from a Medicinal Plant *Prunus africana* (Hook.f.) Kalkm

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**BACKGROUND:** Herpes simplex virus types one and two (HSV-1 and HSV-2) are among the most common opportunistic infections in immunosuppressed persons especially those with HIV/AIDS. The emergence of resistant strains of HSV to drugs and the high cost of these drugs has worsened the situation. There is therefore need to identify new agents for management of HSV infections. *Prunus africana*, a medicinal plant in Kenya is used in traditional medicine for treatment of bacterial, viral diseases and even benign prostate hyperplasia.

**METHODS:** The *in vitro* anti-HSV activity of the aqueous extract of *prunus africana* was carried using the plaque reduction assay method in Vero E6 cells with 100 PFU of wild type strain of HSV (7401H HSV-1). Cytotoxicity was determined using the trypan blue exclusion test. The *in vivo* activity was examined in a murine model using Balb/C mice cutaneously infected with wild type HSV-1. The extract was administered to mice at an oral dose of 200 mg/kg ( $p < 0.05$  Vs control by Student's t-test). Toxicity in animals was determined in mice using the standard methods.

**RESULTS:** The extract significantly inhibited formation of plaques in Vero E6 cells infected with 100 PFU of wild type strains of HSV-1 with an EC<sub>50</sub> of between 25.2  $\mu$ g/ml with minimal cytotoxicity (CC<sub>50</sub> = 550  $\mu$ g/ml). When the extract was administered in Balb/C mice infected with wild type HSV-1, an oral dose of 200 mg/kg resulted to a significant delay on the onset of HSV infections ( $p < 0.05$  Vs control by Student's t-test for mean time onset of infection). The mean survival time was increased in the treated infected mice by about 10 % compared with the infected untreated mice. The mortality rate was reduced by 30% as opposed to 100% mortality in the infected untreated mice. No toxicity was observed at the oral therapeutic dose of 200mg/ml. A pure compound, triterpenic glycoside, [vitalboside-A, 2'-O-(methyl- $\beta$ -glucuronate)] was isolated as the active anti-HSV compound. This compound had an EC<sub>50</sub> of 3.7  $\mu$ g/ml with minimal toxicity (CC<sub>50</sub> > 150  $\mu$ g/ml).

**Conclusion** The anti-HSV compound, identified will serve as a reference marker for standardization in the formulation of anti-HSV cream from *Prunus africana*.

**123****Two Medicines That Block HIV-1 Gene Expression and Kill HIV-1-Infected Cells Preferentially, Yet Lack Epithelial Toxicity**

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**BACKGROUND:** HIV-1 gene expression and cellular apoptosis involve post-translationally hydroxylated cellular proteins. We hypothesized that protein hydroxylation inhibitors show a novel anti-HIV profile, promoting apoptosis of infected cells by disrupting retroviral control over this innate response. To test our hypothesis, we are using drugs, rationally identified via the active site geometry and/or the catalytic mechanism of protein hydroxylases. We published that two protein hydroxylation-inhibiting drugs, ciclopirox (CPX), the globally available vaginal fungicide, and deferiprone (DEF), a medicinal chelator, interfere with HIV-1 promoter activity and gene expression. We have shown in primary lymphocytes that CPX and DEF block HIV-1 infectivity, kill HIV-infected cells preferentially, and block HIV-1 rebound after drug cessation, an apoptosis-related finding also noted in a pilot DEF trial. We report the cell biology of the drugs' proapoptotic effect and test the epithelial toxicity of DEF.

**METHODS:** Conventional/raft culture of established cell lines; multi-color flow cytometry.

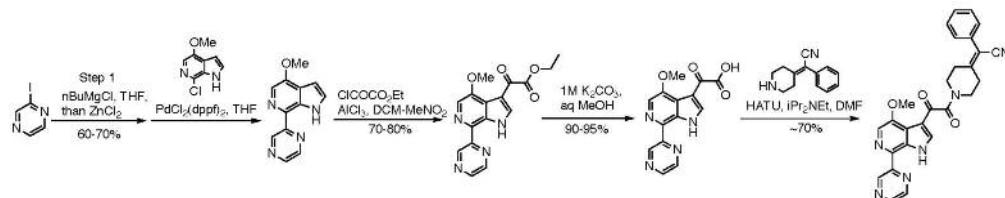
**RESULTS:** HIV-1 infection of CD4+ H9 cells (H9-HIV) markedly stabilized the mitochondrial membrane potential ( $\Delta\Psi$ ), a key endogenous trigger of apoptosis, and significantly reduced apoptosis markers, e.g. the caspase 3-generated 89-kDa fragment of poly (ADP-ribose) polymerase (frag-PARP). CPX and DEF reversed this relation in a dose-dependent manner. Within 24 hours, control-normalized  $\Delta\Psi$  collapse in H9-HIV caused by 30  $\mu$ M CPX was twice that of uninfected H9 ( $P = 0.02$ ); DEF at 200  $\mu$ M gave similar results. CPX increased frag-PARP+ cells by almost tenfold over controls in H9-HIV, but only about twofold in H9. The signal intensity for frag-PARP+ cells was distinctly higher in H9-HIV ( $\geq 1$  log after 24 hours). 30  $\mu$ M CPX did not induce anti-apoptotic Bcl-2; 200  $\mu$ M DEF did so marginally, consistent with a less prominent frag-PARP increase. DEF was as effective as CPX in achieving apoptosis in H9-HIV, measured by annexin / 7-AAD, TUNEL, volume contraction, and cell death. DEF did not degrade the barrier integrity of a tight-junction-linked model mucosa (uterine EEC-1), even after 6 days at 200  $\mu$ M.

**CONCLUSIONS:** CPX and DEF cause preferential apoptotic ablation of HIV-1 infected cells via the mitochondrial pathway. CPX, with its known clinical safety profile as vaginal antifungal, and DEF, which lacks epithelial toxicity in the ECC-1 model, should be considered as candidate microbicides.

ABSTRACT WITHDRAWN BY AUTHORS

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**125****Characterization of HIV-1 Aptamer Degradation in Rectal and Vaginal Lavage Fluid**I. McGowan<sup>\*1,4</sup>, M.D. Moore<sup>2</sup>, B. Sproat<sup>3</sup>, E. Sutton<sup>4</sup>, S. Abo<sup>4</sup>, R. Cranston<sup>4</sup>, W. James<sup>2</sup><sup>1</sup>*Magee-Womens Research Institute, Pittsburgh, PA, US; <sup>2</sup>Sir William Dunn School of Pathology, University of Oxford, Oxford, UK; <sup>3</sup>Chemconsilium GCV, Boisshot, Belgium; <sup>4</sup>University of Pittsburgh School of Medicine, Pittsburgh, PA***BACKGROUND:** Neutralization of HIV infection in intestinal explant systems with aptamers has been modest compared to agents such as tenofovir. We undertook a series of experiments to determine whether exposing aptamers to rectal or vaginal lavage fluid resulted in aptamer degradation.**METHODS:** Vaginal (VL) and rectal lavage (RL) samples were collected from 36 healthy HIV negative participants. Lavage fluid (30 L) was incubated with 20 L of 100nM UCLA 1 aptamer solution. 5 L samples were collected at 0, 5, 10, 20, 40, 80, 160, 320, and 1440 minutes and run on 16% polyacrylamide urea gels. Aptamer degradation was quantified using scanning densitometry. Characterization of lavage induced aptamer cleavage sites was determined by comparison with the aptamer fragmentation pattern induced by alkaline hydrolysis.**RESULTS:** Overall, RL induced more rapid degradation of the aptamers than VL fluid ( $p < 0.0001$ ). However, in paired samples from 16 women the pattern was more heterogeneous: in 7 women there was no difference between RL and VL, in another 7 women RL was more aggressive than VL and in 2 women VL was more aggressive than RL. The samples were heterogeneous not only in the intensity of nuclease activity but also qualitatively: different sites were preferentially cut in different samples. Most obviously, the preferred cleavage site in RL was A9, and A10 with VL. We also observed novel nucleic acid ligase activity in a substantial fraction of RL.**CONCLUSIONS:** VL and RL fluid has the potential to degrade aptamers. The rapidity of aptamer degradation is highly variable and may relate to the endogenous microbiological flora and associated enzymes present in both the rectal and vaginal compartment. The most active nucleases were not dependent on the presence of an adjacent 2'OH moiety, and so were not conventional ribonucleases. The stereotypical nature of the aptamer cleavage suggests the possibility of aptamer modification to prevent enzymatic cleavage. These observations illustrate the challenges of developing nucleic acid based microbicide candidates.**126****Process Development for the Synthesis of DS003, a New Antiretroviral Drug Candidate for the Prevention of HIV Infection**H. Cheng<sup>1</sup>, S. Pukul<sup>2</sup>, J. Romano<sup>\*3</sup>, A. Thompson<sup>1</sup><sup>1</sup>J-Star Research, Inc., South Plainfield, NJ, USA; <sup>2</sup>Princeton API Services, LLC, Princeton, NJ, USA;<sup>3</sup>International Partnership for Microbicides, Silver Spring, MD, USA**BACKGROUND:** DS003 (BMS-793) is a small molecule entry inhibitor that interferes with HIV infection by binding to the gp120 protein. Scale-up synthesis chemistry to Kg quantities, which is described here, was required for continued development of this compound as a microbicide.**METHODS:** Three key raw materials were required for the synthesis of DS003: a substituted azaindole, a substituted piperidine, and iodopyrazine. Optimization of each of the synthetic steps was performed with a particular emphasis on the Negishi coupling reaction. Several reaction parameters were investigated including: reagent types, reagent stoichiometry, and reaction temperatures.**RESULTS:** In order to make the preclinical material, the method of synthesis of DS003 was redesigned and the critical coupling step was moved up in the reaction sequence. The coupling of substituted azaindole and pyrazine based on the Stille conditions was replaced by the Negishi reaction. This method proved optimal from multiple perspectives. The scalability of a new Negishi coupling-based process was demonstrated by the synthesis of 500 g of the API. Additional optimization was performed to develop conditions that ensure the formation of the desired polymorph of DS003, and was employed to produce 2 kg of the target API, which were dedicated to preclinical formulation development and toxicity studies.**CONCLUSIONS:** A new approach to the synthesis of DS003 was developed utilizing the Negishi reaction. As a result, the need for using the expensive and toxic tin reagent in the Stille type coupling reaction was eliminated. Further optimization of the process allowed for full control over the formation of the desired polymorph. The new process was successfully applied to manufacture kilogram quantities of the target active pharmaceutical ingredient.

**127****Full Additivity/Synergy of CCR5 Antagonist RANTES Derivatives When Tested in Combination with Different HIV-1 Blockers**

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**BACKGROUND:** CCR5 ligands inhibiting HIV-1 entry are promising candidates for the development of safe microbicides. However, as in HAART therapy, combination of microbicides with different mechanism of action could enhance protection against HIV-1 infection in genital mucosa.

Two anti-HIV-1 CCR5 antagonists have been developed in our laboratory: Rmax, the most potent peptide derived from the N-loop/β1-strand region of RANTES, and C1C5 RANTES, a full-length RANTES mutant. Here, we report full additivity or synergistic effects on the inhibition of HIV-1 infection *in vitro* when RANTES derivatives were used in combination with maraviroc, cyanovirin-N or T20.

**METHODS:** Rmax was chemically synthesized and then purified by RP-HPLC to >95% purity. C1C5 RANTES was purified to homogeneity by ion exchange chromatography from engineered *Lactobacillus jensenii* culture supernatant. Cell-cell fusion and p24-based assays were performed to determine anti-HIV-1 activity of individual or combined compounds. Combination results were analyzed by using CalcuSyn software for calculating inhibitory concentrations (IC) and combination indexes (CI).

**RESULTS:** Rmax inhibits HIV-1<sub>Bal</sub> infection with an IC<sub>50</sub> similar to that of T20 and exerts its activity against different primary HIV-1 R5 strains of clade B and C. When tested in combination, full additivity was observed with maraviroc and cyanovirin-N and synergy with a CI<sub>50</sub><0.8 was obtained with T20. IC<sub>50</sub> for the combinations were strongly reduced as compared with those corresponding to the single compounds. C1C5 RANTES combination with maraviroc or cyanovirin-N resulted also in a significant synergistic effect on the inhibition of HIV-1<sub>Bal</sub> infection, with a CI<sub>50</sub> of about ~0.8 for both combinations.

**CONCLUSIONS:** RANTES-based CCR5 antagonists are fully suitable to combination with other entry inhibitors, as revealed by potent anti-HIV-1 additivity/synergy effects. All combinations tested significantly reduced HIV-1 infection and no cellular toxicity was observed at the highest concentrations used. These data suggest that RANTES derivatives may be included in the development of a microbicide cocktail to prevent sexual transmission of HIV-1.

**128****Preclinical Safety Assessment of a Novel Silicone Vaginal Ring for Delivery of Dapivirine**

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**BACKGROUND:** Vaginal rings are a promising means of sustained local microbicide delivery. Rings can be made using alternative chemistries and configurations; an optimal ring formulation is determined primarily by the drug being delivered. Dapivirine, a potent NNRTI microbicide currently in clinical trials, was most recently formulated in a Pt-catalyzed cured silicone matrix ring. As this is a novel ring formulation of this drug, pre-clinical safety evaluation was completed to support its use in clinical trials.

**METHODS:** Pre-clinical safety evaluation of the dapivirine ring was performed according to ISO 10993-1 guidelines, and involved biological and chemical assessments. Extracts from rings were prepared using polar and non-polar solvents and subjected to *in vitro* cytotoxicity, genotoxicity, as well as *in vivo* sensitization and subacute/subchronic toxicological evaluations. To further characterize the dapivirine vaginal ring, extracts obtained for biocompatibility tests were analyzed for the presence of oligosiloxanes and trace metals using a combination of GC-MS, LC-MS, and ICP-MS.

**RESULTS:** Ring extracts were shown to be non-genotoxic, and were not sensitizing in the guinea pig model. In 35-day rabbit vaginal dosing studies with saline and sesame oil ring extracts, minimal or no irritation was observed. Although there were some observations in initial cytotoxicity assessments, *in vitro* dose finding and repeated cytotoxicity experiments showed no effects; therefore, the original results were considered spurious. Oligosiloxanes were observed for DMSO and sesame oil extracts; however, only trace amounts were observed in the saline placebo sample (not detected in MEM or RPMI). Extracts were also analyzed for 68 different metals. For polar solvent ring extracts (more indicative of targeted use conditions) the levels of all metals detected were well below current acceptable levels for oral and parenteral dosage forms.

**CONCLUSIONS:** *In vitro* and *in vivo* preclinical safety evaluation of a pt-catalyzed cured silicone dapivirine vaginal ring indicated these rings are appropriate for clinical evaluation. The methods and assessments used here can be applied to future novel microbicide ring formulations.

**129****Preliminary Evaluation of Toxicity and Antiviral Properties of Personal Lubricants**

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**BACKGROUND:** An effective microbicide may have the potential to greatly reduce STI transmission. The failure of recent microbicide clinical trials has strengthened the resolve to identify new microbicide candidates. A recent study concluded that three over-the-counter lubricants were active against HIV *in vitro*. Our lab wanted to verify these findings and assess the toxicity and antiviral activity of additional lubricants.

**METHODS:** Cytotoxicity was assessed using the XTT assay. Antiviral activity was analyzed using the MAGI assay and two different laboratory strains of HIV-1. We also tested the *in vitro* activity against HSV-2 in Vero cells. Additionally, we studied the effect of the lubricants on differentiated Caco-2 cell monolayers measuring transepithelial electrical resistance (TEER) and cell viability at 30min, 1h, and 2h after gel application. Gynol II (2% nonoxynol-9) and Carraguard were used as positive and negative controls respectively for comparison between gels that reduce monolayer resistance and affect cell viability. The raw data from XTT and MAGI assays were used to determine the CC<sub>50</sub>, EC<sub>50</sub> and therapeutic index (TI) values with a non-linear curve fit program (GraphPad Prism version 5.0b).

**RESULTS:** Unlike Carraguard (TI>10000), none of the lubricants tested showed anti-HIV or anti-HSV-2 activity (TI≤ 10) based on the calculation of TI values *in vitro*. Of the 37 water based, non-warming lubricants tested, KY Tingling Jelly, KY Sensual Silk, Durex Soothing, and Durex Pina Colada maintained <80% initial TEER value 1 hour after application. Durex Soothing and Carraguard were the only gels to maintain about 100% initial TEER value after 2 hours. Every other lube produced a drop in resistance similar to Gynol II.

**CONCLUSIONS:** Based on our results, over-the-counter personal lubricants do not show significant antiviral activity *in vitro*, which may indicate poor microbicide candidacy. Furthermore, a great majority of lubricants caused epithelial damage in the Caco-2 cell system comparable to Gynol II, which may increase transmission. More rigorous safety testing should be performed on personal lubricants before they are allowed to go on the market.

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### **In Vitro Anti-HIV-1 Efficacy Profile and Plant-based Recombinant Expression of Actinohivin, an Env Mannose Cluster-specific Lectin**

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**BACKGROUND:** Actinohivin (AH) is an actinomycete-derived, high-mannose glycan cluster-specific lectin that has a potential to inhibit HIV-1 infection. To discern whether this protein can be developed as a prototype or candidate of microbicides, we performed initial feasibility analysis to address the efficacy, safety, and recombinant producibility of AH.

**METHODS:** To reveal AH's anti-HIV-1 characteristics, we employed two validated *in vitro* HIV-1 neutralization assays, i.e., a human peripheral blood mononuclear cell (PBMC)-based assay using primary HIV-1 isolates, and an envelope (Env)-pseudotyped HIV-1 neutralization assay using Envs of A, B and C clade viruses. AH's potential cytotoxicity and mitogenic activity in human PBMCs were assessed by lactate dehydrogenase measurement and carboxyfluorescein succinimidyl ester-based assay, respectively. A tobacco mosaic virus (TMV)-based vector was used to evaluate the expression of rAH in *Nicotiana benthamiana* plants.

**RESULTS:** AH exhibited low- to mid-nanomolar neutralizing effects against primary HIV-1 isolates with diverse cellular tropisms, i.e. R5, R5/X4, and X4 viruses. Meanwhile, unlike many other lectins, AH did not show any sign of mitogenic activity or cytotoxicity in human PBMCs at micromolar concentrations. In an Env-pseudotyped HIV-1 neutralization assay, AH exhibited broad neutralizing activity with IC<sub>50</sub>s overall at the nanomolar range. However, several viruses, particularly those of clade A, showed strong resistance to AH (IC<sub>50</sub>s > 2 μM). A correlation analysis revealed that HIV-1's susceptibility to AH is strongly associated with the total number of N-glycosylation sites at the C2 and V4 regions of Env (Spearman correlation coefficient = -0.65, p = 0.002). In a TMV-based expression system, we obtained efficient expression (up to 120 mg/kg of fresh leaf material) of functional rAH within six days, as demonstrated by a gp120-capture ELISA and a reporter gene syncytium formation assay.

**CONCLUSIONS:** Our results demonstrated AH's broad anti-HIV-1 activity, apparent lack of major side effects in human PBMCs, and high producibility in a system that is potentially more scalable and economical than conventional methods, thereby highlighting the protein's potential as a prototype HIV-1 microbicide.

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### **Cationic Cell-Penetrating Peptides Inhibit HIV-1 Infection**

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**BACKGROUND:** In the absence of an effective microbicide that reduces or eliminates the risk of human immunodeficiency type 1 (HIV-1) transmission, the development of new anti-HIV-1 drugs remains a priority. Our efforts in this area suggest that molecules belonging to the family of cell-penetrating peptides (CPP) may inhibit HIV-1 entry. CPPs are short peptides able to cross the plasma membrane, a formidable barrier to many molecules. Because of this ability, CPPs are being studied as delivery vehicles for therapeutic agents that cannot enter the cell. A 10-amino acid (aa) peptide derived from HIV-1 Tat protein has been well studied as a drug delivery agent. Our studies explore the activity of Tat peptide and other CPPs against HIV-1 infection.

**METHODS:** Peptides used in these studies included: (i) Tat peptide, (ii) R-9 (nona-arginine), and (iii) RG-20 (arginine-rich, 20 aa peptide). P4-R5 MAGI reporter cells were infected with cell-free HIV-1 IIIB (X4) or HIV-1 BaL (R5) in the absence or presence of half-log, serially diluted concentrations of peptide for 2 h. Cells were washed twice, harvested 48 h later and assayed for HIV-1 infection. Peptide cytotoxicity was assessed by MTT assay following 2 h exposure to P4-R5 cells.

**RESULTS:** Tat peptide caused a concentration-dependent inhibition of HIV-1 IIIB infection, but was ineffective against HIV-1 BaL. In contrast, R-9, which differs from Tat by a small number of aa residues, was shown to have activity against HIV-1 IIIB and, surprisingly, concentration-dependent activity against BaL. RG-20 was able to inhibit HIV-1 IIIB and BaL infection comparable to R-9. None of the peptides were cytotoxic at the concentrations used.

**CONCLUSIONS:** Differences in amino acid sequence between the Tat peptide and R-9 peptide contribute to co-receptor-dependent anti-HIV-1 activity. These results suggest dissimilar mechanisms of action against viruses using CXCR4 or CCR5 co-receptors. Additionally, increased peptide length does not appear to be a critical determinant of antiviral activity, since R-9 and RG-20 were similarly active. Using Tat peptide and R-9 as starting points, further studies will identify sequence determinants for CPP biological activity and mechanisms of antiviral activity, and explore the potential of these peptides as novel HIV-1 inhibitors.

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### **Maleic Anhydride-modified Chicken Ovalbumin as an Effective and Inexpensive Anti-HIV Microbicide Candidate for Prevention of HIV Sexual Transmission**

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**BACKGROUND:** Previous studies have shown that 3-hydroxyphthalic anhydride (HP)-modified bovine milk protein, β-lactoglobulin (β-LG), is a promising microbicide candidate. However, concerns regarding the potential risk of prion contamination in bovine products and carcinogenic potential of phthalate derivatives were raised. Here we sought to replace bovine protein with an animal protein of non-bovine origin and substitute HP with other anhydride for development of anti-HIV microbicide for preventing HIV sexual transmission.

**METHODS:** Maleic anhydride (ML), succinic anhydride (SU) and HP at different conditions and variable pH values were used for modification of proteins. The percentages of modified lysine and arginine residues were determined by TNBS and p-HPG assays, respectively. The *in vitro* anti-HIV-1 activities of the modified proteins were detected by measuring p24 production, luciferase responses, and cell-cell fusion. Their binding activities on sCD4 or gp120 and their inhibitory activities on the interaction between sCD4 and the HIV envelope proteins were determined by ELISA.

**RESULTS:** All the anhydride-modified globulin-like proteins showed potent anti-HIV activity, which is correlated with the percentage of modified lysine and arginine residues in the modified protein. We selected maleic anhydride-modified ovalbumin (ML-OVA) for further study because OVA has more abundant resource than β-LG and ML is safer than HP. ML-OVA exhibited broad antiviral activities against HIV-1 (including different subtypes) HIV-2, SHIV and SIV, with IC<sub>50</sub> in a range from 20 nM to 12 μM. Particularly, ML-OVA could inhibit both X4 and R5 laboratory-adapted HIV-1 strains and clinical isolates. This modified protein has no or low cytotoxicity on women vaginal cells and is resistant to trypsin hydrolysis because the lysine and arginine residues in OVA were modified by ML. Mechanism studies suggest that ML-OVA inhibit entry/fusion by targeting both gp120 and CD4.

**CONCLUSIONS:** ML-OVA is a potent HIV fusion/entry inhibitor with the potential to be developed as an effective, safe and inexpensive anti-HIV microbicide.

**133****A Natural Product from Los Andes with Promising Activity as Microbicide Prevent Cell-free and Cell-associated Herpes Simplex Type 2 Infection**

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**BACKGROUND:** The infection by Herpes simplex type 2 (HSV-2) has been strongly associated with HIV acquisition, thus preventing HSV-2 may reduce HIV sexual transmission. We examined whether *Opuntia spp* (Osp), an aqueous extract of medicinal plant from Los Andes, used topically by native population to heal eruptive febrile processes and applied on herpes lesions induce faster resolution, could prevent cell-free and cell-associated HSV-2 infection, using an *in vitro* model of epithelial HeLa cell culture representing primary target cells.

**METHODS:** A dilution series of Osp was present before and during infection of HeLa cells with cell-free or cell-associated HSV-2 clinical isolates [genital HSV isolates were typed by PCR and cell-associated HSV-2 was prepared by overnight incubation of peripheral blood mononuclear cells with 10<sup>3</sup> multiplicity of infection (MOI) of cell-free virus]. After 1 hour of infection, HeLa cells were washed and subsequently cultured for 7 days. Compounds were simultaneously restored to the cultures, but were gradually diluted by refreshing the medium at 3rd and 5th day of culture. Subliminal infection was detected by subculturing supernatants with Vero cells. At 96 hours, the minimum inhibitory concentration (MIC) was determined by Tetrazolium salt (MTT)-reduction assay. Cytotoxic activity was evaluated by cell viability on treated but non-infected cultured cells.

**RESULTS:** The Osp blocked cell-free or cell associated HSV-2 infection with different potency. At 10<sup>4</sup> MOI, 0.020 mg/ml of Osp inhibited 80% of cell-free virus infection, whereas at 10<sup>3</sup> MOI 10-fold of Osp concentration (0.20 mg/ml) inhibited only about 40% infection and showed no activity against cell-associated virus. The MIC value of Osp varied between 2–20 mg/ml to block both cell-free and cell associated viral infection. The potency of Osp was similar for all three cell-free clinical isolates. Furthermore, none of the Osp concentrations showed acute (3 days-treatment) or delayed (7 days-treatment) cytotoxic effect.

**CONCLUSIONS:** The Osp shows blocking activity against cell-free and cell-associated HSV-2 cell infection with non-toxic activity. Thus it may represent a potential candidate microbicide for further development for prevention of HSV-2 transmission.

**134****RVF H a Promising Protein Having Anti-STI and Anti-HIV Activities Identified in Rabbit Vaginal Fluid**

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**BACKGROUND:** The HIV/AIDS epidemic continues to have a devastating impact on the lives of millions of people worldwide. The failures of microbicide products such as cellulose sulphate, carraguard and PRO-2000 has prompted to identify new and safe alternatives to these polymer/chemical based microbicides. In the absence of an effective prophylactic anti-HIV therapy or vaccine, existing methods failing to halt the epidemic, current efforts are aimed at developing intra-vaginal topical formulations of anti-HIV agents such as microbicides.

Vaginal epithelial cells are constantly exposed to bacteria and virus or their products. Several vaginal secretory proteins reported to have antimicrobial properties. In the present study attempts were made to identify host defense molecules having antimicrobial activities from rabbit vaginal fluid.

**METHODS:** Vaginal fluids were collected from normal healthy rabbits. Vagina fluid proteome profile was generated by employing various conventional purification approaches (RP-HPLC, 2D, LC-MS, Acid-Urea PAGE Far Western Blot and Homology Modeling). A 15 kDa protein having antimicrobial activities has been identified and designated as rabbit vaginal fluid  $\alpha$ -hemoglobin (RVF $\alpha$ H15). Using Bioinformatic tools a 25 mer domain which binds to gp120 was identified and synthesized. The antibacterial activity was determined using the two-layer radial diffusion and the minimal inhibitory concentration (MIC) assays. The interaction between RVF $\alpha$ H15 with gp120 protein was determined by ELISA. RVF $\alpha$ H has been sequenced and the gene encoding this protein was identified. The recombinant RVF $\alpha$ H (rRVF $\alpha$ H) protein was produced using *Pichia pastoris*. Cytotoxicity of rRVF $\alpha$ H was determined on TZM-bl cells with Kinteic Blue assay. The anti-HIV activity of rRVF $\alpha$ H was carried out using  $\beta$ -galactosidase on TZM-bl cell line.

**RESULTS:** The RVF proteome mainly consist of molecules involved in vaginal immunity which play a role in host defense against invading pathogens. RVF $\alpha$ H15 showed activity against several STI causing pathogens and HIV. Using the RVF $\alpha$ H sequence a primer set was designed and RT-PCR was carried out and amplified a 470 bp product, which was cloned and sequenced. The rRVF $\alpha$ H showed antimicrobial activity.

**CONCLUSION:** The study demonstrated that rRVF $\alpha$ H may be explored further as an alternative to chemical and/or polymer based microbicides for the prevention of STIs (Funded by IPM, USA).

**135****Identification of Anti-lipoplysaccharide Factor (ALF) from the Hemocytes of Mud Crab *Scylla serrata*: A Promising Molecule Having Anti-STI and Anti-HIV Activities**

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**BACKGROUND:** The HIV/AIDS epidemic continues to have a devastating impact on the lives of millions of people worldwide. In the absence of an effective prophylactic anti-HIV therapy, current efforts are aimed at developing safe and effective microbicides.

The bacterial endotoxin also known as lipopolysaccharide (LPS) is a major cause of inflammation regulated by release of pro-inflammatory cytokines. LPS is known to induce TLR4 induced HIV-LTR transactivation. Hence molecules that bind to LPS (ALF) and prevent the release of pro-inflammatory cytokines and inhibit HIV transcription are considered important candidates for microbicide development. This study describes the identification of full length cDNA coding for *Scylla serrata* anti-lipoplysaccharide factor (SsALF) protein having antibacterial and anti-HIV activities.

**METHODS:** Using degenerate and RACE-PCR approaches a full length cDNA coding for SsALF protein has been identified. The recombinant SsALF (rSsALF) has been produced in *E.coli* system and characterized. Initially a microtiter plate based assay was performed to determine the ability of the rSsALF (6.25–200 g ml<sup>-1</sup>) to bind to bacterial LPS. Later the MIC of the rSsALF was evaluated against vaginal pathogens and commensals lactobacilli. The ability of rSsALF to inhibit the LPS mediated release of TNF- $\alpha$  in U937 cells was evaluated by ELISA. The ability of rSsALF to inhibit LPS mediated HIV-LTR activation was evaluated on HeLa cell lines transfected with HIV-LTR-GFP reporter plasmid. Finally the anti-HIV activity of rSsALF was carried out using TZM-bl cell line. Virus was quantitated by the NIH-ELISA kit for p24<sup>gag</sup>.

**RESULTS:** rSsALF protein demonstrated antibacterial activity against vaginal isolates (*E. coli*, *P. aeruginosa*, *S. aureus*, *C. albicans*, *N. gonorrhoeae* and *S. pyogenes*) at concentrations 100–200 g ml<sup>-1</sup>. rSsALF exhibited a significant binding to LPS at 100 g ml<sup>-1</sup> as observed by a microtiter plate assay. Upto 2 folds decrease in TNF- $\alpha$  levels was observed in U937 cells incubated with LPS along with rSsALF (200 g ml<sup>-1</sup>). rSsALF caused a 70–80% decrease in HIV-LTR-GFP promoter activity. rSsALF also exhibited anti-HIV activity.

**CONCLUSIONS:** From the above studies it is clear that rSsALF can bind to LPS and inhibit TNF- $\alpha$  production and subsequent decrease in LPS regulated HIV infection. Therefore rSsALF can be considered as a promising candidate for microbicide development for the prevention of STIs including HIV / AIDS.

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### Design and Synthesis of Variants of the HIV-1 Aptamer, UCLA1 That Binds to gp-120

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**BACKGROUND:** We have observed that the HIV-1 gp-120-binding aptamer, UCLA1, is sensitive to degradation by nucleases present in vaginal and rectal lavages, limiting its potential as a microbicide compound. Accordingly, we sought to introduce chemical modifications that would protect the most sensitive cleavage sites: residues A9 and A10, which are found in a region of the aptamer lacking secondary structure.

**METHODS:** The starting point was a version of UCLA1 known as UCLA5v1, which was synthesized on solid phase using optimized 2'-O-TBDMS phosphoramidite chemistry, whereby the 3'-terminus was capped with an inverted thymidine residue to block 3'-exonucleases. All pyrimidines were incorporated as 2'-fluoro-2'-deoxyribonucleotides to give stability to RNases, whilst all purines were ribonucleotides. 6 residues in stem I were 2'-O-dimethylallylribonucleotides to enhance the thermal stability of this stem. The 5'-terminus was Cy5 modified to monitor degradation. Two variants, UCLA5v2 and UCLA5v3, were synthesized in which residues A9 and A10 were 2'-O-methyladenosine, respectively.

**RESULTS:** Hypermodified aptamers were obtained in respectable overall yield and purity by chemical synthesis. Alkaline hydrolysis patterns of UCLA5v2 and v3 confirmed the correct location of the 2'-O-methyladenosines. Relative to the parent, UCLA5v1, some limited protection was achieved by these modifications but the effects were far from dramatic and varied according to the lavage tested. In some cases enhanced cleavage was switched to other sites.

**CONCLUSIONS:** Chemical synthesis permits the individual modification of each of the nuclease-sensitive sites of the HIV-1 aptamer to evaluate the optimal chemical modification pattern compatible with maximum activity and stability for eventual *in vivo* use. Our results here suggest that the aggressive nucleases present in rectal and vaginal lavages are not conventional RNases but more general nucleases. The next step forward based on our results is to synthesize variants of UCLA5v1 in which phosphodiester bonds proximal to sensitive residues are modified by sulfurization.

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### New Compound Derived from Seaweed with Antiretroviral Activity, with Prospects of Microbicidal Activity

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**BACKGROUND:** During the Human Immunodeficiency Virus Type 1 (HIV-1) replicative cycle, viral RNA undergoes reverse transcription, by means of the viral enzyme reverse transcriptase (RT), and the cDNA is further integrated in the host genome. The enzyme RT is a major target for antiretroviral therapy, and several RT inhibitors are in clinical use. Recently, we described that the diterpenes inhibits HIV-1 RT enzyme and viral replication in primary cells (Cirne-Santos CC et al, 2008). Now we described a new potent diterpenes that have a different mechanism of action on HIV inhibition, specifically on expression of HIV-VPR protein.

**METHODS AND RESULTS:** We performed kinetic studies and investigated whether D1 could synergize with other antiretrovirals. Initially, we found by PCR methods that D1 (0.8 or 10  $\mu$ M) blocked the synthesis and integration of HIV-1 provirus and completely ablated HIV-1 replication in PBMCs. Next, we observed that D1 Ki value was 1.2  $\mu$ M (AZT Ki = 0.1  $\mu$ M), which is in agreement with D1 EC<sub>50</sub> previously found (1.8  $\mu$ M), indicating that RT is the main target of D1. Studies of kinetic mode of action with respect to dTTP/template-primer detected that D1 is a noncompetitive inhibitor of RT. Thus, D1 might act at the pocket of the palm region of RT, similarly to other non-nucleoside RT inhibitors (NNRTIs). Following, we addressed whether D1 could present additive or synergistic effects with other HIV-1 inhibitors. Thus, HIV-1-infected PBMCs were treated with D1 at EC<sub>50</sub> dose plus sub-optimal concentrations of classical antiretrovirals. D1 presented an additive effect with AZT (HIV-1 inhibition: D1 = 50%; AZT 5 nM = 40%, D1 plus AZT = 90%), and a synergistic effect with Atazanavir (HIV-1 inhibition: D1 = 50%; Atazanavir 5  $\mu$ M = 20%; D1 plus Atazanavir = 100%). D1 plus Nevirapine resulted in no additive effects. Vpr is reported to enhance the transport of the viral DNA into the nucleus of nondividing cells, importantly this compound was able to inhibit 70% of the expression of HIV-1-VPR.

**CONCLUSIONS:** D1 presented a potent antiretroviral activity against a panel of ten HIV-1 isolates carrying common NNRTI-associated resistance mutations. We propose that D1 could be considered as a potential candidate for HIV-1 therapy or prevention, possibly acting as a microbicide. Currently we are carrying out pre-clinical studies on animal models and tissue explants.

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### Driving HIV Into Suicide—A New Approach for a Microbicide

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**BACKGROUND:** We are developing an alternative approach to siRNA, which may be designated as siDNA, small interfering DNA, by using hairpin-loop-structured DNA oligodeoxynucleotides (ODN), targeted to viral or cellular mRNAs. ODNs activate the viral RNase H in retroviral particles and cellular RNases H inside the cell. Also Ago2 may play a role. Other inhibitory mechanisms such as translational arrest may contribute.

**METHODS:** We selected ODNs against various viral and mRNAs of HIV, HSV, Influenza, HCV, HBV, and the terminal repeat of telomeres in malignant melanomas in mice. The ODNs were applied with or without carriers. Furthermore their effects were directly compared to those of single-stranded antisense DNAs and siRNAs to allow comparison of the various efficiencies.

**RESULTS:** The ODNs were most effective in HIV. We are able to induce HIV suicide and inactivate HIV virus particles to prevent infections, inactivate cell-free HIV in the blood from infected individuals, in the vagina of mice, and increase survival time of retroviral-infected mice. We could prevent infection if treated early. 5 out of 6 humanized SCID mice did not get infected with HIV. Furthermore we could reduce other viruses such as HSV. The effects are sequence—and dose—dependent, but the optimal algorithm is not yet known. We are analyzing whether there is a preference for G tracts, which may form higher-ordered structures and enhance uptake.

**CONCLUSIONS:** We can inactivate HIV virus particles before infection without a carrier. Thus HIV is inactivated before infection. The dsODNs are often superior to single-stranded antisense DNA and resemble the effects of siRNAs but with different kinetics. In contrast, we are targeting viral RNA by partially dsDNA. The method may complement existing silencing approaches.

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**Anti-HIV Activity of Novel Dimeric Phloroglucinols and Caffeoyl-Anilide Compounds and Their Potential as Microbicide Candidate**

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**BACKGROUND:** Plants are an important source of a variety of bioactive compounds with different modes of action. These natural product derived compounds can be used in conjunction with existing anti HIV agents and thus possibility of drug resistance could be minimized in novel formulations both for therapeutic and preventive use. Anti-HIV agents from plant sources can also be useful in developing novel microbicides for inhibiting HIV infection. Based on the reported anti-HIV activity of plant derived phloroglucinols and caffeoyl-anilide compounds, we have synthesized and screened a series of novel structurally related compounds in the present study.

**METHODS:** We have screened these compounds at non-cytotoxic concentrations in human CD4+ T cell line CEM-GFP and epithelial reporter cell line TZM-bl using NL<sub>4.3</sub> virus isolate. CC50 and IC50 of active compounds have been identified using a dose response experiment.

**RESULTS:** Some of the synthesized compounds have shown significant HIV inhibitory activity in a human CD4+ T cell line infected with HIV-1 NL4.3 virus isolate. They have also shown significant anti-HIV activity in TZM-bl reporter cell line and human peripheral blood mononuclear cells. Few of them show significant inhibition of virus transmission from epithelium to lymphocytes in trans-well experiments. These compounds seem to target HIV-1 reverse transcriptase and integrase activity respectively.

**CONCLUSIONS:** These two groups of novel compounds show significant anti-HIV activity with good therapeutic index for potential use as a microbicide.

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**Evaluation of Anti-HIV Activity and Cytotoxicity of Retrocyclin RC-101 in PBMC and a Cervical Tissue-based Organ Culture**

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**BACKGROUND:** The antiretroviral peptide retrocyclin RC-101 is known to exert antiviral activity in various cell lines against HIV-1 of different clades. Here we assess the antiviral activity and cytotoxicity in PBMC and in a cervical tissue based organ culture.

**METHODS:** RC-101 was tested for antiviral activity in PBMC against HIV-1 of different clades and of different tropisms by measuring viral HIV-1 p24 in culture supernatant. RC-101 was also tested for cytotoxicity by measuring viability of PBMC using an MTT assay and by measuring immune functions, such as NK cell activity, chemotactic activity and lymphocyte proliferation. RC-101 was next tested for its ability to block HIV-1 transmission in a cervical tissue-based organ culture. Cytotoxicity of RC-101 was also evaluated in organ culture by measuring intracellular level of Ki67, a cellular proliferation marker and cytokeratin, an epithelial marker using a quantitative immunostaining assay, and by measuring proinflammatory cytokine response using real time RT-PCR and Luminex assays.

**RESULTS:** RC-101 inhibited replication of HIV-1 of various clades representing X4 and R5 tropic viruses in a dose-dependent manner. Level of inhibition was between 70% to more than 90% at 40ug/ml. RC-101 showed no cytotoxicity in the MTT assay and had no deleterious effect on NK cell activity, HIV-1 specific lymphoproliferative response of CD8 and CD4 cells and chemotactic activity of lymphocytes. RC-101 blocked HIV-1 transmission in a dose-dependent manner in the organ culture. Such antiviral activity also persisted in the presence of seminal and vaginal fluids. Furthermore, RC-101 did not have any effect on the level of Ki67 and cytokeratin and did not induce proinflammatory cytokines such as IL1 $\beta$ , IL-8, IL-6 and TNF $\alpha$ .

**CONCLUSIONS:** Retrocyclin exhibited antiviral activity in PBMC and organ culture against HIV-1 with varying clades and tropism. Furthermore, RC-101 showed no cytotoxicity in PBMC and organ culture as measured by a variety of immune and non-immune functions. These results suggest RC-101 is a promising candidate microbicide against HIV.

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**A Lactobacilli-based System Molding Screening and Live Microbicide Potential of New RANTES-derived CCR5 Antagonists**

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**BACKGROUND:** Live microbicides based on the engineering of bacteria belonging to the human microbiota is a promising strategy to prevent HIV-1 transmission. Lactic acid bacteria proved to be an excellent secretory platform for relatively complex (disulphide-bonded and multi-domain) proteins. Indeed, engineered lactobacilli revealed a rare case of superior folding (as compared to mammalian cells) for C1C5 RANTES, a difficult to produce protein. This feature is the basis of the system presented here, where the selection of novel RANTES mutants (acting as CCR5 antagonists) for the best candidates as potential live microbicides is performed in lactobacilli. Two lactobacilli strains have been implemented providing vaginal and intestinal applicability, respectively.

**METHODS:** Lactobacilli codon-optimized RANTES mutants were generated and inserted into vectors suitable for protein secretion. Lactobacilli were transformed and recombinant clones' supernatants analyzed by ELISA and Western blot to determine secreted protein quantity and forms, respectively. Qualitative anti-HIV-1 activity was tested by acute infection assays on partially purified proteins. This procedure (mutagenesis, lactobacilli transformation, secretion level and anti-HIV-1 activity assessment) flowed in iterative cycles, with a stepwise integration of successful mutations in single CCR5 antagonist RANTES derivatives.

**RESULTS:** By exploiting an engineered lactobacilli platform, a series of hot spot mutations spanning through RANTES aa sequence were retrieved from previous studies or designed ex novo to achieve CCR5 antagonism and potent anti-HIV-1 activity. The system is designed to collectively yield a small number of lead compounds presenting ideal features as live microbicides. The most effective leads derived from the iterative selection cycles will be purified and accurately tested for: i) CCR5 antagonism, ii) anti-HIV-1 activity, iii) protein full-length, and iv) correct disulphide bonding.

**CONCLUSIONS:** The engineering of lactobacilli to produce RANTES proved to be ideal not only for the live microbicide concept, but also as a recombinant protein platform to screen several mutants. Ultimately, the approach presented here represents an exquisitely compact system molding screening and therapeutic application.

# SUNDAY POSTERS

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### Design and Development of Novel NNRTIs Against HIV-1

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**BACKGROUND:** HIV, an etiological agent for AIDS, has ssRNA as the genetic material which gets converted into dsDNA, the provirus, in the presence of reverse transcriptase (HIV-RT). Inhibition of this reverse transcription process by molecules, like NRTIs and NNRTIs has resulted in inhibition of HIV proliferation. HAART used for AIDS patients' treatment utilizes a combination of NRTIs, NNRTIs and PIs as a potential drug regimen. We report here the designing, synthesis and screening of some novel NNRTIs, the allosteric inhibitors of HIV RT, with promising results under *in vitro* conditions.

**Methods:** We have designed and synthesized a series of novel NNRTIs having isoindolinedione and nitro-/benzimidazole moiety with amide and sulfonamide linkages. The molecules were designed on the basis of extensive literature survey and docking of their energy minimized forms into the allosteric sites of HIV RT using Discovery Studio 2.5 software. These studies revealed very good interactions of the molecules with RT enzyme. These molecules have 5 to 6 hydrogen acceptors and 1 hydrogen donor, and thus formed hydrogen bonds (1–7 in number) with various amino acid residues like K101, K103, Y181, Y188 and P236 present at the allosteric site with bond lengths varying from 2.2 to 3.4 Å. Similarly, we have synthesized some bis-nitro/benzimidazole derivatives also after successful docking of these molecules with HIV-RT. All these molecules have been designed following the Lipinski's rule of five. The molecules were then synthesized and screened against cell associated HIV using TZM-bl assay.

**RESULTS:** Isoindolinedione derivatives with amide linkages have shown 65–71% inhibition of HIV during TZM-bl assay at 15.6 µg/ml concentration. However, these molecules showed only 60–67% cell viability at this concentration during MTT assay. Sulfonamide derivatives of isoindolinedione, on the other hand, showed 33–62% inhibition at 2 µg/ml concentration and 100% cell viability even at 100 µg/ml concentration. Similarly, benzimidazole derivatives too have shown 80–90% HIV inhibition at 15.6 µg/ml concentration with almost nil cytotoxicity.

**CONCLUSIONS:** The isoindolinedione derivatives with sulfonamide linkage and nitro-/benzimidazole derivatives with amide linkage have shown good anti-HIV activity and almost nil cytotoxicity, whereas isoindolinedione derivatives with amide linkage too have also shown activity against HIV-1, but proved toxic.

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### Studies on Molecular Interaction and Molecular Dynamics Simulation of Dual Tropic Small Molecule HIV Co-Receptor Antagonists Against CCR5 and CXCR4

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**BACKGROUND:** The AIDS is a destructive pandemic which very much needs innovative methods of controlling infection. The Chemokine receptors CCR5 and CXCR4, belonging to G-protein coupled receptor super-family have been identified as the major co-receptors for HIV entry into human immune cells during earlier and later stages of infection respectively. Entry inhibitors E913 and NSC651016 are dual tropic antagonists acting against these co-receptors which are undergoing clinical trials. These entry inhibitors could prove to be potential topical microbicides.

**METHODS:** The chemokine receptors CCR5 and CXCR4 are modeled with bovine rhodopsin 1F88 at 2.8 Å resolution as the template and envelope gp120 is modeled with 2B4C G chain at 3.30 Å resolution as the template and CD4 obtained from the same crystal structure. The modeling, pharmacokinetic properties, pharmacophore analysis and molecular properties of the antagonists are studied using Discovery Studio 2.1. The molecular interaction studies of antagonists against the second extracellular loops of both CCR5 and CXCR4 are carried out using Autodock 4.0 program and Discovery studio 2.1 and the molecular dynamics simulation of protein-ligand complexes are studied using GROMACS package.

**RESULTS:** The key residues involved in the binding of CCR5 and CXCR4 to the antagonists responsible for inhibiting gp120/CD4-CXCR4/CCR5 complex formation are studied. The binding affinity of the complexes is determined by the number of hydrogen bonds formed and force field calculations. Molecular dynamics simulation is done to optimize the obtained complexes and the results are viewed in VMD tool. The RMSD graph and RMS fluctuation were obtained to observe the stability and the fluctuation achieved by the protein for the 2ns time period. The deviation of the amino acids from the allowed region during the simulation period was viewed by Ramachandran plot.

**CONCLUSIONS:** The structure prediction and binding mode analysis of the chemokine co-receptors and small molecule inhibitor complexes help us to proceed for further *insilico* studies. The results indicated that E913 and NSC651016 have good binding affinity thus effective in inhibiting the formation of gp120/CD4-Coreceptor complexes.

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### The Antimalarial Action of the Antiretroviral Drugs Lamivudine, Zidovudine, Nevirapine and Stavudine on Plasmodium Berghei in Mice

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**BACKGROUND:** Malaria parasitaemia enhances the replication of the Human Immunodeficiency Virus (HIV). This may increase the HIV prevalence in malarious regions. This is against the backdrop that HIV increases the risk of malaria parasitaemia. It is necessary to investigate the antiplasmoidal effects of antiretroviral drugs commonly used in malaria endemic regions for better control of the two diseases.

**METHODS:** The activity of clinical doses of the antiretroviral drugs (ARVDs), Lamivudine, Zidovudine, Nevirapine, Stavudine on Plasmodium berghei, inoculated into 70 adult albino mice weighing between 15–25g, was investigated. There were 14 groups (I–XIV) of 5 animals per group. Group I, negative control, without parasite was given distilled water. Group II, positive control, was infected with parasite and treated with chloroquine. Eight (8) of the remaining groups were infected with parasites and each group treated with the different ARVDs either as curative (c) or prophylactic (p) therapy. The remaining 4 groups each had triple combinations of Lamivudine, Zidovudine, Nevirapine or Lamivudine, Stavudine, Nevirapine

**RESULTS:** Mean parasite counts decreased with eventual parasite elimination as follows: Zidovudine c group  $38140.7 \pm 21841.8$  SEM,  $1506.8 \pm 1440.2$  SEM, 0 (days 8, 18, and 26 respectively); Zidovudine p group  $27429.47 \pm 9265.9$  SEM,  $7256.15 \pm 4593$  SEM, 0 (days 8, 18 and 26 respectively); Lamivudine/ Zidovudine/ Nevirapine c group  $29059.53 \pm 9018.3$  SEM,  $39.33 \pm 39.4$  SEM, 0 (days 9, 20 and 26 respectively); Lamivudine/ Zidovudine/ Nevirapine p group  $29080.4 \pm 9238.1$  SEM,  $2135.09 \pm 1963.72$  SEM, 0 (days 10, 18 and 26 respectively); The distilled water group increased in values from  $71866.3 \pm 3317.6$  SEM to  $116,225 \pm 44101$  SEM on days 4 and 20 when all the animals died off.

**CONCLUSIONS:** Zidovudine and Lamivudine, Zidovudine, Nevirapine combination at clinical doses were able to eliminate plasmodia berghei. The administration of these drugs on HIV infected patients in malarious regions may modify the epidemiology of malaria and therefore the HIV pandemic.

**145****Hormone Responsive Langerhans Cell (LC) Containing Vaginal Tissue Model**

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**BACKGROUND:** The vaginal mucosa is hormone sensitive and its proliferation and maturation are known to be influenced by reproductive hormones. However, the effect of hormones on vaginal tissue structure and their influence on tissue susceptibility to infection has not been extensively investigated. Here, we report development of a hormone-responsive organotypic human vaginal tissue model containing Langerhans cells.

**METHODS:** The EpiVaginal™ tissue is cultured from primary fibroblasts and epithelial cells derived from non-diseased ectocervical tissue using serum free medium; in addition, normal human Dendritic/Langerhans cells are incorporated into the tissue. Tissue morphology/histology was characterized by H&E staining, ultrastructural features including tight junctions were observed by electron microscopy. Proliferation markers, human β-defensins (HBD), cytokeratin, mucin, hormone receptor, and Toll-Like Receptor (TLR), expression levels were monitored by immunohistochemistry (IHC) and RT-PCR. Inflammatory cytokine/chemokine levels were measured by ELISA. Incorporation of dendritic/Langerhans cells was monitored using confocal microscopy.

**RESULTS:** The EpiVaginal tissue model has phenotypic and architectural similarity to its in vivo counterpart. IHC analysis and RT-PCR showed expression of estrogen receptors (ER $\alpha$  and ER $\beta$ ) and progesterone receptors. Treatment with estradiol resulted in cornification of the epithelial layer. Estradiol treatment also showed increased transepithelial electrical resistance (TEER), Ki67 expression, progesterone receptor B (PRB) levels, and ER- $\alpha$  and  $\beta$  expression. Progesterone treatment enhanced mucin-4 expression but did not produce significant changes in tissue structure or hormone receptor expression levels. The highly differentiated tissue model expresses Toll-like receptors (TLRs 1-6) and human HBD 1 and 2 similar to those observed in vaginal-ectocervical tissue explants. The TLRs are responsive to ligand stimulation resulting in the release of cytokines such as IL-8 and IL-6 and other chemokines/ anti-microbial molecules, including SLPI, MIF, MCP-1, SDF-1 $\alpha$ , and GRO- $\alpha$ , involved in the mucosal innate immune response. Confocal microscopy and IHC also showed the distribution pattern of Langerhans cells in the supra-basal and apical layers of the epithelium.

**CONCLUSIONS:** The model can serve as a valuable tool in the study of hormonal effects of: 1) vaginal mucosal immunology, 2) susceptibility to viral infection including HIV and HSV, and 3) preclinical assessment of toxicity and proinflammatory effects of therapeutics, microbicides, and feminine care products.

**146****Development of a New 3-D Human Endocervical Tissue Model**

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**BACKGROUND:** The endocervical epithelium is a gate-keeper tissue guarding against pathogen invasion and providing a barrier to sperm entry into the uterus. Currently no in vitro reconstructed endocervical tissue is available. Here, we report the reconstruction of the first human organotypic endocervical tissue model generated from primary endocervical epithelial cells using a serum free medium.

**METHODS:** Human endocervical tissues were obtained from Boston Medical Center; endothelial cells were isolated, expanded, and used in the reconstruction of the tissue model. Tissue morphology/histology was characterized by H&E staining and ultrastructural features, including tight junctions, were observed by electron microscopy. Basal cell proliferation markers, cytokeratin, mucin, and hormone receptor expression levels were monitored by immunohistochemistry and RT-PCR. To examine the utility of the tissue for preclinical testing of chemicals, different concentrations of Nonoxynol-9 (N-9) and Benzalkonium chloride (BZK) were topically applied to the tissue model for 24 hr. Tissue viability was assessed by MTT and inflammatory cytokine/chemokine levels were measured by ELISA.

**RESULTS:** Histological and ultrastructural feature analysis of the reconstructed tissue model revealed the presence of columnar epithelial cells with tight junctions. Immunohistochemical staining and RT-PCR showed expression of: a) mucins 1, 4, 5A-C, and 5B, b) cytokeratins 5, 7, 17, 18, and 19, c) estrogen receptor, and d) the progenitor and proliferation cell marker p63 and Ki67, respectively, in a manner similar to that expressed in native endocervical tissue. Exposure of tissue to N-9 at concentrations > 0.002% or BZK at concentrations > 0.125% were found to be toxic to the tissue as measured by the MTT assay (n=3 lots); elevated levels of proinflammatory cytokines including IL-1 alpha, IL-1 beta, IL-6, and IL-8 were also observed following exposure to N-9 and BZK.

**CONCLUSIONS:** The new endocervical tissue model will likely serve as a valuable tool to study mucosal immunology, microbial infection mechanisms, and the effects of topically applied formulations and microbicides in the mucosal microenvironment.

# SUNDAY POSTERS

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### **RNAi Provides a Potential Microbicide in Preventing Sexual Transmission of HIV-1**

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**BACKGROUND:** RNA interference (RNAi) has provided us with an efficient tool of silencing target genes and it holds considerable promise as a therapeutic approach.. siRNAs and shRNAs have been extensively investigated to inhibit HIV-1 infection. Considerable efforts have been paid to innovative design of more effective siRNA with lower silencing concentration, prolonged stability and less possibility of sense strand incorporation, and more and more effective delivery vehicles are getting access to clinical trials. We aim to utilize siRNA as a topical microbicide and designed a number of siRNAs targeting HIV-1 coreceptor CCR5 and compared their ability to downregulate the expression of CCR5 and investigated the effect on HIV-1 infection. In this study, the sense strand of the siRNA was shortened and a mismatch was incorporated into the first nucleotide of the anti-sense strand (guide strand). Then we translated this construction into plasmid expressed shRNAs to check the potential for vector delivery. It was anticipated that the side effects caused by sense strand and non-specific interruption of cellular function through RISC occupation should be maximally avoided. To improve the stability of siRNA, a number of technologies, including cholesterol-tagging, PEGylation combining with beta-cyclodextrin were investigated for vaginal delivery.

#### **METHODS:**

**RNA AND PLASMID:** The RNAs were designed based on two highly effective RNA sequences reported previously by other researchers. The sense strands were shortened from the 5' end, so the remaining length of sense strand was left 13nt to 18nt, and a mismatch of the 5' first nucleotide was incorporated. Then each sequence was submitted to manufacturer for synthesis and annealing.

To construct the plasmid expressing shRNA, the cDNA sequences of the two strands of siRNA were connected by a nine-nucleotide link and cloned into the restriction site of a plasmid under the control of a RNA polymerase III promoter.

**CELL LINES AND PSEUDOVIRUS:** TZM-b1 is a Hela derived cell line stably transfected with human CCR5 and CD4, moreover genes of luciferase and galactosidase under the control of HIV-1 LTR were permanently integrated into its genome.

GHOST R5/X4/R5X4 cells were derived from human osteosarcoma and stably transfected with human CCR5 and/or CXCR4.

Env gene were clone from patients infected with B', B'C and AE clade viruses and inserted into a plasmid, then it was used to cotransfected 293T and 293FT cells with another plasmid encoding NL4-3 backbone to package pseudovirus particles.

All these cell lines and pseudovirus plasmid were generous gifts from Hong Kong University and Tsinghua University.

**TRANSFECTION AND INFECTION:** siRNAs and plasmids were all transfected with cationic liposome transfection reagents. Cells were infected with transfected 293 cells supernatant containing pseudovirus particles, and infection was promoted by DEAE.

**DETECTION OF RNAI EFFECTS:** The downregulation of mRNAs was detected by RT-QPCR. The inhibition of CCR5 protein expression was investigated by Western-Blot and Flowcytometry. The suppression of pseudovirus infection was accomplished by Britelite Luminescence Reported Gene Assay System.

**RESULTS:** For each siRNA sequence, one or two chimeric siRNAs were demonstrated to show comparable or even more potent silencing effects than traditional 19+2 siRNA and this was substantiated by their inhibitory activities on pseudovirus infection. The results demonstrated that the new designs could achieve effective downregulation of CCR5 with less off-target effects and at low RNA concentrations. Some researchers have reported that vector expressed shRNAs showed more potent effect than synthesized siRNAs, but our results showed only comparable activity between vector expressed shRNAs and siRNAs, except for the prolonged time course of shRNAs. This phenomenon may be caused by the high efficiency of the selected target sequences.

Cholesterol conjugation has showed effective systemic activity in previous studies. In our experiments, all cholesterol conjugated siRNAs could penetrate into cells and silence CCR5 without the presence of tranfection reagent.

**CONCLUSIONS:** The greatest hindrance to the clinical use of RNAi is the delivery system. Recent years, many new delivery methods have arisen and several have showed promising clinical results, which opens the door for more and more forms of siRNA to enter clinical trial from bench. The shortened siRNA tested in our experiments may be vulnerable to degradation, but this can be overcome by rational design of delivery system. Now there are several choices for our siRNA delivery, besides lentiviral vector, a few nonviral methods are available, including chemical modification, stable nucleic acid-lipid particle, Fab complex, Aptamer and nanoparticles. Combined with these tools, our findings and the works of other researchers in RNA interference may introduce a new member to the family of anti-HIV microbicide, and could be similarly applied to other STDs.

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### **Twenty-Eight Day Repeat Dose Toxicology Studies of UC781 Vaginal Gel in Sprague-Dawley Rats and New Zealand White Rabbits Following Rectal Administration**

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**BACKGROUND:** The objective of these studies was to determine the potential toxicity of UC781 vaginal gel following single rectal administration daily for 28 days to male and female rats and rabbits. These animal studies were used to support the first Phase I rectal safety study of the vaginal formulation of UC781 microbicide gel (supported by DAIDS IPCP #A1060614).

**METHODS:** Rats (5 animals/sex/group) and rabbits (4 animals/sex/group) were assigned to six treatment groups and received a single daily rectally administered dose (0.1 mL for rats and 1.0 mL for rabbits) of test article, placebo, or control for 28 days. The six treatment groups included: three test article groups of UC781 vaginal gel (0.25%, 0.75%, and 2.5% w/w), one vehicle placebo gel group, and a sham control group. Cageside observations were performed daily and body weights were recorded weekly. Blood samples for assessment of clinical pathology parameters were collected just prior to necropsy on Day 29. Blood for pharmacokinetic analysis was collected at 0 (pre-dose), 1, 2, 4, 8, and 24 hours on Days 1 and 28. On Day 29, animals were euthanized and gross necropsy was performed. Selected organs were weighed and absolute and relative weights were recorded. Also, specified tissues (rectum, anus, and descending colon) were collected for histological evaluation.

**RESULTS:** No mortalities were reported during the course of these studies. No test-article related findings were observed in clinical observations, body weights, food consumption, or clinical pathology following 28 days of rectal administration of UC781 vaginal gel. Also, no significant findings were noted upon necropsy or histological evaluation of specified tissues. Analysis of the Day 1 and 28 rabbit plasma samples and the Day 1 rat plasma samples indicated that UC781 was either not detected or was below the lower limit of quantitation (LLOQ) of 25 ng/mL. For the Day 28 rat plasma samples UC781 was detected in the plasma of nine rats at concentrations ranging from 26–89 ng/mL. However, these findings appear to be toxicologically irrelevant and no clear dose response was observed.

**CONCLUSIONS:** Based upon the results of these studies, the apparent no-observable-adverse-effect-level (NOAEL) of UC781 vaginal gel is 2.5% (w/w) in Sprague-Dawley rats and New Zealand White rabbits treated once daily via the rectal route of administration for 28 days.

**149****Development and Validation of a Stability-indicating HPLC Method for Simultaneous Determination of Dapivirine NNRTI and BMS-599793 (DS003) Entry Inhibitor**J. Gupt<sup>\*1</sup>, A. Othman<sup>1</sup>, S. Singh<sup>2</sup>, J.Q.Tao<sup>2</sup>, S. Garg<sup>1</sup><sup>1</sup>School of Pharmacy, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand; <sup>2</sup>International Partnership for Microbicides (IPM) 8401 Colesville Road

**BACKGROUND:** Dapivirine and BMS-599793 (DS003) are new investigational drugs being developed as anti-HIV microbicides. Dapivirine and BMS-599793 (DS003) are being developed in combination as a long acting, rapidly disintegrating, and bioadhesive vaginal tablet. A stability indicating reverse phase liquid chromatographic method was developed and validated for the simultaneous determination of Dapivirine and BMS-599793 (DS003).

**METHODS:** Chromatographic separation was achieved using Luna 3 $\mu$  PFP (2) (150 mm x 4.6 mm) column with a gradient method using 0.05 % v/v trifluoroacetic acid in water and 100% acetonitrile as mobile phase at a flow rate of 0.5 ml/min. Dapivirine and BMS-599793 (DS003) were quantified with a UV detector at 245 nm. The total chromatographic run time was 40 minutes. The method was partially validated for linearity, specificity, system suitability, system precision and accuracy. Forced degradation studies were performed on the drug sample of Dapivirine and BMS-599793 (DS003) in solution form using acid and base hydrolysis, oxidation and thermal stress to show the stability indicating power of the method. Oxidative studies were carried out by incubating the drug solutions at 40°C for 4 hours and hydrolysis and thermal studies were carried out at 70°C for 24 hours.

**RESULTS:** Calibration curves were linear for BMS-599793 (DS003) and Dapivirine with a correlation coefficient of 0.9997 and 0.9992 respectively. The % RSD of system precision calculated from six replicate injections was less than 2% for both the analytes. The accuracy of the assay method for both the analytes was found to be within the range of 98 to 102%. The formulation excipients did not interfere with the determination of the two active ingredients. Significant degradation of both the analytes was observed during oxidative stress, slight degradation of both the analytes was observed in acidic and thermal stress conditions. BMS-599793 (DS003) was significantly affected by base hydrolysis. None of the degradation peaks was found to co-elute with Dapivirine and BMS-599793 (DS003) peaks. The peak purity profile for both the analytes indicated 100% purity.

**CONCLUSIONS:** A new method has been developed for simultaneous determination of Dapivirine and BMS-599793 (DS003) which is not only specific, accurate and precise but also has stability indicating capability.

**150****In Vitro Study of Toxicological Properties of Griffithsin, A Potent Anti-HIV Lectin from the Red Alga *Griffithsia* sp.**

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**BACKGROUND:** Griffithsin (GRFT) is a recently discovered carbohydrate binding protein that was first identified in the red alga *Griffithsia* sp. Since its discovery, GRFT has been considered a potential candidate microbicide to prevent the sexual transmission of HIV and AIDS in part because of its pronounced anti-viral activity in the picomolar range against both laboratory and primary isolates of HIV. In addition, the mode of action of GRFT favors its eventual use in the treatment and/or prevention of other diseases caused by enveloped viruses. Recently, we reported a manufacturing breakthrough of plant produced GRFT where *N. benthamiana* seedlings were transduced with a tobacco mosaic virus expressing GRFT. This would enable bulk, cost-effective production of the lectin.

**METHODS:**

- Cell viability was accessed using an MTT kit (Biotium) after 72 hours of treatment.
- The mitogenic effect on cervico-vaginal cell lines (VK2/E6E7, Ect1/E6E7 and End1/E6E7) was evaluated by the BrdU incorporation using a Cell Proliferation ELISA kit from Roche. For human PBMCs, cells were treated and analyzed by flow-cytometry using forward scatter (FSC) and side scatter (SSC).
- Immuno-assays for cytokine/chemokine detection were carried out on culture supernatants collected from cells treated with GRFT or controls for 24-72 h. The Multiplex immuno-assays were done using a Bio-Plex instrument (Bio-Rad) and Milliplex Human Cytokine/Chemokine 42-plex assay (Millipore).
- The effect of GRFT on cellular activation markers in PBMCs was studied by flow-cytometry using monoclonal anti- CD4, -CD25, -CD69 and -HLA-DR antibodies
- Lectins Concanavalin A (ConA) and phytohemagglutinin (PHA) were used as comparative controls.

**RESULTS:** In cervico-vaginal cell lines, GRFT (up to 8  $\mu$ M, ie at least 13,000 times the EC<sub>50</sub>) did not affect cell viability, did not induce any mitogenic effect and did not induce key cytokine (IL-2, IL-1b, IL-6 and IL-8) release as assessed by single ELISA experiments. In addition, little to no change was observed in the cytokine release profile of Ect1/E6E7 treated with 4  $\mu$ M GRFT using a 42 plex cytokine and chemokine multiplex immuno assay. In hPBMCs, up to 4  $\mu$ M GRFT did not affect significantly either cell proliferation or the expression of activation markers including CD25, CD69 and HLA-DR.

**CONCLUSIONS:** High concentrations of GRFT show low to no toxicity in vitro, measuring different experimental parameters including cell viability, cell proliferation, cytokine/chemokine release and activation of cellular markers of immune cells. Further characterization of GRFT toxicology is under way in our laboratory and others.

**151****Stability Indicating HPLC Validated Assay of a Non-Nucleoside Reverse Transcriptase Inhibitor, MIV-150, in PC-815 Formulation**S. Seidor\*, T. Henares, T. Zydowsky  
*The Population Council, New York, NY*

**BACKGROUND:** Incorporation of a non-nucleoside reverse transcriptase inhibitor (NNRTI) like MIV-150 into a gel formulation is one of many strategies for developing a microbicide gel that may prevent HIV transmission. The development of this formulation requires having an accurate, precise and reproducible analytical method to ensure a quality product. Here, we developed and validated a stability indicating assay using reverse phase high performance liquid chromatography (HPLC). We have analyzed different analytical parameters such as degradation, precision, accuracy, specificity and ruggedness.

**METHODS:** An Agilent 1100 series HPLC system with an ACE C-18, column was used to analyze MIV-150 in PC-815 formulation. The mobile phase ran at a flow of 1.0ml/min and was composed of 50% 200mM ammonium acetate buffer at a pH of 5.0 and 50% acetonitrile. MIV-150 was detected at 260 nm. Degradation was induced with strong acid and base, heat, oxidant and light to observe the effect on quantitation of MIV-150. Moreover, the accuracy was performed by comparing 3 trials of 5 different standard concentrations with 3 trials of 5 different concentrations of spiked placebo. Eventually, ruggedness was done by studying the effects of slight method alterations on quantification of MIV-150 peak. These method alterations included: change of mobile phase pH and salt concentration, diluent composition, HPLC system, and column.

**RESULTS:** The degradation study resulted in no degradent peak overlap with MIV-150. Furthermore, accuracy testing resulted in 99.3% recovery. Both system and method precisions were reliable with RSDs of 0.1%, and 1.4% respectively. The response of MIV-150 is statistically correlated for both standard and spiked placebo between concentrations of 0.25 to 1.23 ng/mL MIV-150. Slight method alterations did not affect quantification of MIV-150.

**CONCLUSIONS:** We have developed a consistent accurate and precise method for the separation, identification, and quantification of MIV-150 in PC-815 microbicide gel that will ensure a good quality of formulation. The method will attain reliable results for future in-vitro release studies. The method meets all ICH and FDA guidelines for validation and can be used for routine laboratory testing.

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### **Development and Validation of a Radioimmunoassay to Quantify MIV-150 in Blood and Tissues from Pharmacokinetic Studies**

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**BACKGROUND:** The quality of pharmacokinetic (PK) studies is directly related to the quality of the underlying bioanalytical data. The most known methods for molecular analyses are HPLC, LC/MS, ELISA and Radioimmunoassay (RIA). The RIA is a competitive assay with high sensitivity and specificity as well as low cost. We optimized the RIA for rat and monkey samples and conducted PK studies with MIV-150 intravaginal gel in rats.

**METHODS:** RIA of MIV-150 was an indirect extraction based assay. The sensitivity was estimated by multireplicate analysis of the zero standard. Specificity was assessed by cross-reactivity with other NNRTIs. A set of quality controls was used to measure precision of the method. The analysis was done with a curve fitting procedure (logistic 4-parameter model). Employing this RIA, we executed PK studies in female Sprague-Dawley rats, administering Carraguard-based gels with different concentrations of MIV-150 intravaginally. The levels of MIV-150 in blood and cervicovaginal tissues were measured over time following a single or repeated application. PK data was analyzed and  $C_{max}$ ,  $T_{max}$  and AUC were estimated.

**RESULTS:** An RIA was developed to detect MIV-150 with a limit of detection of 0.7 ng/mL and 2 ng/mL in rat and monkey samples respectively. In PK studies performed in rats, high levels of MIV-150 were observed in blood and cervicovaginal tissue after applying a gel formulation containing 5000 M of MIV-150. However, after administering gels containing less than 500  $\mu$ M, low or undetectable levels were found in both blood and tissues samples.

**CONCLUSIONS:** The RIA method discussed here is an excellent tool for detection of MIV-150 in PK studies. An NNRTI to be used as a topical microbicide may need to be absorbed into the surrounding tissues to effectively prevent infection. Data obtained here show that a dose dependent absorption of MIV-150 was observed following a single gel application. Additional studies are being planned to compare the efficacy and absorption (PK) studies in monkeys in order to define optimum levels in tissues versus blood that are needed for protection from SHIV infection.

## 153

### **Preclinical Toxicological Evaluation of the Dapivirine Vaginal Ring in the Sheep**

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**BACKGROUND:** Vaginal rings represent a promising technology for the sustained delivery of antiretroviral drugs as microbicides. However, the preclinical safety evaluation of vaginal rings needs to address the regulatory requirements for both pharmaceuticals and medical devices, and this presents challenges for toxicological testing because of the small size of the species traditionally used for such studies. The sheep has potential as an alternative species because the dimensions of the vagina are adequate to allow insertion of rings intended for human use. As is the case in humans, the cervicovaginal tract of the sheep is composed of a stratified squamous epithelium, the degree of keratinisation and epithelial thickness vary with the stage of estrus, and there are also similar anatomical features.

**METHODS:** A study was conducted to evaluate the Dapivirine Vaginal Ring containing 25 mg dapivirine for local and systemic toxicity. Five female sheep received a single ring for 30 days, and another 5 sheep received 3 rings, each for a period of 30 days, after which the ring was removed and replaced with a fresh ring for a total exposure of 90 days. Similar groups received a placebo ring for the same durations. In order to bridge to data obtained in chronic toxicity studies in rabbits previously conducted with dapivirine gel, 5 female sheep received 0.2% dapivirine gel intravaginally once daily (dose volume = 2.5 mL) for 30 days, and another 5 sheep received the same dosage for 90 days. Similar groups received a placebo gel for the same durations. Investigations included toxicokinetics, clinical signs, body weight, food consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, organ weights, and macroscopic and microscopic pathology.

**RESULTS:** There were no effects of treatment on any of the parameters investigated in animals treated with the dapivirine ring or gel. All changes observed were minor and consistent with normal background variations. Although histopathological examination identified a variety of incidental findings, the sheep was considered to be an appropriate model for the evaluation of the potential of microbicides to cause local vaginal irritation.

**CONCLUSIONS:** Dapivirine did not cause any vaginal irritancy or provide any evidence of local or systemic toxicity when administered to sheep in a vaginal ring or gel for up to 90 days. The sheep is a suitable species for the toxicological evaluation of microbicides delivered in vaginal rings.

## 154

### **PC-815 Blocks Rectal Transmission of SHIV-RT When Applied 4 Hours Prior to Virus Challenge**

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**BACKGROUND:** Testing anti-HIV microbicides in rectal transmission models is important due to the higher efficiency of rectal relative to vaginal transmission. Earlier research revealed that Carraguard (CG) is safe when applied rectally, unlike commonly used lubricants. In addition, CG has been shown to be safe after repeated usage in women, although the anti-HIV effects were limited. We evaluated the rectal efficacy of a topical microbicide, PC-815, comprising CG to deliver an NNRTI, MIV-150, in rhesus macaques. PC-815 has shown partial efficacy against vaginal transmission in rhesus macaques. To evaluate the durability of protection, we challenged either 0.5 or 4h after gel application.

**METHODS:** Rhesus macaques were treated rectally with 5mL of PC-815 (n=8) or methyl cellulose (MC) placebo (n=4) gel either 0.5 or 4h prior to rectal challenge with 1000 TCID<sub>50</sub> of SHIV-RT (SIVmac239 with HIV RT). Infection was assessed by testing for plasma virus RNA, PBMC virus DNA, IFN- $\gamma$  ELISPOT, and seroconversion. Statistical significance was determined with Fisher's exact test.

**RESULTS:** All PC-815-treated animals (4 of 4 in each group) were protected from rectal SHIV-RT infection when gel was applied either 0.5 or 4h prior to rectal challenge, while 100% of the control MC-treated animals (4 of 4) became infected. Thus, PC-815 afforded statistically significant protection against rectal challenge, even when administered 4h prior to exposure ( $p=0.029$ ). This study utilized animals recycled from other studies where they had been previously exposed to SHIV-RT. While the animals were evenly distributed between the test and control groups, we examined whether the uninfected animals might be inherently resistant to SHIV-RT infection. Activated PBMCs from the uninfected animals were all susceptible to SHIV-RT infection *in vitro* (additional controls of PBMCs from animals that resisted both *in vivo* and *in vitro* infection were included). Analysis of PBMCs confirmed that the uninfected animals were also negative for SIV DNA. All infected animals had normal viremia and developed SIV-specific T and B cell responses.

**CONCLUSIONS:** PC-815 blocked rectal SHIV-RT infection when applied up to 4h prior to virus challenge ( $p<0.03$ ). These data are promising for the development of NNRTI-containing gels for the prevention of rectal transmission. Future studies are needed to determine how long this protective effect lasts and to ensure that repeated application of the gels remains safe.

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### Sexual Practices of MSM in Nigeria and Interest in Microbicides

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**BACKGROUND:** To assess the sexual practices of MSM and their acceptability of rectal microbicide

**METHODS:** Questionnaire based cross-sectional study among persons with same sex practice in Eastern Nigeria on sexual behaviour, knowledge and attitude on microbicides. The data on MSM was extracted from the entire data for reporting. Data with STATA version 10.0

**RESULTS:** A questionnaire was administered to 498 persons of same practice in Ebonyi and Enugu states of Nigeria. There were 293 (58.8%) female and 205 male (41.2%) respondents. Respondents age ranged from 16–43 years with mean of 25.4 years. 359(72.7%), were unmarried, 110 (22.3%) were married and 25 (5.0%) are planning to get married. Only 55 (27.4%) of the men respondents were married. Four were planning to get married.

Of the 205 male respondent, 107 (52.2%) had had sex before while 98 (47.8%) had not. 101 (49.3%) had engaged in vaginal sex and 51 (24.9%) in anal sex with a woman. 69 (33.7%) males were bisexual, 180 (87.8%) had more than one sexual partner. 80 (41.9%) respondents had a stable female relationship out of which 33 (41.3%) had shared information with their female partners.

Only 4 (0.8%) of respondent use lubricant. Other use lubrications in the form of saliva (27), baby oil and vaseline (187). Four use cotrimozazole cream. Lubricants are often use so as to make penetration easy and reduce pain.

390 (83.7%) persons had learnt of microbicide mostly through health seminars. 250 (73.5%) are willing to use a microbicide when developed. Respondents are willing to insert the microbicide into the anus, vagina and apply it around the penis. 64 would apply to both vagina and anus. Most respondent noted they will want the microbicide to protect them from HIV infection and STIs.

**CONCLUSIONS:** The development of a microbicide for prevention of sexual transmission of HIV infection is of equally high importance to men in Africa. Microbicide development should not be marketed as a gender specific commodity

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### Sexual Behaviour, Knowledge and Attitude to Microbicide Development and Use among MSM in Eastern Nigeria

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**BACKGROUND:** Sexual behaviour and attitude of MSM towards rectal microbicide are important for development, deployment and eventual use. In this study we assessed the sexual behaviour and attitude of MSM in Eastern Nigeria.

**METHODS:** Questionnaire based cross-sectional study among MSM in Eastern Nigeria on sexual behaviour, knowledge and attitude on microbicides. Data with SPSS for windows version 10.0

**RESULTS:** 206 MSM participated in the study, of which 151(73.3%) were single. The ages ranged from 16 to 43 with a mean of  $26.6 \pm 7.4$ . 49.0% were bisexuals and 50.5% of the bisexuals have anal intercourse with their female partners. Multiple sexual partnerships were common among both the strict homosexual (54.9%) and the bisexuals (50.5%). The condom uses among these men were low with consistent condom use rate of 9.7%. Lubricant use rate was 82.1% and 37.3% during anal sex and vaginal sex respectively. Body cream/lotions (34.4%), petroleum jelly (27.3%), baby oil (22.7%) and KY gel (3.1%) were commonly used lubricants. 82.0% of MSM have heard of microbicides and willing to use if found effective (67.9%). Products that can be applied both in the anus and the penis are preferred.

**CONCLUSIONS:** Bisexual anal multiple sexual partnerships are common with a low condom use rate of 9.7%. However, knowledge of microbicide, willingness to use the product and use of lubricant during sex is high. Design of microbicide should target product similar to commonly used lubricants that will be easy to apply to both anus and penis for high acceptability.

## 157

### Practice of Anal Sex and Awareness of Rectal Microbicides among Sex Workers

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**BACKGROUND:** People with low levels of education, who are also not computer literate, lack adequate access to HIV prevention information including information about rectal microbicides, this is especially true in low-resource countries. Methods of HIV prevention—the female condom, which is available, and microbicides, which are being tested have the potential to provide women with greater control over sexual relations. Anal sex is carried out in both homogenous and heterogeneous relationships; therefore the availability of rectal microbicides will help in preventing new HIV infections especially among women with multiple sexual partners that are projected to occur in this decade

**METHODS:** A cross-sectional study was conducted on participants at an HIV/AIDS sensitization workshop, the participants were brothel-based female sex workers from the 5 brothels located in an urban town in Ota Ogun State. A structured interviewer-administered questionnaire was used because of the low level of education of the attendees. Data was analyzed using epi info statistical software

**RESULTS:** All the 159 female sex workers who attended the sensitization program on HIV were involved in the survey, 27% (43) of them had anal sex in the past one month, out of this number 34 (79%) had anal sex with their boyfriend while the remaining 9 (21%) had anal sex with customers. The frequency of anal sex compared to vaginal sex among this group was 2:25 respectively, barrier method (condoms) was used only for vaginal sex while no form of protection was used for oral sex and petroleum jelly was used as lubricants for anal sex. Only 15% of the attendees had attended secondary school and they did not complete secondary school education, none of the attendees read newspaper or are computer literate but they all claim to listen to radio and watch TV. The sensitization workshop is their first attendance at any HIV/AIDS related program and none of the respondents had heard of microbicides/rectal microbicides in the past.

**CONCLUSIONS:** The participants in the study have low literacy level, are not computer literate, lack access to adequate HIV/AIDS prevention information, practice anal sex without prevention, have multiple sexual partners but have no knowledge about microbicides/rectal microbicides

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### **Development of the First Rectal Microbicide Will Require a Four-fold Increase in Funding: Tracking Global Funding for Rectal Microbicides Research**

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**BACKGROUND:** In 2006, the International Rectal Microbicide Advocates (IRMA) completed a resource tracking exercise to determine both the total level of funding provided globally for rectal microbicide (RM) research between 2000 and 2006, and to estimate the level of funding required to bring a small number of candidates through all stages of research over the following 10 years. IRMA found that only US\$7.2 million was spent on RM research in 2006, while IRMA estimated that annual spending of US\$35 million would be required over 10 years to realize a comprehensive RM research program. In other words, yearly investments would need to increase five-fold from 2006 levels. The US public sector contributed 97.4% of overall monies contributed between 2000 and 2006.

**METHODS:** In 2010, IRMA updated its resource tracking, and revised its estimates of funding required to maintain a viable RM research agenda. IRMA confirmed RM research funding through snowball sampling, starting with RM researchers known through the IRMA network; searching for all relevant projects through the NIH Research Portfolio Online Reporting Tool (RePORT); directly contacting the funders identified through the last resource tracking exercise; contacting the European Research Directorate-General; and, confirming with each of these stakeholders and the IRMA Steering Committee that the compiled list of funding was accurate and complete.

**RESULTS:** Between 2007-2010, US\$23.4 million has been spent on RM research, or an average of US\$5.9 million per year. This represents an 18% reduction from annual levels observed in 2006. Between 2007-2010, the US public sector contributed 96.3% of global funding for RM research, the philanthropic sector contributed 3.2% and European funding sources contributed 0.4%. According to conservative estimates developed by IRMA, at least US\$125 million are needed over the next 5 years to ensure a minimal number of candidates are pursued from bench to clinical efficacy trials.

**CONCLUSIONS:** Current annual funding levels for RM research must increase four-fold to ensure that at least one candidate is developed, according to revised IRMA estimates. A greater diversity of funders would not only increase the amount of resources available, but would ensure greater sustainability for the RM research field.

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### **Using Drug Combinations to Design Effective Colorectal Microbicides: Where is the Limit?**

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**BACKGROUND:** Receptive anal intercourse is associated with the highest probability for sexual HIV infection. The aim of the study was to assess the importance of reverse transcriptase inhibitor (RTI) combinations in the design of colorectal microbicides and the number of drugs required to obtain maximum efficacy against wild type isolates and RTI-escape mutants.

**METHODS:** The antiviral efficacy of two nucleos(tides) reverse transcriptase inhibitors (NRTI) PMPA and FTC, and two non-NRTIs (NNRTI) UC-781 and TMC120, used in double, triple and quadruple combinations, was assessed in colorectal explants. Pre-incubation with the drugs individually or in combination, for one hour was followed by addition of virus. Infection was determined by measurement of virion protein (p24 antigen) in colorectal explants supernatants.

**RESULTS:** All combinations inhibited the isolates tested in colorectal explants, and produced, for at least one of the compounds, a change in the dose response curve. Double and triple combinations incrementally augmented activity, even against RTI escape mutants, whereas quadruple combinations conferred little further advantage. The colorectal explant model is key to identification of the best candidate molecules and their combinations at the preclinical stage.

**CONCLUSIONS:** Triple combinations based on RTIs have potential as colorectal microbicides to prevent the transmission of wild type and resistant isolates.

## 160

### **Preclinical Evaluation of Aptamers as Candidate Rectal Microbicides**

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**BACKGROUND:** An aptamer is a short RNA nucleotide sequence specific to a target, in this case the HIV gp-120 glycoprotein, for which activity has been seen in a PBMC model. We present our evaluation for efficacy and toxicity of aptamers.

**METHODS:** 3 aptamers at 100nM and 500nM concentrations were evaluated against HIV-1 BaL ( $10^5$  infectious units/mL). Nonoxynol-9 (N-9), tenofovir, TAK-799 and MAb-12 were used as controls. Most experiments were conducted in triplicate. In the colorectal explant, biopsies obtained by sigmoidoscopy had a 2 to 4-hour exposure to virus and drugs, and were maintained on gel rafts. Supernatant was collected at 4 or 16 hr, 3, 7, 10 and 14 days, and evaluated for p24 by EIA. TZM-bl cells were incubated at a density of  $10^4$  cells per well, with infection and toxicity quantified by Tat transactivated luciferase gene luminescence and ATP production, respectively. In the transwell model, CaCo2 cells ( $3 \times 10^5$  cells/mL) grown to confluence as measured following the transepithelial electrical gradient (TEER) to a plateau were exposed to aptamers for 18 hr. TEER was measured for up to 7 days afterwards. Additionally, we evaluated the ability of HIV to freely cross the CaCo2 and infect PM-1 cells in the lower chamber (by p24 quantification) at one and 4 days after exposure to aptamers. Fluorescence microscopy was performed to evaluate for integrity by staining for zonula occludens (ZO-1).

**RESULTS:** The explant model showed variable results with a moderate, yet significant ( $p \leq 0.05$ ), mean reduction of infection with aptamers at 100nM concentration at day 14. A significant dose dependent inhibition of infection was noted in the TZM-bl model (up to 90% reduction with 500nM). No toxicity was seen with any aptamer at 100 or 500nM. The TEER remained stable. The architecture was preserved in fluorescence microscopy, and no increased viral translocation was noted in the transwell model. No decrease in ATP production was seen.

**CONCLUSIONS:** Aptamers show moderate efficacy in the TZM-bl and colorectal explant models, although a wide range of activity was noted in the last platform. No toxicity was seen. Additional experiments need to be conducted at higher doses and with newer aptamers. Experiments to evaluate for aptamer-induced inflammation and apoptosis are needed as well.

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### Rectal Microbicide Trials in Nigeria: Potential Challenges for Engaging MSM in Trials

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**BACKGROUND:** HIV intervention in Nigeria pay little attention to the needs of high risk groups (MSMs, SWs, IDUs) due partly to stigma and discrimination by health service providers. Past biomedical HIV Prevention research had focused on vaginal products with little or no information collected about anal sex. Yet data shows as high as 40% of some populations in Nigeria engaging in anal sex. This study evaluates the place and importance of rectal microbicides for MSMs who are in heterosexual relationships and practice anal sex.

**METHODS:** MAN conducted a FGD among 12 MSM in Abuja, Nigeria to discuss their perspectives on rectal microbicide, possibility of participating in rectal microbicide trials, and to identify and better understand challenges they may face if they are to participate in rectal microbicide studies. Responses were recorded by a note taker and voice recorder and analysed by two behavioral researchers.

**RESULTS:** While respondents identified the need for products to reduce anal transmission of HIV for use by both their male and female sex partners, they had varied perceptions about the use of placebos (especially about the selection criteria for participants on placebo). Participation in trials may be limited by community stigma of MSM. If such trials are openly labeled as recruiting MSM, participation may be poor. Members of the MSM community will need to be engaged in the planning and implementation of such trials to ensure that the needs of the community are met. Participants also complained about other trials looking only like a money making venture because investigators spend little time explaining the trials, but entice the trial participants with incentives provided.

**CONCLUSIONS:** MSM in Nigeria are well aware of rectal microbicide trials due to the increasing advocacy efforts and discussions. While the community understands a lot about microbicide trials, the potentials for low participation in such trials exist due to the current unfavourable legal and social environment for open labeled studies engaging MSM. Such trials will therefore need to consult and engage the MSM community to ensure their buy in and support, as well as identify appropriate mechanisms for engaging with such trials. Studies to understand challenges for MSM engagement in trials need to be conducted.

## 162

### The Use of Rectal Douches among Peruvian MSM: Implications for Rectal Microbicides

J. Galea, J. Kinsler, P. Segura, J. Peinado, J. Sanchez

University of California, Los Angeles, CA, USA

**BACKGROUND:** Rectal douching prior to receptive anal intercourse (RAI) is common among men who have sex with men (MSM) in the USA, and a rectal microbicide (RM) delivered as such could therefore be acceptable among this population. Nevertheless, equivalent data from non-US populations are virtually nonexistent yet critical if RMs are to have applicability in other settings such as Latin America where the majority of HIV is transmitted via unprotected RAI among MSM.

**METHODS:** A convenience sample of 1,342 Peruvian MSM reporting anal intercourse with another male in the 12 months prior to the study was recruited to participate in the 2008 National Peruvian HIV Sentinel Surveillance. Consenting participants completed a structured survey administered via computer-assisted self-interviewing. The interview included questions on demographics, sexual behaviors and rectal douching. Blood samples were collected and tested for anti-HIV-1 antibodies using Determine HIV-1/2 rapid assay with Western Blot confirmation. Bivariate analyses was used to assess the association of rectal douching with demographics, behavioral characteristics, HIV status, and willingness to use a RM formulated as a rectal douche (RD) to protect against HIV infection. Multivariate analyses were conducted to examine the relationship between RD use and willingness to use a hypothetical RD adjusting for demographics, behavioral characteristics, and HIV status.

**RESULTS:** Nineteen percent of respondents had ever used a RD specifically to clean their rectum prior to AI. In bivariate analyses, RD use was significantly associated with being 25 to 29 years of age ( $p < 0.01$ ) and 35 years or older ( $p < 0.001$ ); being homosexual/gay ( $p < 0.001$ ); passive/receptive role during AI ( $p < 0.001$ ); having exclusively male sex partners ( $p < 0.01$ ); and, condom use during AI with last partner ( $p < 0.05$ ). Among all study participants, 70% reported willingness to use an RM formulated as a RD to prevent HIV infection if one were available. Ever using an RD was significantly associated with willingness to use a RM formulated as a RD in both bivariate and multivariate analyses ( $p < 0.001$ ).

**CONCLUSIONS:** While 19% of our sample reported ever using an RD, 70% reported they would use a future RD to protect against HIV. These findings suggest that an RD holds promise as a delivery method for future RMs in Peruvian MSM. Further research is warranted to understand the use and acceptability of RD in this population.

## 163

### Cost-Effectiveness of Prescreening VCT During HPTN 035

W. Phiri<sup>\*1</sup>, C. Reid<sup>1,2</sup>, S. Roberts<sup>1,2</sup>, M.B.Guffey<sup>1,2</sup>

<sup>1</sup>Centre for Infectious Disease Research in Zambia, Lusaka, Zambia; <sup>2</sup>University of Alabama at Birmingham, Alabama, USA

**BACKGROUND:** In the microbicide preparedness study HPTN 055, 28% of women presenting for study screening in Lusaka, Zambia were excluded because of positive HIV status. For the subsequent microbicide trial HPTN 035, we hypothesized that the introduction of a pre-screening visit for voluntary HIV counseling and testing (PSVCT) prior to study screening would reduce the overall cost of identifying women otherwise eligible for the study.

**METHODS:** The actual expenses incurred during PSVCT in Lusaka during HPTN 035 were compared with the anticipated scenario of screening those same individuals without PSVCT. Determination of costs included calculation of staffing time, material and laboratory costs, and transport reimbursements from the time a woman presented to clinic until HIV status was determined during the first screening visit.

**RESULTS:** 697 women presented to the study clinic intending to screen for HPTN 035. All women underwent PSVCT prior to initiating screening. Cost of PSVCT was \$4,500 USD during which 138 women (20%) were found to be HIV positive. Of the 559 HIV negative women, 445 returned and proceeded with study screening at a cost of \$10,500. Only 1 was found to be HIV positive at that time leaving 444 women to continue with screening. The anticipated cost of processing all 697 women through a traditional screening visit up to the point of determining HIV status was \$17,200. The cost benefit of PSVCT in our research unit was a minimum of 12% and was primarily the result of time saved during informed consent (which ranged 5 to 15 minutes in PSVCT versus 50 to 59 minutes for study screening) and a less expensive HIV testing algorithm (\$4.69 versus \$8.69 per woman) utilized during PSVCT compared to traditional screening. Additional benefits included identifying 114 eligible women that were not going to continue with study screening prior to the additional expense of processing those women through the remainder of screening and dedicated time for counseling and referral of 138 HIV positive women.

**CONCLUSIONS:** Identification of women at high risk for HIV infection is important during implementation of HIV prevention trials; however, the process of identifying eligible women is costly, especially in settings with high background HIV prevalence. Introduction of PSVCT for the identification and counseling of HIV positive women is beneficial for those found to be HIV positive and introduces cost savings during the overall screening process.

# SUNDAY POSTERS

## 164

### Cost Analysis of Conducting Clinical Trials in Resource-Poor Settings

K. Haripersad\*, G. Ramjee  
South African Medical Research Council

**BACKGROUND:** HIV prevention research is urgently needed to address this devastating epidemic. Current prevention options are not suitable or available to those most vulnerable to HIV. Biomedical research of new prevention technologies is urgently needed. One of the challenges in conducting HIV prevention trials is to identify regions with high HIV incidence. These regions are mostly in the Sub-Saharan Africa which are more often than not poorly resourced. The Medical Research Council conducted 5 Phase III trials in a period of 6 years of HIV prevention technologies. We undertook a cost analysis of each trial taking into consideration, HIV testing, laboratory costs, trial duration, follow-up periods, visit schedules, participant reimbursements, infrastructure, operational and human resources cost treatment of STIs.

**METHODS:** An average cost method was utilised in the calculation of the cost per participant. Trial A was conducted at one site in Durban with 600 participants enrolled and followed up for a 12-month period. The study was conducted over 23 months. 1,054 Participants were enrolled into Trial B which was conducted across two sites. Participants were followed up for 30 months and the trial was carried out for 51 months. Trial C enrolled 2,392 participants over a 54 month period with a follow up period of 12 months. The trial was conducted at 3 sites. Quarterly follow up for 24 months were carried out in Trial D. 1,515 Participants were enrolled. The trial was conducted over a 46 month period across 2 research sites. 1,485 Participants were enrolled in Trial E with quarterly follow up visits for 24 months. It was conducted at one site in Durban for a total period of 42 months.

**RESULTS:** Our analysis of trial expenses highlighted that it will cost approximately \$5,853 per participant to fund a Phase III Microbicide trial in KwaZulu Natal in order to achieve trial objectives. Depending on the design of the Phase III trial, it is estimated that it will cost approximately \$12 million to conduct a Phase III Microbicide trial in KwaZulu, South Africa over a four year period enrolling 2,300 women.

**CONCLUSION:** This cost analysis suggests that per patient cost of clinical trial in a middle-income country is fairly cost-effective. As we move forward with larger sample size due to falling HIV incidence, the cost of conducting trials in this region is likely to increase. Building laboratory capacity at researching institutions would cut down the cost enormously.

## 165

### How Different Efficacy, Consistency of Usage and Uptake/Distribution Factors Affect the Relative Impact of Microbicide Interventions: Analytical Insights

A.M. Foss\*, A.P. Cox, P.T. Vickerman,  
C.H. Watts  
London School of Hygiene and Tropical Medicine

**BACKGROUND:** The HIV epidemic in India remains concentrated in high-risk groups including men who have sex with men (MSM). Given the challenges of condom use, interest has been growing in the potential role of a rectal microbicide for MSM. Prior to this study, there were no estimates of the likely public health impact of a rectal microbicide in any low- or middle-income country. This research project aimed to model the potential impact of a rectal microbicide on the HIV epidemic among MSM in Bangalore, India.

**METHODS:** A deterministic compartmental model was developed, parameterised and fitted to detailed epidemiological and behavioural data from MSM in Bangalore. The model was used to simulate the joint transmission dynamics of HIV, syphilis and herpes between three subgroups of MSM, based on sexual identities/risk behaviour. Potential evolution of the HIV epidemic was investigated in the absence and presence of a 5-year microbicide intervention, with moderate underlying levels of condom use. It was assumed that 30% of the total MSM population of Bangalore Urban District would be reached by the intervention and use the microbicide between half and all the time when a condom is not used during anal sex (allowing 20% fewer sex acts to be covered by a condom following access to microbicide). Microbicide efficacy scenarios of a 35%, 60% or 85% reduction in the probability of HIV transmission to the receptive partner per anal sex act were considered.

**RESULTS:** For a microbicide with 35% HIV-efficacy per sex act, preliminary model findings suggest that approximately 4-8% of HIV infections among MSM in Bangalore could be averted within a 5-year period, given the assumptions stated above. Preliminary results imply that this impact may increase to averting about 10-20% of HIV infections if the microbicide's per sex act HIV-efficacy is 85%. In all these scenarios described, approximately 800 to 4,000 infections among MSM in Bangalore could be averted over 5 years through the use of an effective rectal microbicide.

**CONCLUSIONS:** This study highlights the importance of pursuing further research and investment for developing rectal microbicides. The public health benefit from an effective rectal microbicide could be considerable. These preliminary findings are in some sense conservative since they do not include the onward infections averted among the female partners of MSM.

## 166

### Need We Worry About Condom Substitution for High-Efficacy ARV-based Microbicides? Model Findings from Southern India

A.M. Foss<sup>1</sup>, A.P. Cox<sup>1</sup>, P.T. Vickerman<sup>1</sup>,  
L. Heise<sup>1</sup>, S. Reza-Paul<sup>2</sup>, B.M. Ramesh<sup>2,3</sup>,  
M. Alary<sup>4</sup>, S. Moses<sup>4</sup>, C.H. Watts<sup>1</sup>

<sup>1</sup>London School of Hygiene & Tropical Medicine, London, UK; <sup>2</sup>University of Manitoba, Canada; <sup>3</sup>Karnataka Health Promotion Trust, Bangalore, India; <sup>4</sup>Centre hospitalier affilié universitaire de Québec, Canada

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**167****Structure-Property Relationships for Computational Molecular Design of Microbicide Formulations**T. Wilson, A.M. Markey, K.V. Camarda,  
S.L. Kieweg\*  
*University of Kansas, Lawrence, KS, USA*

**BACKGROUND:** The long-term objective is to demonstrate a method to computationally design new microbicide delivery systems, specific to selected female populations, with simultaneously-optimized physicochemical properties, biocompatibility, and innate microbicidal activity. The objective of this study is to measure a set of physicochemical properties for a range of cellulose structure/formulation combinations. These property data are used to create Quantitative Structure-Property Relationships (QSPR), the first step towards computational molecular design of microbicide formulations. Shear-thinning viscosity, viscoelasticity, and compressibility data were collected for a set of formulations in order to build a numerical QSPR model. These properties represent characteristics that impact formulation spreading when compressed between vaginal epithelial surfaces.

**METHODS:** 18 structure/formulation combinations were created that spanned a range of cellulose structures with varied concentrations, molecular weight, degree of substitution, and molar substitution. The cellulose structures were hydroxyethylcellulose, methylcellulose, and binary mixtures. We varied concentration depending upon polymer molecular weight to yield properties that both compared to the Universal Placebo and created a large property range needed for the QSPR model. Shear-thinning index and consistency were measured on a rheometer (TA Instrument AR2000, 40mm 2° cone). Oscillatory frequency sweeps (40 mm plate) were performed at an applied stress in the linear viscoelastic regime to find G' (storage modulus), G'' (loss modulus), and loss tangent; three representative frequencies (0.01, 0.1, 1.0 Hz) were used. Work of compression (compressibility) was defined as the area under a force-distance curve from a 40 mm plate pressed into a sample (7 formulations, 2 compression rates). All data were median of n=3 samples at 37°C.

**RESULTS:** Six physicochemical properties of structure/formulation combinations were tabulated and min-max ranges were: consistency (30.18-655.57 Psecn<sup>-1</sup>), shear-thinning index (0.49-0.95), G' (0.00285-169.5 Pa), G'' (0.187-140.2 Pa), loss tangent (0.248-68.5), work of compression (0.6-8.5 N mm).

**CONCLUSIONS:** The physicochemical properties of 18 cellulose structure/formulation combinations were tabulated and produced a wide range of values needed for a mathematical QSPR model, necessary for our ongoing work in computational molecular design of microbicide formulations.

**208****Molecular Mechanisms Mediating Proinflammatory Responses in Human Vaginal Epithelium**T. Joseph\*, I. Zalenskaya, G. Doncel  
*CONRAD-Eastern Virginia Medical School, Norfolk, Virginia, USA*

**BACKGROUND:** The cervicovaginal epithelium of the lower female genital tract actively participates in diverse proinflammatory responses to external pathogenic stimuli and endogenous vaginal microbiota. These responses usually involve the recognition of certain conserved pathogen-associated molecular patterns by specific receptors called Toll-like receptors (TLRs). Most of these TLRs activate MAP kinases (MAPK) and NF-κB signal transduction pathways. These pathways are also involved in the expression of proinflammatory mediators like the prostaglandin synthase COX-2. In this study, we evaluated key signal transduction mechanisms leading to COX-2 expression by human vaginal cells exposed to TLR ligands, nonoxynol-9 (N-9), TNF-α and tenofovir.

**METHODS:** Human vaginal epithelial cells (VK2/E6E7), grown to 70–80% confluence, were treated with N-9, TNF-α and TLR ligands such as macrophage activating lipopeptide (MALP2), Pam3CSK4 (Pam), zymosan, polyinosine-polycytidyl acid (poly dI:dC) and lipoteichoic acid (LTA), and dose and time response experiments were performed. Cells were also treated with these proinflammatory stimuli in the presence of pathway inhibitors such as Bay11 7082 (NF-κB), SB202190, SB203580 and U0126 (MAPK). Nuclear, cytoplasmic and total cellular proteins were used to assay for p65 (NF-κB) nuclear translocation, MAPK phosphorylation and COX-2.

**RESULTS:** Stimulation of vaginal cells with TLR ligands, N-9 and TNF-α resulted in COX-2 expression in a dose and time-dependent manner. For example, N-9 caused maximum COX-2 expression at 12.5 μg/ml for 6h. Phosphorylation of MAPKs and p65 nuclear translocation was observed with most TLR ligands, indicating activation of MAPK and NF-κB pathways. Inhibition of these pathways decreased epithelial COX-2 expression. Unlike the above agents, tenofovir did not stimulate proinflammatory pathways or COX-2 expression.

**CONCLUSIONS:** Multiple TLR ligands, TNF-α and N-9 activate vaginal epithelial MAPK and NF-κB pathways and increase COX-2 expression, providing the molecular trigger for a mucosal immunoinflammatory reaction. These findings help understand the molecular mechanisms underlying genital mucosal inflammation and may be used to assist in the rational selection of safe microbicide candidates.

**466****Designs for Future HIV Prevention Trials Once an Effective Intervention is Identified**Y. Huang, B. Mâsse  
*Statistical Center for HIV/AIDS Research and Prevention, Fred Hutchinson Cancer Research Center, Seattle, WA, USA*

**BACKGROUND:** Several on-going or to-be-launched oral pre-exposure prophylaxis (PrEP) and microbicide Phase IIB/III trials provide hope for an effective intervention. Although it is unlikely that a single intervention would be highly effective and be applied universally in all settings and populations, the use of placebo controls in Phase IIB/III will be ethically challenged as the evidence of effective interventions mounts. It is imperative for the HIV prevention field to investigate the design of future trials under such circumstances.

**METHODS:** We investigate the scenario where a microbicide gel is found to be effective and the efficacy of a new gel needs to be evaluated. When there is compelling evidence that a microbicide gel is effective, such gel can be used as an active control to evaluate a new gel by either conducting: 1) a superiority trial to test that the new gel is superior to the active control; or, 2) a non-inferiority (NI) trial to test that the new gel is equivalent or not worse than the active control, by a pre-specified NI margin, δ. We specify δ based on the 95% C.I. of the active control's effect in historical placebo-controlled trials. We also derive δ based on the % of active control effect being retained under the constancy assumption. Both Monte Carlo simulations and standard large-sample approximations for two-sample log-rank tests were used for the sample size calculations.

**RESULTS:** To retain at least 80% of the active control effect in the evaluation of a new gel, a NI trial would be feasible only if the active control effect is at least 70% in the historical placebo-controlled trials. On the other hand, it would be feasible to conduct a superiority trial to detect an efficacy of 30% for a gel over the active control. A trial is defined as feasible if the total estimated person-years is < 9000. These calculations were carried out based on the assumption of a 2-year accrual and a min of 12-month follow-up with an average of 3% annual HIV incidence rate.

**CONCLUSION:** We do not recommend the use of NI trial designs in future microbicide evaluations if the active control gel has a small to moderate effect over the placebo gel. In the near term, we recommend placebo-controlled superiority trials. If feasible, "add-on" trials combining different gels should also be considered to yield high efficacy. Although the results have been derived in the context of microbicides, they can be applied to oral PrEP interventions.



The background of the image is a soft-focus, abstract representation of organic forms in a light green and yellow hue. It features various rounded, bulbous, and elongated shapes that suggest biological structures like cells or microorganisms. Some larger, more complex structures resemble leaves or small plants, while smaller ones look like individual cells or pollen grains. The overall effect is one of a microscopic view of a lush, growing ecosystem.

Abstracts

Monday  
May 24, 2010

# ORAL SESSIONS

## SESSION 18

### Plenary

**Moderators:** Robin Shattock, Roberta Black

Monday, May 24, 8:00 am–9:00 am

Ballroom

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### From Transmission to Targets: How HIV Infects Mucosal Tissue

Ashley T. Haase, M.D.

*Department of Microbiology, University of Minnesota, USA*

The presentation will focus on the small founder populations of infected cells at transmission in the earliest stages in female transmission in both HIV and SIV infections. It will then describe evidence in the SIV-rhesus macaque model of transmission for local expansion as a necessary step in establishing systemic infection, and the critical role the innate response to exposure plays in providing CD4+ T target cells for this expansion. It will conclude with a discussion of how microbicides can intervene in blocking this expansion to thereby prevent systemic infection, using glycerol monolaurate as an example.

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### Not Just a Handmaiden: The Role of Social Science in Microbicides and Other HIV Prevention Research

Judith D. Auerbach, Ph.D.

*San Francisco AIDS Foundation, USA*

This presentation will discuss the contributions of social science to microbicides and other HIV prevention research. It will address the historic ways in which social—often conflated with “behavioral”—science has been employed in the context of clinical trials of candidate technologies, chiefly in the realm of acceptability and use of products by individuals, and the limitations thereof. The presentation will provide a broader view and explication of the role of social science in better understanding the fundamental social drivers of HIV vulnerability and applying that knowledge to interventions and programs. In so doing, it will highlight some of the ways in which a lack of integration of social science knowledge has contributed to problems encountered in microbicides and other prevention trials. It will also discuss the opportunities and challenges of an integrated and comprehensive approach to HIV prevention that links biomedical, behavioral, and social-structural approaches.

## SESSION 19

### Oral Abstracts (OA10): Innovative Preclinical Studies

**Moderators:** Jim Turpin, Victor Garcia-Martinez

Monday, May 24, 9:30 am–11:00 am

Ballroom

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#### DC-SIGN Increases the Affinity of HIV-1 Envelope Glycoprotein Interaction with CD4

K. Hijazi<sup>1</sup>, Y. Wang<sup>1</sup>, S. Jeffs<sup>2</sup>, C. Longstaff<sup>3</sup>, B. Haggarty<sup>4</sup>, G. Vanham<sup>5</sup>, J. Hoxie<sup>6</sup>, R. Shattock<sup>6</sup>, C. Kelly<sup>\*1</sup>

<sup>1</sup>King's College London, London, United Kingdom;

<sup>2</sup>Imperial College, London, United Kingdom;

<sup>3</sup>National Institute for Biological Standards and Control, Hertfordshire, United Kingdom; <sup>4</sup>University of Pennsylvania, Philadelphia, Pennsylvania, USA;

<sup>5</sup>Institute of Tropical Medicine, B-2000 Antwerp, Belgium and University of Antwerp, Antwerp, Belgium; <sup>6</sup>St George's, University of London, London, United Kingdom

**BACKGROUND:** Mannose-binding C-type lectin receptors, expressed on Langerhans cells and subepithelial dendritic cells (DCs) of cervico-vaginal tissues play an important role in HIV-1 capture and subsequent dissemination to lymph nodes. DC-SIGN has been implicated in both productive infection of DCs and the DC-mediated *trans* infection of CD4<sup>+</sup> T cells which occurs in the absence of replication. However, the molecular events which underlie this efficient transmission have not been fully defined. In this study, we examined the effect of the extracellular domains of DC-SIGN and Langerin on the stability of the interaction of the HIV-1 envelope glycoprotein with CD4 and also on replication in permissive cells.

**METHODS:** Surface plasmon resonance (SPR) was used to determine the effect of C-type lectins on CD4 binding affinity to trimeric gp140. HIV gp140 (expressed in 293T cells) was captured on the sensorchip using an anti-gp41 monoclonal antibody. CD4 binding was then measured in the absence or presence of soluble DC-SIGN or Langerin. To evaluate the effect of DC-SIGN and Langerin on HIV-1 replication in permissive cells, PM1 cells were infected with virus in the presence or absence of soluble C-type lectins.

**RESULTS:** As measured by SPR, DC-SIGN increased the affinity of binding of trimeric gp140 envelope glycoproteins to CD4, so that the complex dissociated with a rate constant at least two orders of magnitude lower than that observed in the absence of DC-SIGN. In contrast, Langerin did not show any significant effect on the stability of the gp140:CD4 complex. HIV-1 replication in PM1 cells (CD4<sup>+</sup>/CCR5<sup>+</sup>/DC-SIGN<sup>+</sup>) was significantly raised when cells were infected in the presence of soluble DC-SIGN, with a greater enhancing effect of a CXCR4-tropic opposed to a CCR5-tropic strain, whereas Langerin showed no effect. Experiments where infection of Thp-1 cells was compared with that of Thp-1 DC-SIGN (where DC-SIGN is expressed in *cis* on the cell membrane surface) confirmed *cis*-enhancement of infection by the CXCR4-tropic strain IIIB. When the same infections were carried out using a variant of HIV-1 IIIB that acquired the ability to utilize CXCR4 without CD4, lower levels of *cis*-enhancement of infection were observed.

**CONCLUSIONS:** These findings suggest that DC-SIGN, and not Langerin, facilitates viral replication in CD4<sup>+</sup> cells by stabilizing interaction of the envelope glycoprotein with CD4. This study provides further insight into DC-mediated mechanisms of HIV-1.

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#### Electrophoretic Mobility Reveals Conformational Changes in HIV-1 Env That Model Male-to-Female Transmission Events

D. Stieh<sup>\*1</sup>, D. Fairhurst<sup>2</sup>, I. Monahan<sup>1</sup>, R. Rowell<sup>3</sup>, R. Shattock<sup>1</sup>

<sup>1</sup>Division of Cellular and Molecular Medicine, St. George's University of London, UK; <sup>2</sup>International Partnership for Microbicides, MD, USA; <sup>3</sup>University of Massachusetts, MA, USA

**BACKGROUND:** An understanding of the chemical nature of events that take place during HIV-1 transmission enables the rational design of microbicides. Dynamic electrophoretic mobility measurements allow characterization of the surface charge of colloidal particles and determination of their attractive or repulsive properties. It is hypothesized that measurement of surface chemistry of biological surfaces can be used to define conditions in which a microbicide will be most effective.

**Methods:** Electrophoretic mobility was measured for resting and activated CD4<sup>+</sup> T cells. Two viral strains, one R5- (HIV-1<sub>BaL</sub>) and one X4-(HIV-1<sub>IIIB</sub>) tropic, were purified and subsequently analyzed for their surface charge. The Env spike, represented by a BX08 gp120 monomer as well as CN54 and BX08 gp140 trimers were analyzed for complete electrophoretic fingerprints. Binding measurements of soluble CD4 (sCD4) interacting with gp140 trimers at a range of pH values were made using an acoustic biosensor.

**RESULTS:** Activated CD4<sup>+</sup> T cells are more negatively charged at low pH values than resting ones. The R5-tropic virions exhibit a negative mobility over all pH values examined. The X4-tropic virions have an isoelectric point at pH 4.5, above which the mobility is negative. BX08 gp120 has an electrophoretic fingerprint shape similar to that of a mixed carboxyl and amino coated particle. Gp140 trimers demonstrated a distinct profile, whereby their surface charge increases from pH 6.0 to 7.5, forming a ridge in the fingerprint. sCD4 bound the most to gp140 trimers at pH 4.5, and had decreased affinity at neutral pH.

**CONCLUSIONS:** These data suggest that there is a change in the surface chemistry as T cells are activated. This may be a factor in increased susceptibility to infection. The charge characteristics of purified X4-tropic HIV-1 resemble those of the progenitor T cells from which it was derived, while R5-tropic varies in that it is more negative under low pH conditions. The gp140 trimers from two different clades demonstrate an increase in mobility as pH approaches neutral that likely reflects a conformational rearrangement. This change is paralleled by a decrease in affinity for primary HIV-1 receptor, CD4. Given that vaginal pH can range from 4–7, these data suggest that there may be different conformations of Env present at mucosal pH ranges that influence the efficiency of sexual transmission.

# ORAL SESSIONS

**172**

## Evaluation of ARV-based Microbicides, Alone and in Combination, Against Drug-resistant HIV-1 Transmission *In Vitro*

S.M. Schader<sup>\*1,2</sup>, H. Xu<sup>2</sup>, S.P. Colby-Germinario<sup>2</sup>, M. Oliveira<sup>2</sup>, D. Moisi<sup>2</sup>, B.J. Scarth<sup>1</sup>, M.A. Wainberg<sup>2</sup>

<sup>1</sup>Department of Microbiology and Immunology, McGill University, Montreal QC, Canada; <sup>2</sup>McGill AIDS Centre, Lady Davis Institute, Jewish General Hospital, Montreal QC, Canada

**BACKGROUND:** Transmission of drug resistant (DR) non-subtype B HIV-1 is increasing. Thus, it is necessary to evaluate the efficacy of candidate ARV-based microbicides against the transmission of DR HIV-1 from multiple subtypes. We determined single drug efficacy and the combined effects of entry inhibitors, DS001 and DS003, and reverse transcriptase (RT) inhibitors, tenofovir (TNV) and dapivirine (DAP), against B and non-B subtype DR HIV-1 *in vitro*. We also evaluated the capacity for candidate microbicide ARVs to select for DR HIV-1 to anticipate the impact of microbicide use on ART efficacy.

**METHODS:** To assess breadth of activity, compounds were tested alone and in combination against subtype A, B, C, & CRFO2\_AG primary DR HIV-1 isolates. Infection was quantified by counting light units emitted from TZM-bl cells <48h post-infection. Combination ratios were based on drug IC<sub>50</sub>s and combined effects were determined by calculating combination indices (Cl<sub>i</sub>s). Clonal wild type (WT) and DR HIV-1 were used to control for viral variation. Mechanisms underpinning effects observed in cell culture were elucidated by molecular based approaches. To evaluate the capacity for candidate microbicide ARVs to select for DR HIV-1, CBMCs were infected with WT HIV-1 from assorted subtypes and cultured under increasing drug pressure over time. Replicating virus was genotyped and phenotyped routinely.

**RESULTS:** All candidate microbicide ARVs demonstrated potent anti-DR HIV-1 activity *in vitro*, albeit combinations protected better than single drug treatments. Of particular interest, the DAP+TNV combination exhibited synergy (Cl<sub>50</sub>=0.567) against a subtype C DAP-resistant HIV-1 while additivity (Cl<sub>50</sub>=0.99) was observed against the WT counterpart from the same patient. The effect was confirmed using complimentary clonal subtype C WT (Cl<sub>50</sub>=0.97) and DR (Cl<sub>50</sub>=0.67) HIV-1 in lieu of the patient quasispecies and, further, in a cell/virus-free assay utilizing complimentary WT (Cl<sub>50</sub>=1.0) and DR (Cl<sub>50</sub>=0.43) recombinant RT. Interclass combinations were also effective against WT (Cl<sub>50</sub>=0.70-0.90) and DR (Cl<sub>50</sub>=0.90-1.0) HIV-1.

**CONCLUSIONS:** Results indicate that combined effects may be accounted for at the molecular level and are largely subtype independent. Drug selection experiments showed that DR HIV-1 emerged only with pre-established HIV-1 and detection required many rounds of viral replication to occur. These results suggest that ARV based microbicides will likely not select for incoming DR HIV-1.

**173**

## A Preclinical Evaluation of the Small Molecule Entry Inhibitor DS003 as a Potential Microbicide

S. Harman<sup>\*1</sup>, J. Nuttall<sup>2</sup>, R. Shattock<sup>2</sup>

<sup>1</sup>St. Georges, University of London, London, UK;  
<sup>2</sup>International Partnership for Microbicides, MD, USA

**BACKGROUND:** DS003 is a small molecule entry inhibitor that blocks binding of host cell CD4 with viral gp120. To be effective as microbicides, entry inhibitors must be able to block infection of mucosal tissue under all circumstances, particularly when mucosal integrity has been compromised by epithelial trauma. Therefore we have devised a tissue challenge model to mimic a worse case scenario, in which viral inoculum has free access to sub epithelial target cells. In addition, potential microbicides must demonstrate good tissue biocompatibility; to this end, we have assessed the entry inhibitor for toxicity.

**METHODS:** DS003 was tested for efficacy in TZM-bl cells and human genital tissue explants, and results assessed by p24 ELISA or luciferase assay. The effect of various concentrations of compound on the viability of human genital tissue was determined using MTT assay, with Nonoxynol-9 as a control.

**RESULTS:** Using TZM-bl cells, DS003 demonstrated an IC<sub>50</sub> of 2.5nM against HIV<sub>Bal</sub>. DS003 also demonstrated good inhibition of HIV<sub>Bal</sub> infection in human genital tissue explant models with IC<sub>50</sub> values of 27nM when included for the first 24 hours of culture and 18nM when maintained throughout the culture period. Good activity was also observed against the dissemination of virus from migratory cells emanating from explants, to co-cultured T cells, although this was only effective when the tissue was exposed to the compound for the duration of the experiment. Interestingly, some inhibition was also observed when the entry inhibitor was used to pre-treat the tissue for 2 hours and then removed by washing prior to viral exposure. Here the drug was still active against direct infection, with an IC<sub>50</sub> of 120nM, suggesting drug may bind or be sequestered within tissue. No toxicity was observed at the highest concentrations tested.

**CONCLUSIONS:** DS003 demonstrates good anti-viral activity in cellular and human genital tissue explant models, and shows no toxicity at the highest concentrations tested, making it a good candidate microbicide.

**174****Degradation of Naturally Occurring and Engineered Antimicrobial Peptides by Proteases**B.J. Moncla<sup>\*1,2</sup>, L.C. Rohan<sup>1,2</sup>, K. Pryke<sup>2</sup>, P.W. Graebing<sup>2</sup><sup>1</sup>University of Pittsburgh, Pittsburgh, PA, USA;<sup>2</sup>Magee-Womens Research Institute, Pittsburgh, PA, USA

**BACKGROUND:** Clinical and preclinical trials are expensive and time consuming. It is therefore important to screen potential molecules to ensure they will persist and remain active *in vivo*. Many groups are developing peptide based microbicides using naturally occurring and engineered antimicrobial peptides or proteins (ATM). Peptidases are ubiquitous in the human body and result from natural human metabolism, tissue injury and the microflora in the mouth, vagina and GI track, all sites of potential HIV infection. Although peptidases are many, their mechanisms of action are few; therefore, we selected peptidases to represent the different enzyme classes and determined their effectiveness in degrading different ATMs.

**METHODS:** The peptides studied were: Griffithinsin (GRFT); RC101, a retocycline derivative, LL-37, LSA-5 an engineered cathelicidin, PSC-RANTES and DS007, a modified HIV derived protein. The proteinases studied: Trypsin; Papain; Endoproteinase Lys-C; Leucine aminopeptidase; Elastase (human leukocyte); Proteinase K;  $\alpha$ -Chymotrypsin and Pronase (*Streptococcus grieus*). ATMs, 5 to 1,000  $\mu$ g, were diluted with buffer to 15  $\mu$ l and 15  $\mu$ l enzyme solution was added and mixed for 1 hr at 37C. Samples were withdrawn for HPLC analysis (10  $\mu$ l) and the remainder prepared for evaluation by SDS-PAGE. Peptide degradation was considered positive if the peptide bands disappeared from the gel after proteinase treatment and/or HPLC analysis demonstrated reduced quantities of drug after enzyme treatment.

**RESULTS:** GRFT was only degraded by elastase while LL-37 was degraded by all enzymes tested. PSC-RANTES was degraded by all enzymes except Leucine aminopeptidase. LSA-5 was resistant to 2 of the enzymes tested and the RC101 and DS007 were degraded by one half of the proteases. These results were confirmed by quantitative analysis using HPLC.

**CONCLUSIONS:** There is great abundance and variety of protease types at sites intended for microbicide use. These preliminary results suggest that peptide based microbicides should be thoroughly investigated regarding their susceptibility to degradation by proteases. The GRFT was the most promising material tested; however, degradation by human derived Leukocyte elastase is cause for concern. Additional studies are required to determine the acceptability of such compounds for further development.

**175****Dual-chamber Model for Assessment of Pharmacodynamics and Bioavailability of Candidate Microbicides**P.M. Mesquita<sup>\*</sup>, N.M. Torres<sup>1</sup>, M.S. Kay<sup>2</sup>, R.W. Buckheit Jr.<sup>3</sup>, P.F. Kiser<sup>2</sup>, B.C. Herold<sup>1</sup><sup>1</sup>Albert Einstein College of Medicine, Bronx, NY, USA; <sup>2</sup>University of Utah School of Medicine, Salt Lake City, UT, USA; <sup>3</sup>ImQuest BioSciences

**BACKGROUND:** HIV infects intraepithelial immune cells, traverses a disrupted epithelial barrier and/or is transported by dendritic cells across the epithelium to infect submucosal T cells. Whether or not a microbicide is present at sufficient concentrations at each of these sites will determine its efficacy. Therefore, it is essential to evaluate the ability of candidate microbicides to cross the epithelial barrier, particularly for drugs that act intracellularly.

**METHODS:** We developed a dual-chamber model to evaluate the ability of unformulated and formulated (gel or ring) microbicides to cross the mucosal barrier. Human epithelial cells or tissue are cultured on Transwell inserts and allowed to polarize before being exposed apically to compounds in the absence or presence of human semen. Supernatants are collected from the lower chamber and the concentration of drug (PK) and the ability to inhibit HIV-1<sub>BaL</sub> infection of TZM-bl cells (PD) determined. Controls included Transwell inserts where no cells had been cultured to permit drug to freely diffuse across the pores of the inserts.

**RESULTS:** Table 175.1 summarizes inhibitory activity (IC<sub>50</sub>) of basolateral supernatants 3 days after apical exposure to unformulated microbicides in the absence of semen. In addition to the results with the compounds, we also found that IQP0528 released from a miniature polyurethane ring readily crossed the epithelium, leading to complete inhibition of HIV infection. PK will be correlated with PD values.

TABLE 175.1

DRUG	IC <sub>50</sub> (NO CELLS)	IC <sub>50</sub> (WITH CELLS)	FOLD DIFFERENCE
PRO 2000 (G/ML)	78.6	654	8.32
PMPA (G/ML)	43.2	48.7	1.13
IQP0528 (NM)	50	51	1.01
MARAVIROC (G/ML)	0.14	0.42	3.00
PIE12 TRIMER (NM)	46	68	1.48

**CONCLUSIONS:** This simple inexpensive *in vitro* model provides insights into the ability of drugs to remain within the epithelium and/or traverse the epithelial barrier to reach immune cells within the lamina propria. Both sites may be important for microbicide activity, depending on drug target. Combinations of compounds with different rates of permeation, affording protection to both sites, may be optimal.

# ORAL SESSIONS

## SESSION 20

### Mini-Symposia (MS4): How Good is Good Enough?

**Moderators:** Jeanne Marrazzo, Barbara Mensch

Monday, May 24, 9:30 am–11:00 am

Rooms 301–304

# 176

### Population Effectiveness and Public Health

Marie-Claude Boily

*Department of Infectious Disease Epidemiology,  
Imperial College, UK*

The design and evaluation of an effective microbicide trial to prevent HIV infection is challenging for many reasons. In order to design a trial, one must decide what *magnitude of effect* is of “clinical” relevance to individuals. However, for a product that aims to prevent infectious diseases, it is also important to take into consideration, at the design stage, what *magnitude of effect* would be of public health relevance since a product with an apparently modest efficacy at the individual-level could be useful at the population-level. In this talk, we present results and implications of mathematical modeling studies that look at the potential population-level benefits and risks of microbicides.

# 177

### Balancing Microbicide Efficacy with Community Availability

Samukeliso Dube

*Global Campaign for Microbicides, South Africa*

The efficacy of a microbicide needed for robust public health impact against HIV has been a subject for deliberation for policy makers, researchers and community advocates. The current microbicides pipeline provides a compelling case for potential efficacy given the use of antiretrovirals and different strategies for dosing. Even with this scientific promise, the need to consider impact through the ultimate use and availability in the community remains a challenge. The balance between protection for the individual based on consistency of use and impact on population level use will depend upon effective messaging and access.

Sharing learning from community consultations conducted by the Global Campaign for Microbicides, this presentation will highlight issues raised by potential end-users—women in the settings where microbicide trials are taking place, when asked the question “how good is good enough?” The discussion will be supported by public health debates taking place in the African region including those communities and policy makers who can draw upon their experiences of introducing partially-effective technologies such as male circumcision. The presentation will conclude with a profile of factors that affect efficacy and availability such as demand-creation, provider logistics, end-user acceptability and possible cost implications.

# 178

### How Good is Good Enough—A Regulatory Perspective

Rajen Misra

*Clinical Evaluations & Trials Medicines Regulator Authority, National Department of Health, South Africa*

Because there are no microbicides yet which have been approved by regulatory authorities for prevention of HIV, new drug applications seeking regulatory approval for microbicides will be viewed with caution. When considering the risk and benefit profile, the efficacy profile of the microbicide will have to be balanced against the burden of HIV. With all prevention products to be used by healthy people, safety will be prioritized over efficacy. Consideration will also be given to how well the clinical trial populations represent the populations for the intended use of the products. It is possible that the first microbicide could be given a conditional registration, which would require that additional safety data be collected post registration (Monitored Phase 4). In summary, the safety and efficacy of any new microbicide product will be critical components of any regulatory approval, but additional data derived from post-licensing studies may be needed in order to gain safety data supporting broader use.

## 179

### User-Identified Vaginal Gel Characteristics: A Qualitative Exploration of Perceived Product Efficacy

K. Morrow<sup>\*1</sup>, C. Barroso<sup>5</sup>, D. Katz<sup>3</sup>, P. Kiser<sup>1</sup>, R. Rosen<sup>2</sup>, S. Vargas<sup>4</sup>

<sup>1</sup>Department of Bioengineering, University of Utah, Salt Lake City, UT, USA; <sup>2</sup>Department of Community Health, Brown University and Centers for Behavioral & Preventive Medicine, The Miriam Hospital, Providence RI, USA; <sup>3</sup>Department of Biomedical Engineering and Department of Obstetrics and Gynecology, Duke University, Durham, NC, USA; <sup>4</sup>Centers for Behavioral & Preventive Medicine, The Miriam Hospital, Providence, RI, USA; <sup>5</sup>Centers for Behavioral & Preventive Medicine, The Miriam Hospital, Providence, RI, USA

### SESSION 21

#### Oral Abstracts (OA11): Formulation Science

**Moderators:** David Katz, Lisa Rohan

Monday, May 24, 9:30 am–11:00 am

Rooms 319–321

**BACKGROUND:** To date, microbicides have been developed with little attention to how products feel and whether those perceptions impact use and efficacy. In this study, bioengineers and behavioral scientists explored 1) whether women can discern differences in vaginal products based on their perceptions of gels properties; and 2) how those perceptions impact perceived product efficacy (PPE).

**METHODS:** Qualitative in-depth interviews captured women's experiences manipulating gels in their hands, during insertion and ambulation, and during/after simulated coitus. 16 women used 2 vaginal gels (each with distinct biophysical profiles) (total=32 interviews). Narrative data were conceptually and thematically analyzed for gel descriptions, including how each felt and "behaved" as women manipulated them in their hands or felt them in their vaginas. Further analyses compared sensorial distinctions between the products and effects of particular perceptions on women's opinions of PPE.

**RESULTS:** Qualitative analyses revealed that most participants (pts) were able to perceive differences between the two products (e.g., *"there was definitely a difference between the two"*) and evaluated products based on those characteristics (e.g., *"it seemed to act less like a lubricant than the last one"* and *"I liked last week's better"*). A few pts felt there was little or no difference. Descriptions captured whether or not the gels stayed in place, and pts' awareness of the product and its perceived movement in the vagina (e.g., *"when I stand up it'll probably move but I don't think it's moving right now"*). Emergent data suggest that perceptions such as leakage and the timing of leakage, a characteristic related to rheological properties of yield stress and shear rate-dependent viscosity, were associated with pts' judgements about gel efficacy, some directly linking certain gel behaviors to their ability to prevent HIV (e.g., *"I would feel scared when it came out the next day, like did it work? Was it effective?"*).

**CONCLUSIONS:** Women were able to discern differences between gel properties. Emergent data provide insight into how gel properties may impact PPE, and ultimately, acceptability and adherence. Understanding how candidate products are sensorially perceived and what meanings are ascribed to those perceptions is important to users' PPE. Because women can identify differences between gel properties, rational microbicide design must consider users' perceptions of products' properties.

## 180

### Use of a Weak Buffer Does Not Adversely Impact Osmolality and Rheological Properties of a Vaginal Gel

D. Fairhurst<sup>1</sup>, S. Singh<sup>1</sup>, J.Q. Tao<sup>1</sup>, S. Kepka<sup>2</sup>, K. Chubb<sup>2</sup>, J. Nuttall<sup>\*1</sup>

<sup>1</sup>International Partnership for Microbicides, Silver Spring, MD, USA; <sup>2</sup>Particle Sciences, Bethlehem, PA, USA

**BACKGROUND:** The physico-chemical and mechanical characteristics of a vaginal gel are critical to both effective use and acceptability. Excipients can directly impact the characteristics of the gel, such as rheological behavior (delivery from an applicator and spreadability within the vaginal lumens) and osmolality (an important metric in body fluid maintenance and in the therapeutic action of drugs). In addition, use of a buffer system, to control gel pH, could alter the overall pH of the vagina and adversely affect the homeostasis of vaginal health. We report on a study of these factors using a series of combination microbicide gels containing the CCR5 antagonist maraviroc and the NNRTI dapivirine.

**METHODS:** A simple Brookfield viscometer (Model DV-II) was used to measure the viscosity of HEC-based maraviroc and dapivirine gels. Osmolality was measured using an Advanced Instruments Model 3320 Osmometer that uses the depression of freezing point technique (considered to be the method of choice in pharmaceutical quality control). Buffer capacity was determined from pH titrations based on USP guidelines but using a validated procedure developed specifically for undiluted gels. The gels were formulated to contain between 1 and 10mM citrate buffer and a fixed composition of dapivirine (0.05%) and maraviroc (0.1%).

**RESULTS:** Viscosity values ranged from ca 500Kcps to 900Kcps; the presence of citrate buffer had no effect. Although these values are greater than that for the Universal Placebo (UP; ca 200Kcps), they are within the range for OTC vaginal products. However, unlike some commercial vaginal gels, these combination gel formulations are not hyperosmolar. Osmolalities were all <100 mOsm/kg—less than that for the UP (at 350 mOsm/kg) but similar to that for the 0.5% active Pro2000 microbicide gel and other HEC-based gels containing dapivirine alone. The buffer capacity of the gels containing the highest concentration of citrate buffer was significantly lower than that reported in the literature for vaginal fluid. Thus, use of a weak buffer system is unlikely to be detrimental to gel performance.

**CONCLUSIONS:** The incorporation of a weak (citrate) buffer system in these formulations is advantageous because of the basic nature of maraviroc and the need to maintain a low pH in the vaginal lumen. The results clearly demonstrate that the presence of the buffer does not adversely impact characteristics critical to effective use and acceptability such as viscosity and osmolality.

# ORAL SESSIONS

## 181

### Design of Novel Solid Vaginal Dosage Form of Dapivirine NNRTI and BMS-599793 (DS003) Entry Inhibitor in Combination

S. Garg<sup>\*1</sup>, J. Gupta<sup>1</sup>, A. Othmana<sup>1</sup>, S. Singh<sup>2</sup>

<sup>1</sup>School of Pharmacy, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand; <sup>2</sup>International Partnership for Microbicides (IPM) 8401 Colesville Road, Suite 200, Silver Spring, MD 20910, USA

**BACKGROUND:** Dapivirine and BMS-599793 (DS003) are new investigational drugs being developed as topical microbicides for prevention of HIV infection. The main aim of this study was to develop and evaluate novel rapidly disintegrating bioadhesive vaginal tablets of Dapivirine and BMS-599793 (DS003) in combination.

**METHODS:** The formulation comprised of super disintegrants, bioadhesive polymer, soluble excipients, diluents and lubricants. The almond-shaped tablets were prepared by direct compression at an average weight of 1 gm with a target dose of 1.25 mg of Dapivirine and 2.5 mg of BMS-599793 (DS003). A reversed phase HPLC method was developed for simultaneous determination of the drugs in pure and tablet form. Compatibility of drugs with each other and with excipients was conducted using the developed HPLC method. Stability study of the tablets was conducted at three conditions of 4°C, 30°C/65% RH and 40°C/75% RH for six months. A new *in vitro* release method was designed to study the release behaviour of the drugs from vaginal formulation. The method employed existing USP dissolution apparatus in conjunction with Enhancer cell in small volume flasks (150 ml) and mini paddles.

**RESULTS:** The tablets were successfully prepared with desired disintegration time (< 3 min), optimum moisture content (2.4% m/m), hardness (13.7 Kp) and thickness (5.2 mm). The content uniformity of the powder blend and tablet assay were found to be within the range of 98–101%. Drug-drug and drug-excipient compatibility was confirmed by HPLC method. The formulation was found to be stable for six months at 40°C/75% RH (99.4% of Dapivirine and BMS-599793 (DS003) 98.8%). The designed dissolution system showed a slow and linear release profile consistent with the expected release behaviour of drugs from bioadhesive formulations. Dapivirine and DS003 showed a release of 38% and 26% after 8 hours of study.

**CONCLUSIONS:** This project has led to development of novel rapidly disintegrating bioadhesive vaginal tablets of Dapivirine and BMS-599793 (DS003) for prevention of HIV infection. The tablets exhibited rapid disintegration and sustained release of drugs. After toxicology studies and necessary regulatory approvals, the formulation may be ready for clinical trials.

## 182

### Simultaneous Optical Detection of Intravaginal Gel Coating, Dilution, Drug Distribution, and Tissue Integrity in Women

T. Drake\*, M. Rinehart, K.H. Kim, J. LaCroix, M. Henderson, M. DeSoto, D.F. Katz, A. Wax  
Duke University, Durham, NC, USA

**BACKGROUND:** Microbicide effectiveness is governed by many factors, including extent of gel coverage, transport of active pharmaceutical ingredients (APIs) to target luminal fluids and tissues, and structure and integrity of vaginal epithelium. Current animal models are helpful, but *in vivo* human measurements of gel performance are necessary. Biopsies provide information about local drug delivery and tissue structure. But *in vivo* imaging modalities are preferable, to obtain objective 3D data on API distribution (in luminal fluids and tissue, for any dosage form), gel coating distribution, and tissue integrity.

**METHODS:** We are developing multiple optical imaging methods to be applied intravaginally in women to obtain data on distributions of drug concentration and gel coating, and tissue integrity. Each is implemented using a custom-built fiber optical system; all can be incorporated into the same probe that is inserted into the human vagina and, while held stationary, gives automated 3D scanning of the vaginal luminal surface and fluids. Vaginal gel coating thickness distribution is measured using Low Coherence Interferometry (LCI), a label-free imaging method. Local concentrations of target molecules (in both fluids and tissue) are measured using *in vivo* confocal imaging of API fluorescence. Structure and integrity of the vaginal epithelium are imaged using Optical Coherence Tomography (OCT) linked to automatic imaging processing/analysis.

**RESULTS:** Our LCI gives 7 µm resolution *in vitro* to measure gel coating thickness; it is now being compared with simultaneous measurements of vaginal gel coating by our fluorometric method in a clinical trial. The confocal technique is being tested *in vitro* for measuring 3D drug concentrations in fluids and tissue. For example, it can detect UC781 *in vitro*—in fluids and tissue phantoms. The OCT method obtains histological information co-registered with confocal imaging with higher spatial resolution and scan rate than commercial systems.

**CONCLUSIONS:** The co-registered measurements in women of API distribution, gel distribution/dilution and epithelial integrity will provide valuable data about microbicide product performance. Because LCI is label free, it can obtain gel distribution data at extended times after application. The confocal technique will obtain unprecedented direct *in vivo* data characterizing vaginal drug delivery and applicable to IVRs as well. The OCT data are objective and high resolution and robust to a range of vaginal conditions.

## 183

### Multivariate Computational Model To Help Rate Microbicide Gel Performance: Combining Vaginal Distribution, Drug Delivery and Acceptability

D.F. Katz<sup>\*1</sup>, J. Peters<sup>1</sup>, M. Clark<sup>2</sup>, D. Friend<sup>2</sup>, P. Kiser<sup>3</sup>

<sup>1</sup>Duke University, Durham, NC, USA; <sup>2</sup>CONRAD, Arlington, VA, USA; <sup>3</sup>University of Utah, Salt Lake City, UT, USA

**BACKGROUND:** Successful microbicides require effective APIs delivered by dosage forms that achieve targeted pharmacokinetics (PK). Drug delivery (and therefore PK) by microbicide gels relates to vaginal distribution (epithelial surface area coated, Ac) coupled to drug release rate. If vaginal distribution leads to excess leakage, acceptability can decrease.

**METHODS:** We created an analytical method to interpret vaginal deployment of microbicide gels (computed or measured) using a mathematical expression—the Scoring Function (SF)—which computes a continuous number (0–1) that gives merit to gel performance. Drug delivery increases with extent of epithelial coating, and SF increases from 0 to 1 as Ac increases from its value at insertion to total vaginal surface area. Spreading in excess of this causes gel leakage, and therefore SF decreases to zero as leakage increases, at a rate defined by specifying the maximum volume of leaked gel. We initially applied the SF approach using biomechanics-based computational estimates of vaginal deployment by gels. Inputs were gel rheological properties, applied volume, vaginal geometry and forces. Initial computations did not vary API release rate. Microbicide gels and placebos (at their volumes in trials: 3.5–4.2 mL), and commercial vaginal lubricants and moisturizers (at 3.5 mL) were evaluated.

**RESULTS:** Clinical gels had values in two groups: 1% Tenovor (CAPRISA and prototype gels) (0.96), HEC Placebo (0.82); versus Carraguard (0.67); 2 Cellulose sulfate gels (0.58, 0.62), UC781 prototype clinical gel (0.44). Commercial gels varied: KY Jelly (0.79); Astroglide (0.78); Replens (0.57); RepHresh (0.24). Initial analysis of within-gel variability over different lots defined 95% confidence limits to be ~ SF ± (0.1)SF.

**CONCLUSIONS:** Use of the SF consolidates, in an objective, mechanistic manner, information about many factors that influence microbicide gel drug delivery and acceptability. Low SF values can be due to poor spreading that limits drug delivery, or excess spreading that causes leakage. SF values depend upon a tradeoff between gel properties (especially viscosity vs. yield stress) and volume. We found conspicuous differences across the gels studied. This methodology can compare prototype gels during initial design and also help evaluate performance of existing gels in the microbicide pipeline.

## SESSION 22

### Oral Abstracts (OA12): Preclinical Studies of Ring and Film Formulations

**Moderators:** Dorothy Patton, Joseph Romano

Monday, May 24, 9:30 am–11:00 am

Rooms 403–405

**184**

#### Formulation Development of Matrix-Type Silicone Elastomer Vaginal Rings Containing Protease Inhibitors

S. Fetherston<sup>\*1</sup>, K. Malcolm<sup>1</sup>, D. Woolfson<sup>1</sup>, C. Lacey<sup>2</sup>, R. Shattock<sup>3</sup>

<sup>1</sup>Queen's University Belfast, Belfast, UK; <sup>2</sup>Hull-York Medical School, Hull, UK; <sup>3</sup>St George's Hospital Medical School, London, UK

**BODYBACKGROUND:** Protease inhibitors (PIs) are currently used in the treatment of HIV infection as part of HAART, i.e. in combination with other antiretroviral drugs from different therapeutic classes. A rationale exists for their use as vaginal microbicides, most likely in co-formulations with other microbicides. This study describes the development of sustained release silicone elastomer matrix-type vaginal rings (VRs) containing saquinavir (SAQ), ritonavir (RIT), and lopinavir (LOP), and various combinations.

**METHODS:** Macaque-sized matrix-type silicone elastomer VRs were manufactured by reaction injection molding at 80°C. Single-PI formulations were prepared, containing SAQ, RIT or LOP at loadings of 1, 3, 5 and 10% w/w. Dual (RIT and LOP) and triple (SAQ, RIT and LOP) formulations were also manufactured, containing each PI at a concentration of 3% w/w. *In vitro* PI release from the VRs (n=4 per formulation) was evaluated for 15 days using a one-compartment sink condition model. Compression strength of the VRs and PI melting temperature (in powdered form and within cured silicone elastomer) were also assessed. Mean cumulative mass of PI released, and PI melting temperature, were compared for each formulation using a one-way ANOVA and the Tukey-Kramer multiple comparisons test.

**RESULTS:** Each PI was released *in vitro* according to  $t^{1/2}$  kinetics. A burst release was observed on day 1 followed by steadily declining daily release. Cumulative release increased significantly with drug loading ( $p < 0.001$  in all cases) and release rates were directly proportional to the log P value of the PI. LOP was released at the highest rate, followed by RIT and then SAQ (representative release rates: 2.65, 1.36 and 0.93 mg/day $^{1/2}$  from 10% w/w LOP, RIT and SAQ VRs respectively) ( $p < 0.001$  in all cases when formulations of equivalent loadings compared). Ring compression strength increased with PI loading. The presence of multiple PIs in the formulations had no significant effect on individual melting temperatures within silicone elastomer ( $p > 0.05$ ).

**CONCLUSIONS:** Protease inhibitors were successfully formulated in silicone elastomer vaginal rings, and should be considered in future work investigating controlled vaginal delivery of HIV microbicides, most likely in combination products containing drugs from other therapeutic classes.

**185**

#### Combination Ethylene Vinyl Acetate Intravaginal Vaginal Rings Containing Dapivirine and Maraviroc

A. Loxley<sup>\*1</sup>, A. Gokhale<sup>1</sup>, J. McConnell<sup>1</sup>, O. Okoh<sup>1</sup>, K.L. Edwards<sup>2</sup>, M. Mitchnick<sup>1</sup>

<sup>1</sup>Particle Sciences Inc, Bethlehem, PA, USA;

<sup>2</sup>International Partnership for Microbicides, Silver Spring, MD, USA

**BACKGROUND:** Microbicides have historically been incorporated into daily use vaginal gels. We report here the development of combination intravaginal rings (IVRs), based on ethylene-vinyl acetate copolymer (EVA), containing the microbicides Dapivirine (Tibotec, 25 mg) and Maraviroc (Pfizer, 200 mg or 300 mg). These IVRs are designed for 30-day use and are considered a viable non-coitally dependent dosage form for the prolonged release of Dapivirine and Maraviroc.

**METHODS:** IVRs were prepared by hot-melt batch mixing or hot-melt extrusion, followed by injection molding. Durometer hardness, and flexibility were measured and IVRs were assayed by HPLC. To determine API-release kinetics, IVRs were incubated in 100 mL isopropanol-water mixture at 37°C. The medium was replaced periodically over 30 days to maintain sink conditions, and aliquots were analyzed by HPLC. The stability of compounded API/EVA blend, and finished IVRs, was tested for up to six months at 40°C.

**RESULTS:** IVRs have similar properties to currently marketed contraceptive IVRs (4 mm cross-sectional diameter and 54 mm overall diameter, shore hardness 20D–25D, and 0.74–1.07 N bending force). API assays show ~100% recovery of both APIs, with minimal related substances formed during processing. Dapivirine release follows approximately first-order kinetics, whereas after an initial peak release, Maraviroc follows close to zero-order release kinetics. The *in vitro* release of each API is loading-dependent (linearly in the case of Maraviroc), independent of the loading of the other API, and relatively insensitive to EVA type. The day 15 *in vitro* release of APIs from optimally formulated IVRs is on the order 500 µg/d (Dapivirine) and > 1000 µg/d (Maraviroc). IVRs and EVA/API compound was stable at 40°C for at least three and six months respectively. IVRs made with pilot-scale equipment showed equivalent properties and performance to lab-scale IVRs.

**CONCLUSIONS:** A viable, non-coitally dependent Dapivirine/Maraviroc combination microbicide device has been developed as an alternative to daily use vaginal gels, and the IVRs have similar physical properties to marketed contraceptive devices, assuring good tolerability. The production processes are facile and scalable, and can be performed at any suitably equipped location. The *in vitro* API release from the IVRs is considered sufficiently high over a 30-day period to be practical, and clinical trials for these devices are planned for 2010.

# ORAL SESSIONS

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### **In Vitro and In Vivo Release of Tenofovir from Silicone Intravaginal Rings**

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**BACKGROUND:** We seek to empower women to protect themselves from HIV infection through development of a sustained-release microbicide suitable for the developing world. We report the *in vitro* and *in vivo* investigation of an intravaginal ring (IVR) microbicide formulation for the reverse transcriptase inhibitor tenofovir (TFV). This IVR platform is based on technology developed for the intraocular implant which was FDA approved for the treatment of AIDS-related CMV retinitis and releases ganciclovir into the eye for 8 months.

**METHODS:** The technology platform is based on a polymer-coated solid core of TFV incorporated into a silicone ring and exhibiting pseudo-zero order release kinetics. Drug cores (4 mg) were coated with layers of semi-permeable polylactic acid polymer. Coated drug pods were incorporated during an injection molding process into 25 mm diameter silicone rings for macaque studies or 2 cm ring segments for rabbit studies. Drug is released through a delivery window in the silicone ring, with the release rate determined by the window diameter. For these studies, rabbit segments (1 TFV pod) and macaque rings (4 pods) were designed to release 40 and 160 µg/day, respectively. Implants were inserted into rabbits (n=6) for 14 days and macaques (n=4) for 28 days. Plasma, vaginal lavage and tissue drug levels were obtained. All data are reported as the average ± SD.

**RESULTS:** *In vitro* experiments verified that the rabbit implants released TFV at an average of  $43 \pm 9$  µg/day for 30 days (n=10) and macaque implants released TFV at  $118 \pm 27$  µg/day (n=4) for 30 days. *In vivo* lavage levels for rabbits averaged  $1190 \pm 1480$  ng/ml with plasma levels of  $2.7 \pm 6.6$  ng/ml over the 14 days. Vaginal tissue levels at sacrifice were  $3597 \pm 1678$  ng/g. *In vivo* lavage levels for macaques averaged  $3319 \pm 3021$  ng/ml (daily average range 2708-5263 ng/ml) with plasma levels BLQ over the 28 days. These levels are 5.8 times the IC50 of tenofovir (2 µM). No localized inflammation or altered vaginal microflora was observed.

**CONCLUSIONS:** We have demonstrated sustained pseudo-zero order release in vaginal lavage of tenofovir for 14 days in rabbits and 28 days in macaques. This demonstrates feasibility of a one-month tenofovir microbicide ring for women. To our knowledge this is the first time that one-month *in vivo* data has been obtained for release of tenofovir from vaginal rings.

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### **In Vivo Safety Study in *Macaca fascicularis* on Matrix-Type Silicone Elastomer Vaginal Rings Loaded With Saquinavir**

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**BACKGROUND:** Saquinavir (SAQ) is a protease inhibitor currently used as part of HAART regimens for the management of HIV infection. It is therefore reasonable to investigate the use of SAQ as an HIV microbicide, preferably in combination with other microbicides having alternate mechanisms of action. Sustained release of combination microbicides from vaginal rings (VRs) could provide women with a female-controlled, coitally independent means of protection from heterosexual transmission of HIV. In this study, we report the *in vivo* safety testing of a matrix-type silicone elastomer VR formulation containing SAQ in cynomolgus macaques (*Macaca fascicularis*).

**METHODS:** Macaque-sized matrix-type silicone elastomer VRs loaded with 0, 1, 5 and 10% w/w SAQ were manufactured by reaction injection molding at 80°C. VRs were inserted into the vaginas of adult female *Macaca fascicularis* for 14 days (n=2) that had been treated with Depo-Provera 20–35 days prior to ring placement. Vaginal fluid and serum were sampled at regular intervals and quantified by ELISA. Parallel *in vitro* release studies on similar rings were also conducted using a one-compartment sink condition model. All rings were evaluated for residual SAQ content at the end of the study to aid *in vitro*-*in vivo* comparison of SAQ release.

**RESULTS:** Results from the *in vivo* safety study indicated that ring formulations were safe. SAQ was detected in the vaginal fluid of macaques (peak ~2000ng/mL), although the levels were highly variable and showed no distinct trend with SAQ ring loading. For all formulations, SAQ was not detected in serum. SAQ was released *in vitro* according to  $t^{1/2}$  kinetics with a burst release on day 1. The mean *in vitro* cumulative mass of SAQ released over 14 days was 0.92, 2.38 and 3.57 mg from rings loaded with 1, 5 and 10% w/w SAQ, respectively. Quantification and comparison of residual SAQ content in rings post-release indicated poor *in vivo* release compared to *in vitro* data. The poor correlation is likely attributed to the Depo-Provera treatment and/or the unsuitability of current *in vitro* release models.

**CONCLUSIONS:** SAQ-loaded matrix-type silicone elastomer VRs are safe *in vivo*. However, compared with *in vivo* data, SAQ did not release appreciably *in vivo*. The results may indicate that alternative *in vitro* release models need to be developed, or that Depo-Provera treatment is adversely affecting release, as demonstrated in more recent studies with CMPD167.

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### **Dapivirine Vaginal Ring Design and Drug Load Effects on *In Vitro* Drug Release and Intravaginal Drug Distribution in Women**

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**BACKGROUND:** Vaginal rings can be fabricated from multiple polymer types and in different configurations. This versatility allows for targeted ring development for different microbicide candidate drugs. This report summarizes *in vitro* drug release and vaginal distribution kinetics in women achieved with different configurations and drug loads of dapivirine silicone rings.

**METHODS:** Silicone vaginal rings were produced in either matrix or reservoir configurations, using Sn-catalyzed chemistry. Drug loads ranged from 25–200 mg/ring. *In vitro* drug release was measured for up to 28 days duration using HPLC analysis of samples collected in a 50:50 isopropanol:water mix. Vaginal fluid samples were collected in independent studies from multiple locations in the vagina using absorbent strips and were analyzed with tandem MS-LC technology.

**RESULTS:** Minimal to no difference in drug release was seen *in vitro* with reservoir rings independent of dapivirine drug load. Concentration trends in vaginal fluids obtained with 25 or 200 mg of dapivirine reservoir rings were similar despite the drug load difference. Matrix configuration rings with 25 mg of dapivirine released significantly more drug *in vitro* than either 25 or 200 mg dapivirine reservoir rings. Although basic trends in vaginal distribution of dapivirine were similar with matrix and reservoir rings (ring area>cervix>introitus), absolute drug levels were significantly higher with matrix rings. Drug dissolution kinetics in vaginal fluids after ring removal were similar for different ring types, although again levels were higher with matrix rings. Day one burst levels *in vitro* and *in vivo* were higher with matrix rings than with reservoir rings. All ring configurations and drug loads were safe and well tolerated in these trials up through 28 days of use.

**CONCLUSIONS:** Multiple vaginal ring configurations and chemistries are available for the development of sustained release dosage forms for microbicides. Although individual drugs will likely perform differently *in vivo* and *in vitro*, results presented here indicate *in vitro* results can inform *in vivo* studies, and multiple configurations of silicone rings can effectively deliver dapivirine at different levels in women.

# ORAL SESSIONS

SESSION 22/23  
Monday, May 24, 2010

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## Vaginal Film Drug Delivery of the Pyrimidinedione IQP-0528 for the Prevention of HIV Infection

A.S. Ham<sup>\*1</sup>, L.C. Rohan<sup>2</sup>, A.D. Boczar<sup>1</sup>,  
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**PURPOSE:** The pyrimidinedione (PYD) IQP-0528 is a highly potent non-toxic, dual-acting HIV nonnucleoside reverse transcriptase inhibitor that targets both virus entry and reverse transcription. Preformulation studies have shown the molecule to be highly stable and biological evaluations have demonstrated that the compound possesses a high genetic barrier to resistance selection. The poor solubility of IQP-0528 presents formulation issues in traditional microbicide drug delivery systems. Therefore, IQP-0528 is being investigated and developed into a solid vaginal film dosage formulation as a topical microbicide.

**METHODS:** *In vitro* evaluations of antiviral activity, mechanism of action, and toxicity against primary and established cells and normal vaginal flora were performed in vaginal and seminal fluid simulants to evaluate the efficacy and toxicity of IQP-0528. IQP-0528 was formulated into polyvinyl alcohol polymer vaginal films via solvent cast manufacturing. Film *in vitro* characterizations evaluating drug content uniformity, film disintegration, drug release, and product stability were conducted.

**RESULTS:** In standard *in vitro* assays, IQP-0528 was active against all clinical strains of virus studied in the nanomolar to sub-nanomolar concentration range with therapeutic indices greater than one million. IQP-0528 film formulations resulted in no toxicity to CEM-SS and HeLa-CD4-LTR βgal cells or to human PMBCs. Films containing 1.25% IQP-0528 resulted in uniform distribution of IQP-0528 with a %RSD of 6.24 when the film was subdivided into four sections. The films visually disintegrated within 10 minutes with a release of 75% of IQP-0528 and with 90% release after 25 minutes. When formulated into a film and packaged into air tight pouches, IQP-0528 demonstrated no significant degradation at regular (35°C / 65%RH) and accelerated stability conditions (40°C / 75%RH) for 7 days.

**CONCLUSIONS:** IQP-0528 is a novel drug candidate for development as a vaginal topical microbicide based on its dual mechanism of action, high level of potency, and lack of toxicity. We have demonstrated that IQP-0528 is capable of being formulated into a non-toxic solid film dosage with near complete drug release. Such novel formulations may be necessary for the success of pyrimidinediones as a topical microbicide product. This work was supported through NIH MIP I Grant 8R33A1076967-03 and ImQuest BioSciences.

## SESSION 23

### Oral Abstracts (OA13): Vaginal Practices and Barriers

**Moderators:** Janneke van de Wijgert, Cynthia Woodsong

Monday, May 24, 11:30 am–1:00 pm

Ballroom

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## Intravaginal Practices Among Female Sex Workers in Kibera, Kenya

M.F. Gallo<sup>\*1</sup>, A. Sharma<sup>2,3,4</sup>, E.A. Bukusi<sup>2,3,5</sup>,  
B. Njoroge<sup>2,5</sup>, R. Ngut<sup>5</sup>, D.J. Jamieson<sup>1</sup>,  
A.J. Bell<sup>1</sup>, D.A. Eschenbach<sup>3</sup>

<sup>1</sup>Centers for Disease Control and Prevention, Atlanta, GA, USA; <sup>2</sup>Kenya Medical Research Institute, Nairobi, Kenya; <sup>3</sup>University of Washington, Seattle, WA, USA; <sup>4</sup>Independent consultant, Liverpool, England; <sup>5</sup>University of Nairobi, Nairobi, Kenya (affiliations as of time of research)

**BACKGROUND:** Intravaginal practices (e.g., vaginal cleansing and lubrication) could affect the development and evaluation of female-initiated methods of HIV/STI prevention. For example, the evaluation of a candidate microbicide to prevent the acquisition of HIV could be compromised if women prematurely remove or dilute the product by cleansing internally either before or after coitus. Further, an eventual effective microbicide could be limited if women outside of the research setting continue to cleanse vaginally.

**METHODS:** We assessed predictors of intravaginal practices among 140 female sex workers (FSWs) in the Kibera settlement in Nairobi who participated in a 6-month, prospective study of the acceptability and safety of the diaphragm. Women were instructed to wear the diaphragm for all coital acts during follow-up and to refrain from vaginal cleansing while wearing the diaphragm. We used logistic regression to identify predictors of recent vaginal cleansing to "tighten" the vagina reported at baseline; recent vaginal cleansing to prevent infection reported at baseline; recent vaginal cleansing with the diaphragm in place reported during follow-up; and recent use of oil-based lubricant during coitus reported at baseline.

**RESULTS:** Participants completed 140 baseline visits and 390 bi-monthly follow-up visits. At baseline, 99% of women reported vaginal cleansing in the prior 2 weeks for purposes of hygiene or to remove evidence of past coitus. About 41% of women also reported cleansing in the past 2 weeks to "tighten" the vagina. Women reported vaginal cleansing with the diaphragm in place in the past 2 weeks at 14% of follow-up visits in which the diaphragm was used. Predictors of such cleansing included young age, 6-month study visit, being divorced or widowed, and higher educational level. At baseline, almost half of the women (48%) reported the use of a lubricant during recent coitus. The most common lubricant was petroleum jelly (n=60) followed by saliva, oil, water and water-based commercial lubricant. High coital frequency was the only predictor of recent use of oil-based lubricant during coitus.

**CONCLUSIONS:** While vaginal cleansing is a modifiable behavior, given that cleansing for hygiene was almost universal among this study population at baseline and that more women reported cleansing while wearing the diaphragm as the study progressed, the complete eradication of the practice likely would be difficult.

# ORAL SESSIONS

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### The Duet Cervical Barrier Used Continuously or Pre-coitally for HIV Prevention in Zimbabwean Women: An Acceptability and Safety Study

E.T. Montgomery<sup>\*1,2</sup>, C. Woodsong<sup>3</sup>, P. Musara<sup>4</sup>, H. Cheng<sup>1</sup>, T. Chipato<sup>4</sup>, T.R. Moench<sup>5</sup>, A. van der Straten<sup>1,6</sup>

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**BACKGROUND:** Adherence problems with coitally dependent female-initiated HIV prevention methods have contributed to several trials' failure to establish efficacy. Continuous use of a cervical barrier with once-daily cleaning may improve adherence. We assessed the acceptability and safety of pre-coital and continuous use of the Duet, a cervical barrier and gel-delivery system, in Zimbabwean women.

**METHODS:** Using a two-arm cross-over design with a parallel observation group, we randomized 103 women in a 2:2:1 ratio to: 1) 14-day continuous Duet use, followed by 2-week wash-out and 14 day pre-coital use; 2) the reverse Duet regimen; or 3) observation only. Women were 18–40; and recruited evenly from a previous diaphragm study and the general community. Acceptability and adherence were assessed by face-to-face interview, safety was monitored by speculum exam and AE reports.

**RESULTS:** The proportion of women consistently using Duet during sex was identical during both Duet regimens (89%). Most were "very comfortable" using it continuously (86%) and pre-coitally (93%). There were no SAEs, and 90 mild or moderate AEs among 57 women. There were no significant differences in: 1) the proportion of women reporting AEs by group; 2) event severity among Duet users vs. controls; nor 3) event severity during each Duet regimen.

**CONCLUSIONS:** This study found the Duet to be acceptable and safe when used pre-coitally or continuously for 14 days. Continuous use did not increase adherence to Duet during sex. Future HIV prevention trials should evaluate use of the Duet with promising microbicide candidates.

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### Vaginal Practices, Gel and Barrier Methods Use Among HIV Prevention Trial Participants in Southern Africa

A. van der Straten<sup>\*1,2</sup>, H. Cheng<sup>1</sup>, A. Chidanyika<sup>3</sup>, G. De Bruyn<sup>4</sup>, N. Padian<sup>3</sup>, and the MIRA Team

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**BACKGROUND:** In Southern Africa, vaginal practices (VP) are common. We investigated if they are associated or interfered with the use of female-initiated methods being evaluated for HIV prevention.

**METHODS:** Southern-African women aged 18–49, were recruited into a phase III trial of the diaphragm (MIRA), randomized to the intervention (diaphragm; vaginal gel and condoms) or the control group (condoms only), and followed for a median of 21 months. VP and study methods use were assessed by Audio-Computer Assisted Self-Interviewing at baseline and quarterly thereafter. We used generalized estimating equation multivariable logistic regression to assess whether VP differed by randomization group, and if they were associated with "always" use (since last visit) of study methods.

**RESULTS:** In our baseline sample (N= 4925), VP were commonly reported over the previous 3 months: vaginal washing (82.8%), wiping (56.5%) and insertion of dry or absorbent materials (20.6%). VP decreased to 74.3%, 35.9%, and 11.5%, respectively, by month-24 visit, but less so in the intervention compared to the control group. During follow-up, women in the intervention were significantly more likely to report washing (AOR 1.35; 95% CI 1.22-1.50) and wiping (AOR 1.14; 95% CI 1.05-1.24) compared to those in the control group. Furthermore, washing (AOR 0.88; 95%CI 0.79-0.98), wiping (AOR 0.90; 95%CI 0.83-0.97), and insertion (AOR 0.83; 95%CI 0.74-0.93), were all independently and inversely associated with "always" use of the diaphragm and gel in the intervention group. These VP were also inversely associated with "always" use of condoms in both groups.

**CONCLUSION:** VP remained higher in the intervention group, who received diaphragm and gel. VP were inversely associated with consistent methods use and thus may have limited their effectiveness. Improved educational strategies to address these modifiable behaviors may benefit future HIV prevention interventions of vaginal methods.

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### How Many Women Really Achieve Consistent Condom Use Over the Course of a Year? Evidence from Rural KwaZulu-Natal

M. Gafos\*, H. Ndlovu, M. Mzimela and the MDP team

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**BACKGROUND:** The Africa Centre for Health and Population Studies was one of six sites participating in the Microbicides Development Programme MDP301 clinical trial. Local evidence suggests that nearly half of women aged 15 to 25 report using condoms, but there is no evidence estimating consistent condom use longitudinally. In this study we use longitudinal data on condom use to approximate the proportion of HIV negative women enrolled in MDP301 who sustained consistent condom use over a year.

**METHODS:** Data were collected on condom use at each sex act in the last week at clinic visits 4, 24, 40 and 52 weeks after enrolment. Consistent condom use was defined as a condom being used at every reported sex act in the week. At other 4-weekly visits data on condom use at last sex were collected. Participants underwent testing for pregnancy, HIV and STIs. We calculated the proportion of women who reported consistent condom use in the previous week. Of these, we identified women who reported not using a condom at last sex at any other visit. Finally among consistent condom users, we identified any women who had a biomarker (HIV, pregnancy or STI) during the course of the trial which suggested that condoms may not have been used consistently.

**RESULTS:** Of 1177 women enrolled, 850 women provided detailed sexual behaviour data at weeks 4, 24, 40 and 52. The proportion who reported consistently using condoms in the last week varied from 52% at week 4 to 66% at week 52 with a linear increase over time. However only 342 (40%) reported consistent condom use at all 4 time points. Of these 59 had reported not using a condom at their last sex at some stage after week 4, leaving 283 consistent users. Of these who consistently reported using condoms, 70 had a laboratory biomarker event indicating unprotected sex. Consequently we estimate that a maximum of 213 women could be considered consistent condom users, equivalent to 25% (CI: 22-28%) of the cohort, and less than 50% of those reporting consistent condom use at a single time point.

**CONCLUSIONS:** Cross sectional condom use data probably overestimates consistent condom use by at least 100%. In this cohort, less than a quarter of women achieved consistent condom use during the year. It is important to note that the lack of a biomarker event does not prove consistent condom use, and this primary analysis does not account for sustained changes in behaviour during the course of the trial.

## SESSION 24

### Oral Abstracts (OA14): Looking for Answers: Trial Design, Outcomes, and Mathematical Modeling

**Moderators:** Benoît Mâsse, Regina Osih

Monday, May 24, 11:30 am–1:00 pm

Rooms 301–304

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#### Adaptive Design Considerations in Planning the CAPRISA 004 Study

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**BACKGROUND:** One microbicide trial with the strength of evidence of two independent trials, each significant at the 0.025 level, may suffice for registration in the USA. Undertaking a large study risks wasting resources if there are scant data to inform a plausible effectiveness level. Conducting an initial phase 2b screening trial has been proposed to address this concern (Fleming and Richardson, 2004). Alternatively, an adaptive design where study size is allowed to increase if an interim estimate of effectiveness achieves pre-specified criteria could improve the likelihood of obtaining evidence for licensure from a single trial.

**METHODS:** We compared two trial designs under various assumptions about product effectiveness and feasible study size when planning CAPRISA 004. The first was a fixed 68 endpoint trial with alpha=0.025 and 80% power to conclude a non-zero reduction in HIV risk, and pre-planned interim analyses (IA) after 22 and 44 endpoints. The alternative approach was an adaptive design in which the estimate of effectiveness at the second IA determines whether the trial should be expanded to 88 endpoints, improving the chance of concluding risk is reduced by 25% (equivalent to evidence of a non-zero effect from two trials).

**RESULTS:** Assuming a 60% effective product, conditional power to obtain licensure levels of evidence in an 88 endpoint trial is < 55% if the observed interim effectiveness estimate is < 40%. Conditional power in a 68 endpoint trial is > 95% if the interim estimate is > 70%. This informed our hypothetical adaptive trial design: if interim effectiveness estimate is between 40% and 70%, then the trial is expanded to 88 endpoints; otherwise, it stops at 68.

Based on simulations, power to obtain licensure-levels of evidence using the adaptive design is approximately 75%, compared to 80% for an 88 endpoint trial and 60% for a 68 endpoint trial. However, there is a 65% chance that the adaptive trial will need to be expanded to 88 events, suggesting that committing to the larger trial from the beginning is the more sensible strategy. For the adaptive study Type I error was reasonably controlled.

**CONCLUSION:** Adaptive trials may improve the chance of a positive outcome in a clinical trial. We found the utility of one such design for the CAPRISA004 study to be limited, primarily due to serious constraints on maximum study size. Whether or not the approach has merit in settings where there is greater flexibility in setting maximum study size are also explored.

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#### Appropriateness of Hydroxyethylcellulose Gel as a Placebo Control in Vaginal Microbicide Trials: A Comparison of the Two Control Arms of HPTN 035

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**BACKGROUND:** The hydroxyethylcellulose (HEC) gel, formulated to minimize impact on vaginal mucosa, acquisition of HIV-1 and other sexually transmitted infections (STIs), and pregnancy, has been proposed as a "universal placebo" for vaginal microbicide trials. HPTN 035, a double-blinded randomized controlled trial (RCT) was designed with a HEC gel control arm and a no gel control arm, to address both the potential behavioral effects associated with gel use, and the potential direct causal effects of the HEC gel with respect to multiple outcome measures including acquisition of HIV-1 and other STIs, pregnancy, and genital safety.

**METHODS:** HIV-uninfected sexually active women in four African countries and the U.S. were randomized between 4 arms: between two active microbicide gel arms (n=775 and n=769) and the HEC gel arm (n=771) in a blinded manner, and to an unblinded no gel arm (n=772). All groups of women received safer sex counseling, condoms, and STI management/treatment. Participants in the blinded gel arms were instructed to insert the study gel intravaginally <1 hour before each vaginal sex act. Data on sexual behavior, adherence, genital safety, pregnancy, and STIs were collected at least quarterly for 12 to 30 months of follow-up. Pearson chi-square tests and Cox proportional hazards models were used in the analysis.

**RESULTS:** When comparing the HEC gel and no gel control arms, baseline characteristics were similar. During follow-up, the mean reported percentage of vaginal sex acts with a condom in the past week was significantly lower in the HEC gel arm (70% versus 81%, p<0.001). The (HEC gel versus no gel) hazard ratio for the rate of HIV-1 acquisition was 0.97 (95% Confidence Interval: (0.66, 1.44)), and was similar after adjustment for differential condom use post baseline. In addition, the two arms had similar rates of genital safety events, pregnancy outcomes, and incidence of other STIs.

**CONCLUSIONS:** In HPTN 035, the blinded HEC gel control arm and the unblinded no gel control arm had similar rates of genital safety events, pregnancy outcomes, and acquisition of HIV-1 and other STIs, suggesting that the HEC gel is suitable as a control for ongoing and future vaginal microbicide studies.

# ORAL SESSIONS

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### Discrepancies Between the Outcomes of Two Recent HIV Microbicide Trials Evaluating the Effectiveness of 0.5% PRO 2000

B.R. Mässe<sup>\*1</sup>, D. Dimitrov<sup>1</sup>, M.C. Boily<sup>2</sup><sup>1</sup>Fred Hutchinson Cancer Research Center, Seattle, WA, USA; <sup>2</sup>Imperial College, London, UK

**BACKGROUND:** The HPTN 035 and MDP 301 trials were conducted to evaluate the effectiveness of 0.5% PRO 2000 to prevent HIV. At first glance, the effectiveness of the 2 trials appears discrepant as the HPTN 035 and MDP 301 observed an effectiveness of 30% (95% CI [-8%; 54%],  $p=0.10$ ) and 0% (95% CI [-26%; 21%],  $p=0.99$ ), resp. Although both results are not significant, the estimates are substantially different. A simple explanation for this difference can be attributed to random variability since the two 95% CIs overlap substantially. However, the magnitude of different sources of efficacy dilution (e.g. adherence, unprotected anal intercourse [UAI]) may have differed between settings and trial conditions.

**METHODS:** The impact of differential trial conditions, observed between the HPTN 035 and MDP 301, on the magnitude of efficacy dilution was explored using a previously published efficacy dilution model and standard large-sample approximations for the distribution of hazard ratios. First, the likelihood of observing differences in effectiveness as large as the one reported between the 2 trials, in absence of differential dilution effects was evaluated assuming different magnitude of true effectiveness. Second, the impact of dilution on the effectiveness was investigated with the dilution model under different trial conditions in absence of random variability associated with the size of each trial.

**RESULTS:** In absence of differential dilution effects, the likelihood that 2 trials similar to HPTN 035 and MDP 301 will observe a difference in effectiveness of  $\geq 30\%$  is about 23% and 16% assuming a true efficacy of 0% and 15%, resp. As both trials have used the same placebo and observed similar adherence and pregnancy rates, UAI is one major source of potential differential efficacy dilution. Assuming a true efficacy of 40% and similar dilution effects from adherence, pregnancy, and placebo between trials, the expected effectiveness decreases from 23% to 18% if the proportion of HIV infections from UAI in one of the trial is substantially larger (a 2-fold increase: 20% vs 40%).

**CONCLUSIONS:** Observed differences between trials can be attributed to random variability and differential trial conditions. For the former, the likelihood of observing differences as the one observed between HPTN 035 and MDP 301 is relatively high. However, it is unlikely that this difference can be attributed solely to differential dilution effects although it could have been a contributing factor.

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### Should Oral PrEP and Microbicide Randomised Trials be Powered to Rule Out Products with Efficacy of 0% or greater?

D. Dimitrov<sup>\*1</sup>, B.R. Mässe<sup>1</sup>, M.C. Boily<sup>2</sup><sup>1</sup>Fred Hutchinson Cancer Research Center, Seattle, WA, USA; <sup>2</sup>Imperial College, London, UK

**BACKGROUND:** Several phase IIb/III vaginal microbicides (VMB) and oral pre-exposure prophylaxis (PrEP) trials to prevent HIV transmission are currently ongoing or planned. Most of these individual-based randomized controlled trials (I-RCT) have been powered to rule out products with minimum individual-level efficacy (MIE) varying between 0%–30% (i.e. lower bound of the 95% CI). However, the MIE is often chosen arbitrarily despite having a substantial effect on the sample size. Ideally, the MIE should be chosen to reduce uncertainties in the estimation of population-level effects to help identifying interventions of public-health relevance. We are presenting the effect of MIE on estimates of population-level effects.

**METHODS:** We developed deterministic models of HIV transmission to quantify the population-level impact of a wide-scale usage of VMB and PrEP under different efficacy and behavioral/biological characteristics. The theoretical efficacy is sampled from log-normal distributions for each of 9 hypothetical I-RCTs with different design and size. For each trial, the influence of different factors (coverage, adherence, rates of resistance development, etc.) on population-level benefits (e.g. infections prevented and HIV-incidence) and risks (resistance prevalence) is studied through simulations, using 10,000 parameter combinations sampled from ranges representative of the developing countries.

**RESULTS:** The expected population level benefits and risks over 20 years for different PrEP and VMB trials assuming 70–80% coverage and 80% adherence are summarized below:

TABLE 197.1

	75L	75M	75S	50L	50M	50S	33L	33M	33S
<b>DESIGN-NULL MIE</b>	20%	10%	0%	20%	10%	0%	20%	10%	0%
<b>HIV ENDPOINTS (ACTIVE/PLACEBO)</b>	31/124	20/80	14/56	51/102	33/66	22/44	62/93	40/60	28/42
<b>TRIAL SIZE</b>	7000	4500	3100	5700	3700	2400	5100	3300	2200
<b>OBSERVED EFFICACY [95% CI]</b>	75% [63,83]	75% [59,85]	75% [55,86]	50% [30,64]	50% [24,67]	50% [16.5,70]	33% [8,52]	33% [0.5,55]	33% [-7,59]
<b>SIMULATION RESULTS OF POULATION EFFECTS (50<sup>TH</sup> [5<sup>TH</sup>,95<sup>TH</sup>] PERCENTILES)</b>									
<b>INFECTIONS PREVENTED (PrEP)</b>	71.3% [63,78]	71.0% [62,83]	71.0% [60.5,78.5]	49.9% [36,61]	49.9% [35.5,63]	49.9% [30,63.5]	32.0% [14.5,47]	31.9% [10.5,50]	32.0% [6,51]
<b>RESISTANCE PREVALENCE (PrEP)</b>	0.30% [0,0.7]	0.31% [0,0.7]	0.31% [0,0.7]	0.41% [0,0.9]	0.41% [0,0.9]	0.41% [0,0.9]	0.45% [0,1]	0.46% [0,1]	0.46% [0,1]
<b>INFECTIONS PREVENTED (VMB)</b>	38.5 [30.5,47]	38.6% [29.5,47]	38.4% [28.5,48]	24.5% [15,34]	24.4% [13.5,35]	24.3% [10.5,36]	14.9% [4.1,25]	14.7% [1.4,26]	14.8% [-1.5,28]
<b>RESISTANCE PREVELENCE (VMB)</b>	1.0% [0.7,1.4]	1.0% [0.7,1.4]	1.0% [0.7,1.4]	1.1% [0.8,1.6]	1.1% [0.8,1.6]	1.1% [0.8,1.6]	1.2% [0.8,1.7]	1.2% [0.8,1.7]	1.2% [0.8,1.7]

**CONCLUSIONS:** Trials with large MIE require substantially more resources than those with small MIE. While trials with larger MIE reduce uncertainties on estimates of population effects, the enhanced precision is not substantial, especially for better products. Additional resources needed for increasing the MIE of trials should be used instead for estimating other intervention and/or local parameters which strongly influence the population-level benefits and risks.

**198****Are Vaginal Microbicides Cost-effective?**S. Verguet<sup>\*1</sup>, J.A. Walsh<sup>2</sup><sup>1</sup>Institute for Health Metrics and Evaluation, 2301 5th Avenue, Suite 600, Seattle, WA, USA, 98121; <sup>2</sup>School of Public Health, University of California at Berkeley, Berkeley, CA 94720, USA

**BACKGROUND:** Little research addresses epidemiologic and economic impacts once a safe and efficacious microbicide becomes available. We assess here the cost-effectiveness of a prospective HIV prevention intervention including an anti-HIV vaginal microbicide.

**METHODS:** We use mathematical modeling and data from South Africa and the United States to look at a hypothetical microbicide intervention that lasts 1 year. The intervention uses existing infrastructures for condom interventions and targets women in a 1,000,000-inhabitant city. We explore two distinct settings: South Africa, a developing country severely touched by the HIV epidemic, and the United States, a developed country with a low level HIV epidemic. The base case scenario retains a 55% efficacy, 30% adherence and a price per use of \$0.51 and \$2.23 for the South African and American public sectors respectively. Supplemental work studies the same intervention within the setting of Washington, D.C. where the AIDS diagnoses rate is the highest in the United States. We estimate the uncertainty from the model inputs through multivariate and univariate sensitivity analyses.

**RESULTS:** In South Africa, over 1 year, the intervention would cost less than antiretroviral therapy and be cost-effective at \$65/DALY, which places it within the range of cost-effective interventions in Sub-Saharan Africa. Even a lower efficacy of 30% would keep the intervention cost-effective at \$119\$/DALY. In the United States, the intervention would cost more than antiretroviral therapy at 21,986\$/DALY. However, in Washington D.C. with a higher HIV prevalence, the same intervention would cost less than antiretroviral therapy and be cost-effective at 2,965\$/DALY. Above all, HIV prevalence drives the cost-effectiveness of the intervention: the intervention breaks even when the male HIV prevalence drops to 2.9% in South Africa and rises to 2.4% in the United States. Within each setting, microbicide price and efficacy drive the results: the intervention breaks even when efficacy drops to 11% or the price increases to \$2.50 in South Africa and when the price drops to \$0.67 in the United States (it never breaks even as effectiveness increases in the United States).

**CONCLUSIONS:** A microbicide intervention would be cost-effective in South Africa and in most Sub-Saharan African settings. However, it would not be cost-effective in the United States or in most developed country settings.

**199****Emergence and Spread of HIV Drug Resistance Arising from Rollout of Antiretroviral Pre-Exposure Prophylaxis (PrEP)**U. Abbas<sup>\*1</sup>, G. Hood<sup>2</sup>, A. Wetzel<sup>2</sup>, J. Mellors<sup>3</sup><sup>1</sup>Cleveland Clinic Foundation, Cleveland, OH, USA;  
<sup>2</sup>Pittsburgh Supercomputing Center, Pittsburgh, PA, USA;  
<sup>3</sup>University of Pittsburgh, Pittsburgh, PA, USA

**BACKGROUND:** The potential for emergence and spread of HIV drug resistance from rollout of antiretroviral (ARV) pre-exposure prophylaxis (PrEP) is an important public health concern. We investigated determinants of HIV drug resistance prevalence after PrEP implementation through mathematical modeling.

**METHODS:** A model incorporating heterogeneity in age, gender, sexual activity, HIV infection status, stage of disease, PrEP coverage/discontinuation, and HIV drug susceptibility, was designed to simulate the impact of PrEP on HIV prevention and drug resistance in a sub-Saharan epidemic.

**RESULTS:** The prevalence of HIV drug resistance was influenced most by the duration and extent of inadvertent use of PrEP in previously-infected individuals. Other key factors influencing resistance prevalence included the duration of PrEP use in individuals who become infected on PrEP, the persistence time of transmitted resistance, the rate of PrEP discontinuation in susceptible individuals, and the persistence time of acquired resistance after PrEP discontinuation. An optimistic scenario of 75% PrEP efficacy, 60% coverage of the population, and 5% inadvertent PrEP use predicted a rise in the prevalence of HIV drug resistance to only 2.5% after 10 years. By contrast, in a pessimistic scenario of 25% PrEP efficacy, 15% population coverage, and 25% inadvertent PrEP use, resistance prevalence increased to over 40%

**CONCLUSIONS:** Inadvertent PrEP use in previously-infected individuals is the major determinant of HIV drug resistance prevalence arising from PrEP. Both the rate and duration of inadvertent PrEP use are key factors. PrEP rollout programs should include routine monitoring of HIV infection status to limit the spread of drug resistance.

# ORAL SESSIONS

## SESSION 25

### Mini-Symposia (MS5): A Tale of Two Compartments

**Moderators:** Craig Hendrix, Sharon Hillier

Monday, May 24, 11:30 am–1:00 pm

Rooms 319–321

# 200

### Non-Human Primate Studies of Rectal and Vaginal Safety and Efficacy

Dorothy Patton

*University of Washington, USA*

The development of topical microbicides represents a new and exciting field in the prevention of sexually transmitted diseases, and it is especially important that candidate products undergo rigorous preclinical safety and efficacy testing in the vaginal and rectal compartments before advancing to clinical trials. We have developed a clinically relevant animal model (pigtailed macaque) that has been used to evaluate the impact of formulated microbicidal compounds on vaginal/rectal microflora, pH and cervicovaginal/rectal tissues and to investigate their chlamydiacidal activity. The US Food and Drug Administration requires that safety, and whenever possible, *in vivo* activity, of candidate topical microbicide products be evaluated in animals before their use in humans. Preclinical animal studies are particularly useful in that both acute and cumulative effects of product exposures can be assessed, and to a certain extent, the behavior of the test subjects can be controlled (e.g., timed product administration). The standardized nature of our particular animal studies allows for findings from any single test product to be compared with those of another. The purpose of these preclinical studies is to provide evidence, which helps to move promising products forward in development, and perhaps more importantly, to recommend reformulation for products which are observed to cause significant, deleterious changes or toxicity to product-exposed cervicovaginal/rectal tissues.

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# 201

### Compartment Specific Pharmacokinetics

Angela Kashuba

*University of North Carolina at Chapel Hill, USA*

Antiretroviral drugs hold significant promise for prevention. However, reliable, biologically relevant pharmacologic data for the efficacious use of these medications must be obtained to select the best antiretroviral (or combination of antiretrovirals), dose, and dose frequency for clinical studies. Combining an understanding of the cellular pharmacology of antiretroviral drugs in mucosal tissues with the physiology of HIV infection in mucosal surfaces will provide data to develop evidence-based dosing for future clinical trials. This presentation will review what is currently known about extracellular and intracellular antiretroviral drug exposures in vaginal, cervical, and rectosigmoid tissues. Drug exposure between these tissues after topical and oral antiretroviral administration will be compared. Differences between single and multiple doses will be examined, as will the length of tissue drug exposure after cessation of drug dosing. Future directions for pharmacologic investigations for HIV prevention will be summarized.

# 202

### Does Africa Need Rectal Microbicides?

Slim Abdoool Karim

*Centre for the AIDS Programme of Research (CAPRISA), University of KwaZulu-Natal, South Africa*

Men who have sex with men (MSM) are largely ignored in HIV prevention and treatment efforts in Africa. There is an emerging pattern of increasing transmission of HIV in African MSM, with HIV prevalence rates as high as 59%. A particularly high-risk subgroup, with over 60% unprotected anal intercourse, is the rapidly growing group of MSM sex workers, mainly in the big cities in Africa.

It is a common misconception that anal intercourse is an exclusively homosexual male practice, not only in Africa but throughout the world. Women who engage in anal intercourse may be less likely to use condoms and more likely to engage in risky behaviours. In some settings, unprotected anal intercourse is viewed as an alternative to vaginal sex to preserve virginity in young women. In countries in Africa where female genital mutilation (female circumcision) is practised, anal intercourse is often experimented with during the weeks and months before painless vaginal penetration can be achieved.

In female sex workers at truck-stops in KwaZulu-Natal, the increase in the practice of anal sex in this cohort over time—in 1992 the practice of anal sex was rare but by 1996 had increased to 42.8% of women saying that they have ever had anal sex. Based on self-reported, HIV prevalence among sex workers who practised anal sex was 61.3% compared with 42.7% in those who did not.

Clearly there is a need for rectal microbicides in high risk population like MSM and sex workers but further research on the role of anal sex in HIV acquisition in women in the general population in Africa is urgently required.

## SESSION 26

### Oral Abstracts (OA15): Animal Models of Safety and Effectiveness

**Moderators:** Jeremy Nutall, Ronald Veazey

Monday, May 24, 11:30 am–1:00 pm

Rooms 403–405

## 203

### Quantitative Image-based Assessment of Microbicide-induced Damage in the Ovine Vaginal Epithelium Using Confocal Microendoscopy

G. Vargas<sup>\*1</sup>, I. Patrikeev<sup>1</sup>, J. Wei<sup>1</sup>, R.K. Johnston<sup>1</sup>, K. Vincent<sup>3</sup>, B. Bell<sup>1</sup>, N. Bourne<sup>3</sup>, M. Motamedinia<sup>1,4</sup>

The University of Texas Medical Branch: <sup>1</sup>Center for Biomedical Engineering; <sup>2</sup>Department of Neuroscience and Cell Biology; <sup>3</sup>Department of Pediatrics; <sup>4</sup>Department of Ophthalmology

**BACKGROUND:** The development of topical microbicides toward the prevention of HIV and other sexually transmitted infections necessitates accompanying preclinical safety testing approaches. A noninvasive imaging approach could assist in real-time evaluation of microbicide effects on epithelium and facilitate assessment and development of candidate microbicides. Confocal microendoscopy was investigated for assessing microbicide induced injury to the ovine vaginal epithelium.

**METHODS:** Sheep were treated *in vivo* with a 5 cc application of PBS, 0.02% benzalkonium chloride (BZK), 0.2% BZK, Hydroxyethylcellulose (HEC), or Gynol II. After twenty-four hours the vaginal tract was removed, labeled with a fluorescent dead cell indicator dye, propidium iodide, and imaged by confocal microendoscopy (Cell-Vizio, Mauna Kea) at twenty-five sites. The tissue was then fixed and processed for histology and H&E staining. Histological grading was applied to assess degree of damage. An automated quantitative image scoring system based on a mean-to-median image feature computation was developed for evaluating effects on epithelium by confocal imaging using an algorithm written in Matlab.

**RESULTS:** PBS controls had staining of surface nuclei with clearly defined borders and clear separation between nuclei. Epithelial damage induced by was apparent in confocal micrographs by the loss of regular surface nuclear staining seen in PBS controls to that of a disrupted staining pattern which gradually increased with degree of damage judged by histology. Histological scoring revealed damage in 0.02% BZK, and severe damage/epithelial denuding with 0.2% BZK. Degree of damage by Gynol II was comparable to that of 0.02% BZK. Automated scoring by confocal microscopy revealed statistically significant scores for each agent versus PBS controls except for HEC. BZK and Gynol II had above threshold values for injury based on confocal scoring with the degree of damage consistent with histology results.

**CONCLUSION:** Confocal microendoscopy provides a sensitive surface assessment of epithelial integrity of the ovine vagina and could be a highly useful tool for assessing effects of candidate microbicides on epithelial integrity. Moreover, the technique is currently being applied *in vivo* indicating real-time evaluation of vaginal epithelium is feasible.

## 204

### PC-1005, A Novel Broad-Spectrum Microbicide Candidate, Completely Protects Rhesus Macaques Against Vaginal SHIV-RT Challenge

J. Kenney<sup>\*1</sup>, M. Aravantinou<sup>1</sup>, R. Singer<sup>1</sup>, A. Gettie<sup>2</sup>, J. Lifson<sup>3</sup>, M. Piatak Jr.<sup>3</sup>, J. Fernandez-Romero<sup>1</sup>, T. Zydowsky<sup>1</sup>, J. Blanchard<sup>4</sup>, M. Robbiani<sup>1</sup>

<sup>1</sup>Population Council, New York, NY, USA; <sup>2</sup>Aaron Diamond AIDS Research Center, New York, NY, USA; <sup>3</sup>AIDS and Cancer Virus Program, SAIC-Frederick, Inc., NCI Frederick, Frederick, MD, USA; <sup>4</sup>Tulane National Primate Research Center, Covington, LA, USA

**BACKGROUND:** We recently demonstrated partial efficacy of PC-815, a microbicide gel consisting of the NNRTI MIV-150 and Carraguard (CG) against vaginal challenge of rhesus macaques with SHIV-RT (SIVmac239 expressing HIV-1 reverse transcriptase). Here, we aimed to further improve the potency of the gels by adding zinc, which enhances the anti-HSV-2 activity of CG in mice. We tested two candidate microbicides: PC-707 (14mM zinc acetate in CG) and PC-1005 (50μM MIV-150 and 14mM zinc acetate in CG).

**METHODS:** Treatment gels PC-707 (n=14) and PC-1005 (n=21), or methyl cellulose (MC) placebo (n=14), were applied daily (2mL/day) for 14 days to the vaginal epithelium of Depo-Provera-treated rhesus macaques. Animals were vaginally challenged with 10<sup>3</sup> TCID<sub>50</sub> SHIV-RT at 4, 8, or 24h after the last gel application. Follow-up included detection of plasma virus RNA and PBMC virus DNA, seroconversion, and IFN-γ responses. Statistical significance was determined with Fisher's exact test.

**RESULTS:** In the MC group, 12 of 14 animals (85.7%) became infected. Among the PC-707 treatment groups, 1 of 7 (14.3%) and 2 of 7 animals (28.6%), became infected when challenged 8 and 24h after final gel application, respectively. None of the animals treated with PC-1005 became infected with SHIV-RT after challenge at any time point. This contrasts findings from separate studies where we found that in animals treated with CG, 8 (5 of 7 animals or 71.4% infected) or 24h (4 of 7 animals or 57.1% infected) and PC-815 (50μM MIV-150 in CG) in animals challenged 8 (2 of 7 animals or 28.6% infected) or 24h (5 of 7 animals or 71.4% infected) after the last gel application. PC-1005 was significantly more protective than CG (p<0.03, 8h challenge group) and PC-815 (p<0.03, 24h challenge group), as well as the placebo MC control (p>0.0003). Infection typically correlated with the development of SIV-specific Ab and T cell responses.

**CONCLUSIONS:** Repeated daily application of PC-1005, a microbicide containing MIV-150 and zinc acetate delivered in CG, provided complete protection for up to 24h against vaginal SHIV-RT challenge. The enhanced protection mediated by PC-1005 (compared to the partial activity seen when either MIV-150 or zinc acetate alone were delivered in CG) is promising for the development of an effective, coitally-independent gel.

# ORAL SESSIONS

## 205

### Film-formulated Retrocyclin RC-101, Applied Topically to the Vagina of Pigtailed Macaques, is Retained Mucosally and Remains Active Against HIV-1

A.M. Cole<sup>1</sup>, A.L. Cole<sup>1</sup>, D. Patton<sup>2</sup>, L.C. Rohan<sup>3</sup>, D. Ratner<sup>3</sup>, Y. Cosgrove-Sweeney<sup>2</sup>, B. Ramratnam<sup>4</sup>, P. Gupta<sup>3</sup>  
<sup>1</sup>University of Central Florida, Orlando, FL, USA;  
<sup>2</sup>University of Washington, Seattle, WA, US;  
<sup>3</sup>University of Pittsburgh, PA, USA; <sup>4</sup>Brown University, Providence, RI, USA

**BACKGROUND:** RC-101 is a congener of the antiretroviral peptide retrocyclin, which we and others have reported is active against clinical HIV-1 isolates from all clades, does not hemagglutinate, and is non-toxic and non-inflammatory in cervicovaginal cell, tissue and organ cultures. Herein, film-formulated RC-101 was assessed for its ability to be retained in the cervix and vagina and active against HIV-1 after intravaginal application in macaques.

**METHODS:** RC-101 was formulated as a quick-dissolving film (2mg/film), and applied intravaginally in six pigtailed macaques daily for four days. At one and five days following the final application, the presence of RC-101 was assessed in peripheral blood, cervicovaginal lavage, cervicovaginal cells (cytobrush) and biopsied cervical and vaginal tissues by quantitative anti-RC-101 immunodot blot and acid-urea (AU)-PAGE Western blot assays. One day following the last film application, RC-101 exposed cervical biopsies were collected and 24 hrs later were subjected to challenge with HIV-1 BAL in an ex vivo organ culture model. Parallel samples were collected from placebo-controlled macaques which have undergone the same treatment regimen.

**RESULTS:** RC-101 peptide was detected primarily in the cytobrush and biopsied cervical and vaginal tissues, with little to no peptide detected in lavage samples, suggesting that the peptide was associated with the cervicovaginal epithelia. RC-101 remained in the tissues and cytobrush samples up to five days post-application, yet it was not detected in sera or plasma samples collected one day post-application. Importantly, cervical biopsies from RC-101-instilled animals prevented HIV-1 transmission in ex vivo organ culture.

**CONCLUSIONS:** Retrocyclins are remarkably stable peptides both *in vitro* and *in vivo*, and due to their lectin-like properties are likely retained mucosally by binding to the epithelial glycocalyx. The presence of antivirally active RC-101 after five days *in vivo* suggests that RC-101 would be an ideal molecule to develop further as a topical microbicide that could be pre-applied in a manner that is dissociated from coitus.

## 206

### A Macaque Vaginal Explant/RT-SHIV Infection Model for Efficacy Study of Microbicides

C.C. Tsai<sup>\*1</sup>, M. Saifuddin<sup>2</sup>, Y. Jiang<sup>1</sup>, L. Flanary<sup>1</sup>, M.B. Agy<sup>1</sup>  
<sup>1</sup>Washington National Primate Research Center, University of Washington, Box 357330 HSB, Seattle, WA 98195, USA; <sup>2</sup>CONRAD, Eastern Virginia Medical School, Arlington, VA 22209, USA

**BACKGROUND:** Heterosexual intercourse remains the major route of HIV transmission worldwide, and topical microbicides represent an important strategy for preventing sexual transmission of HIV. Although the microbicide development pathways have made a significant progress in the last few years, it should be emphasized to ensure the safety and efficacy of new microbicide products in animal models and tissue explant systems prior to human effectiveness studies. We have developed a macaque vaginal tissue explant system that is susceptible to infection with a CCR5-tropic and NNRTI-sensitive RT-SHIV strain. This vaginal explant/RT-SHIV infection model would be valuable for efficacy evaluation of candidate microbicides.

**METHODS:** Vaginal tissues were obtained from either live or necropsy macaques free of infectious agents. Fresh vaginal tissues were placed in DMEM medium [with FBS, antibiotics and fungizone], washed free of debris and cultured in DMEM complete medium (+PHA + IL-2) at 37°C for 24 h. For efficacy evaluation, tissue explants were washed and incubated with different dilutions of UC781 or Griffithsin gel for 1 h before incubating with RT-SHIV (200 TCID<sub>50</sub>) at 37°C for 2 h. Explant cultures were washed with PBS to remove free virus before culturing in the presence of 7% CO<sub>2</sub> and replacement of fresh medium every 2 to 3 days. Culture supernatants at 7 days post incubation were assessed for viral replication by measuring SIV p27 antigen with ELISA. For toxicity assessment, MTT assay was performed after 24 h incubation of tissue explant with or without the gel.

**RESULTS:** The explant tissues were variable in size due to natural folding of vaginal mucosa and resulted in variable amount of virus production. Vaginal explants prestimulated with PHA and IL-2 showed viral replication in earlier time points and also at a higher level than similar sized explant cultures without PHA/IL-2 pretreatment. Anti-viral efficacy of UC781 and Griffithsin gels were evaluated by measuring RT-SHIV infection in the explant cultures with or without the drug [UC781 10–100 µM and Griffithsin 1–4 µM]. Both UC781 and Griffithsin were highly effective to prevent RT-SHIV infection in explant cultures almost at a comparable level shown in CEMx174 cell culture. However, the overall virus production in explant culture was considerably lower than in the cell line-based system. UC781 (100 µM) and Griffithsin (2 µM) was not toxic to tissue explants.

**CONCLUSIONS:** A macaque vaginal explant culture was developed to infect with RT-SHIV. This combined system of macaque explant culture and RT-SHIV infection would be valuable for evaluating efficacy and toxicity of potential microbicides. Furthermore, this new model can be used for preliminary screening of microbicide candidates.

## 207

### Post Coital Assessments: Potential for Macaque Safety Model Expansion

D. Patton<sup>\*</sup>  
<sup>1</sup>University of Washington, Seattle, WA, USA

**BACKGROUND:** The inclusion of sexual activity in preclinical evaluations of vaginally applied topical microbicides is highly desirable for efficient product testing. Our macaque model currently used to assess preclinical safety of topical microbicide candidates does not reflect the dynamics of sexual intercourse in regards to tissue response, i.e. potential mechanical abrasions, or the presence of seminal fluids in the cervicovaginal environment. Inclusion of sexual activity in animal models would more closely mimic human use of topical microbicides and provide a more rigorous assessment of the safety and effectiveness of topical microbicides.

**METHODS:** These studies assessed the feasibility of incorporating macaque mating behavior into the existing safety protocol. Initially we monitored male-female partners in co-housing arrangements to determine whether macaques would reliably mate when given access to one another for brief periods of time, regardless of the female's stage of menstrual cycle. Additionally, we set out to document the presence or absence of ejaculate with sperm in the cervicovaginal environment after mating visits. Finally, we collected colposcopic, microbiologic and pH assessments from the cervicovaginal environment of female macaques before and after mating.

**RESULTS:** Coital activity (mounting) was reliably observed in 87 of 90 co-housing sessions. Seventy-eight percent of the total mountings (68/87) occurred within the first 15 minutes of partner access. Ejaculation was visually observed in 28 of the 90 sessions. Post-coital colposcopic exams of the females included documentation of ejaculate and sperm contained within a seminal plug in the vagina. Ejaculate was noted in 35 of 88 post-coital vaginal exams. Colposcopies revealed a higher incidence of petechiae and minor tissue abrasions after coital activity. The components of vaginal microbiology were not affected by coitus. In these studies the vaginal pH increased after mating, regardless of whether or not ejaculate was present.

**CONCLUSIONS:** This work clearly demonstrates that it is feasible to incorporate sexual activity in our macaque model for topical microbicide safety assessment.

# POSTER SESSIONS

SESSION 27  
Monday, May 24, 2010

## SESSION 27

### Poster Discussion (PD5): Integrating Contraception and HIV Prevention

**Moderators:** Michael Chirenje, Gina Brown

Monday, May 24, 3:00 pm–4:00 pm

Ballroom

**226**

#### Dual Protection Polyurethane Intravaginal Rings for the Simultaneous Delivery of Levonorgestrel and UC781

F.R. Damian<sup>\*1</sup>, E.A. Ho<sup>1</sup>, M.R. Clark<sup>2</sup>, D.R. Friend<sup>2</sup>, P.F. Kiser<sup>1</sup>

<sup>1</sup>University of Utah, Salt Lake City, Utah, USA;

<sup>2</sup>CONRAD, Arlington, VA 22209, USA

**BACKGROUND:** Most vaginal microbicides in clinical investigation have been formulated as single-drug dosage forms designed for application to the vagina prior to intercourse. However, a compelling rationale exists for providing long-term, controlled release of vaginal microbicides to provide prolonged protection against heterosexually transmitted HIV infection and to improve user adherence. The goal of the present study was to develop a polyurethane intravaginal ring (IVR) capable of sustained delivery of both UC781, a potent anti-HIV agent and levonorgestrel (LNG), a potent contraceptive.

**METHODS:** Polyurethane rods containing 1 or 5%wt LNG and 1–10% UC781 were prepared by melt extrusion at 147°C using a twin-screw extruder to form transparent rods through a 4.2 mm die. Rods were cut into approximately 1.5 cm lengths and were weighed and dimensions measured with digital calipers. Rod segments (end-capped) were placed in 20-mL scintillation vials, and 5 mL of release media (2% Solutol or 0.05% Solutol in acetate buffer, pH 4, N=3) was added to each vial and were incubated at 37°C on a shaker (80 RPM). Release media were replaced daily and analyzed for drug content by HPLC.

**RESULTS:** To confirm the melt-extrusion processing stability of LNG in the polymers, we first evaluated drug-polymer extrusions under elevated stress conditions at 145°C via controlled mixing/cycling in the extruder chamber. The data obtained showed no evidence of drug loss, regardless of exposure time to elevated temperatures. LNG release was detected in all release media conditions tested. Cumulative release from the rod segments in 2% or 0.05% Solutol® (acetate buffer, pH 4.2) appeared to be linear with time and was proportional to the solubility of LNG and UC781 in the release media. LNG release for a full IVR after 30 days were in the following order: 2116 µg (5 wt% LNG in 2% Solutol) > 937 µg (1 wt% LNG in 2% Solutol) > 132 µg (5 wt% LNG in 0.05% Solutol) > 130 µg (1 wt% LNG in 0.05% Solutol).

**CONCLUSIONS:** The data from this study demonstrated controlled released LNG and UC781 from monolithic polyether polyurethane ring segments under *in vitro* conditions. More work on the optimization of drug loading in the formulation and evaluation of proto-type combo-ring formulation is in progress.

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#### Development of Microbicide-Releasing SILCS Diaphragm

I. Major<sup>\*1</sup>, D. Lowry<sup>1</sup>, K. Malcolm<sup>1</sup>,

D. Woolfson<sup>1</sup>, J. Cohen<sup>2</sup>, P. LaBarre<sup>2</sup>

<sup>1</sup>Queen's University Belfast, Belfast, UK; <sup>2</sup>PATH, Seattle, USA

**BACKGROUND:** There is a need for HIV microbicides that simultaneously provide contraception. The SILCS diaphragm is a single-size contraceptive diaphragm developed by PATH and CONRAD. Its polymeric construction (nylon spring core and overmolded silicone elastomer membrane) potentially allows for incorporation and sustained release of HIV microbicides. However, the high processing temperature and poor permeation characteristics of nylon are major obstacles to further development. This study describes the feasibility and preliminary development of a microbicide-releasing SILCS diaphragm.

**METHODS:** Various model diaphragm devices were constructed comprising microbicide-loaded thermoplastic rods inserted into silicone elastomer tubing, and continuous and discontinuous *in vitro* release evaluated over 14 days. Mechanical testing on a range of alternative thermoplastic materials was also performed. Candidate diaphragm devices, prepared by injection molding of POM spring cores and overmolded with silicone elastomer, were assessed for mechanical characteristics and *in vitro* release. User acceptability studies are ongoing.

**RESULTS:** Thermoplastic rods in silicone tubing showed sustained release of UC781 (PEVA and PCL provided cumulative release of 325 and 95 mcg over 14 days into isopropanol:water mixture, respectively). POM copolymer was selected for spring core fabrication, owing to its low processing temperature for compounding and injection molding (140°C), good release characteristics (1, 5 and 10% w/w loadings provided cumulative release over 14 days of 1.1, 6.4 and 30.8mg, respectively) and similar mechanical properties to nylon (flexural modulus 2.9GPa, core compression force 4.4N). Addition of UC781 did not have significantly effect the mechanical performance of spring cores.

**CONCLUSIONS:** The nylon spring core of the current SILCS diaphragms has been successfully replaced with an alternative lower melting POM thermoplastic. Mechanical performance matches the current SILCS device and sustained release of candidate microbicides has been demonstrated.

# POSTER SESSIONS

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## Vaginal Delivery of UC781 in Woman's Condom Capsules

L. Ferguson<sup>\*1,2</sup>, J. Cohen<sup>3</sup>, J. Schubert<sup>3</sup>, T. Gong<sup>2</sup>, C. Dezzuti<sup>2,3</sup>, David Friend<sup>5</sup>, L.C.Rohan<sup>1,2</sup>

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**BACKGROUND:** The female condom provides protection against pregnancy, HIV, and other sexually transmitted diseases. PATH, in collaboration with CONRAD, has developed a new female condom that has been shown to be highly acceptable to a range of users. One modification involves improving insertion with the addition of a rounded polyvinyl alcohol based capsule at the end of the condom, which gathers the condom pouch together. Once the condom is inserted, the capsule quickly disintegrates and the condom unfolds inside the vagina. To further refine the female condom and add a second layer of protection the microbicide drug candidate UC781, a non-nucleoside reverse transcriptase inhibitor, was incorporated into the condom capsule adopting aspects of vaginal thin film technology.

**METHODS:** An appropriate polymer platform for the addition of UC781 to the condom capsules was identified through evaluations of 1) manufacturing process changes [dip-ability, curing, and peel-ability from mandrel]; 2) puncture strength; 3) thickness; and 4) disintegration time. UC781 was incorporated into polymer capsule platforms implementing strategies learned from previous vaginal film formulation efforts with drug candidate. Developed film capsule prototypes were characterized evaluating physical and mechanical properties, drug content uniformity, water content, disintegration time, dissolution, toxicity, and bioactivity.

**RESULTS:** Two condom capsule prototype formulations were identified that retained acceptable manufacturing attributes with respect to product appearance, capsule length, and capsule thickness. Rapid disintegration time was achieved. However, several aspects of the formulation require optimization such as drug content uniformity and mechanical strength.

**CONCLUSIONS:** UC781 can be incorporated into a modified film capsule formulation platform. However, the capsule formulation must undergo further optimization to achieve better drug content uniformity, establish long term stability, and product scalability.

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## Unmet Family Planning Needs Among HIV-Positive Participants Screened for FEM-PrEP

J. Deese<sup>\*1</sup>, K. Agot<sup>2</sup>, K. Ahmed<sup>3</sup>, C. Lie<sup>1</sup>, H. Kiernan<sup>1</sup>, L. Van Damme<sup>1</sup>

<sup>1</sup>Family Health International, USA; <sup>2</sup>Impact Research and Development Organization, Bondo, Kenya; <sup>2</sup>Setshaba Research Centre, Soshanguve, Pretoria, South Africa

**BACKGROUND:** The public health community has recognized the need for integration of family planning and HIV care services for HIV-infected women as a means of reducing mother-to-child HIV transmission (MTCT). The use of effective contraception by HIV-positive women who do not desire pregnancy is a highly efficacious and cost-effective intervention for HIV prevention, yet an often unmet need.

**METHODS:** A multivariate analysis of FEM-PrEP, a randomized placebo-controlled PrEP trial, baseline data was conducted to examine associations between contraceptive attitudes, use and HIV status at two study sites.

**RESULTS:** The analysis included 1086 women screened for FEM-PrEP who also had complete HIV results at screening, including 936 in Bondo, Kenya and 150 in Pretoria, South Africa. Among those screened, 378 (34.8%) were HIV-positive. The majority of HIV-positive women expressed a desire to avoid pregnancy over the next year: 156 (41.3%) reported that they definitely did not want to get pregnant, 211 (55.8%) preferred not to get pregnant, 9 (2.4%) were unsure if they wanted to get pregnant, 2 (0.5%) reported not minding getting pregnant and none reported hoping to get pregnant. While 97.1% reported a wish to avoid pregnancy in the next year, only 168 (44.4%) reported using any method of contraception and only 134 (35.4%) reported using a highly effective contraceptive method (hormonal, IUD or sterilization).

**CONCLUSIONS:** These results reveal a large unmet need for effective contraception among HIV-positive women and an opportunity to prevent MTCT in women with a desire to avoid pregnancy. It may be that a non-negligible number of women identified as HIV-positive at screening were unaware of their status, and thus not currently receiving HIV care. Maximizing the HIV prevention opportunity via contraceptive provision will require integration of family planning and HIV-care services, and also identification of ways to deliver family planning services to the larger community of women desiring contraception, particularly in areas of high HIV incidence.

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## High Uptake of Non-Barrier Contraception by Women Before Randomization into an HIV Prevention Trial in Rural Kenya

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**BACKGROUND:** Women who become pregnant in biomedical HIV prevention clinical trials are often withdrawn from study products. As a result, time off product is increased and trial statistical power may be compromised. High pregnancy incidence has been a challenge in some trials, especially in regions where contraceptive prevalence is low. The pre-randomization period offers a window of opportunity to provide contraception counseling and initiate contraception prior to study enrollment. We initiated pre-randomization contraceptive counseling and on-site provision of contraception for women screened for an HIV prevention clinical trial of antiretroviral pre-exposure prophylaxis (PrEP).

**METHODS:** The Partners PrEP Study is enrolling HIV-1 serodiscordant couples at 9 sites in East Africa, including a site in Thika, Kenya. We provided contraceptive counseling to all HIV positive and HIV negative women during screening and offered methods to women free-of-charge at the research clinic prior to randomization. Contraceptive prevalence data from the screening and randomization visits were analyzed for 260 HIV discordant couples enrolled between October 2008 and November 2009.

**RESULTS:** Of 260 HIV serodiscordant couples enrolled, 59 (22.7%) were couples in which the HIV negative partner was female. Among 59 HIV negative women enrolled, non-barrier contraceptive use increased from 37.3% [22/59] at screening to 71.2% [42/59] at enrollment [ $P<0.001$ ]. The median time between screening and enrollment was 12 days. The 20 HIV negative women who initiated contraception between screening and randomization, used depot medroxyprogesterone acetate 12 [60%], oral contraceptive pills 3 [15%], implants 3 [15%], and intrauterine devices for 2 [10%]. Among the 201 HIV positive women enrolled, non-barrier contraceptive uptake also increased significantly between screening and enrollment: from 34.3% [69/201] to 61.7% [124/201] ( $p<0.001$ ). The 55 HIV positive women who initiated contraception between screening and randomization, used depot medroxyprogesterone acetate 41 [74.5%], oral contraceptive pills 8 [14.5%], implants 4 [7.3%], and bilateral tubal ligation 2 [3.6%].

**CONCLUSIONS:** Providing contraception counseling and access to contraceptive methods before randomization can lead to a significant increase in contraception uptake by women participating in HIV prevention clinical trials. Early initiation of contraception may reduce pregnancies and time off study product.

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### Pregnancy Rates in the Durban Site of the MDP 301 Trial: Implications for VOICE and Other PrEP Studies

Z. Gaffoor\*, Y. Sookrajh, S. Pillay, G. Ramjee, and the MDP Trial Team

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**BACKGROUND:** Women recruited into HIV prevention trials are of childbearing age and at high risk for HIV infection. Since the teratogenicity of microbicides and other interventions are unknown, pregnancy is an exclusion criterion in most prevention studies, and researchers make a concerted effort to provide contraception to women participants. We report here pregnancy rates and contraceptive use among women participating at the MDP 301 sites in Durban, and discuss the implications of such observations for the VOICE study.

**METHODS:** The recently completed MDP 301 trial was an international, multi-centre, randomized, double-blind, placebo controlled trial to evaluate the efficacy and safety of 0.5% and 2% PRO2000/5 gels for the prevention of vaginally acquired HIV infection. Women recruited were tested for pregnancy at every visit, and put on study hold if found to be bHCG positive. They were offered condoms, oral and injectable contraceptives; and contraceptive counselling at every visit. Chi-square tests were used to compare the pregnancy rates between different types of contraception use.

**RESULTS:** Of 2391 women who were enrolled at three centres in Durban, 9.5% (n=228) became pregnant during a one year follow-up period. 72% of pregnant women reported using some form of contraceptive at or before the visit at which a positive pregnancy result was determined. The pregnancy rate in those using oral contraception was 17% (59/353) compared to 3% (1182/1216) in those using injectables ( $p<0.001$ ). Of the 34 women who became pregnant and reported injectable use, only three had received the injectable contraception from study staff.

**CONCLUSIONS:** Our results suggest that injectable contraception administered and monitored by study staff may result in greater compliance, and thus avoid time off-product due to pregnancy.

The VOICE study allows oral pill use as a reliable method of contraception. However, given the higher rate of pregnancies among women who use such pills when compared to injectables, intensive counselling and education on compliance will be necessary, or extensive counselling on an alternative form of contraception should be provided.

## SESSION 28

### Poster Discussion (PD6): Life Beyond Traditional Gels—Upcoming Drug Delivery Systems

**Moderators:** Charles Kelly, Angela Kashuba

Monday, May 24, 3:00 pm–4:00 pm

Rooms 301–304

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### Phenylboronate-Salicylhydroxamate Crosslinked Hydrogels as a pH Responsive Microbicide Vaginal Drug Delivery Vehicle

J.I. Jay<sup>1</sup>, Shetha Shukair<sup>2</sup>, K. Langheinrich<sup>\*3</sup>, G.C. Cianci<sup>2</sup>, M.R. Clark<sup>3</sup>, T.J. Hope<sup>2</sup>, L. Rohan<sup>4</sup>, P.F. Kiser<sup>1,3</sup>

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**BACKGROUND:** New functional materials capable of modulating their properties based on biological cues are a continued focus of study in advanced drug delivery vehicle design. Of particular interest are new materials that sense the presence of HIV-1 or HIV-1 containing fluids and rapidly modulate their transport properties in response to this stimulus thus creating a barrier that inhibits interaction of HIV-1 with susceptible tissues and cells. The reversibility of covalent crosslinks formed by phenylboronate (PBA) and salicylhydroxamate (SHA) has been exploited to provide a pH-responsive gel for application to the vaginal tract. We have examined the properties of both symmetric and asymmetric crosslinked gels to determine their performance under simulated conditions, including rheological properties, HIV-1 transport, and *ex vivo* toxicity.

**METHODS:** Dynamic rheology evaluated the frequency-dependent viscoelastic properties of the gel as a function of pH. This method was also employed to characterize the self-healing properties of the asymmetric covalently crosslinking gels. Nanoparticle tracking assessed the transport of both fluorescently labeled HIV-1 and 100 nm sulfate-modified latex particles in the PBA-SHA crosslinked gel as a function of pH and in the presence of vaginal and penile tissue. Gel toxicity was quantified by MTT and cytokine analysis as well as by exposure to cervical explant tissue.

**RESULTS:** The ensemble-averaged mean squared displacement at lag times greater than three seconds reveals that transport of the HIV-1 and 100 nm particles becomes impeded by the polymer matrix. The viability of vaginal explants exposed to this formulation was 81.18%±0.008. Comparison of tissue histology before and after exposure to the gel showed no evidence of significant morphological changes. The asymmetric crosslinked gel displays self-healing properties after repeated break cycles at both pH 4.5 and 7.5 demonstrating that increasing the ratio of PBA to SHA provides a material that can form a self-healing gel across the full vaginal pH range.

**CONCLUSIONS:** pH responsive gels based on the PBA-SHA crosslink thus display properties of a potentially effective microbicide: biologically safe, the potential to significantly reduce the transport of HIV-1 to susceptible tissues and thus prevent the first stage of male-to-female transmission of HIV-1, and the ability to restructure after exposure to shear.

# POSTER SESSIONS

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## Novel Non-Aqueous Silicone Elastomer Gels for Practical Formulation of HIV Microbicides

C. Forbes\*, D. Lowry, C. McConville,  
D. Woolfson, K. Malcolm  
*Queens University Belfast, Belfast, UK*

**BACKGROUND:** Semi-solid aqueous gels (e.g. hydroxyethylcellulose (HEC) and Carbopol gels) are used routinely for the formulation of HIV microbicides. Such gels are inexpensive, easy to manufacture, provide solubilisation of water-soluble actives, and may be prepared in mucoadhesive forms for enhanced mucosal retention. However, they also require preservation, do not dissolve water-insoluble actives resulting in dispersed gel systems which may provide suboptimal absorption and activity, and are diluted by vaginal fluids leading to decreased retention. It is widely accepted that a more diverse range of formulation options is needed in order to accommodate the varying physicochemical characteristics of microbicide candidates. A particular focus should be directed to the formulation of HIV microbicides with limited water solubility (e.g. dapivirine, UC781). Here we report the potential of non-aqueous, hydrophobic silicone elastomer gels for the formulation of microbicides.

**METHODS:** Silicone elastomer gels were prepared containing different lead candidate microbicide compounds (T-1249 (4995Da), AZT (269Da, logP -0.6), dapivirine (326Da, logP +4.6), CMPD167 (575Da, logP +6.2), UC781 (336, logP +5.4) and various excipients to modify release. *In vitro* release studies were performed in simulated vaginal fluid (SVF) using both single and dual-compartment models.

**RESULTS:** The silicone elastomer gels were not dissolved or diluted by SVF during release testing. All the gels tested provided measurable release of each microbicide candidate in quantities reflecting the relative hydrophilic/hydrophobic character of the microbicide. For example T-1249 released up to 32 $\mu$ g, AZT released 9.5 mg and dapivirine released 9.8 mg, CMPD167 released 38 mg over 8 hours. The incorporation of hydrophilic excipients into the silicone elastomer gels modified the release rates, and also increased the mucoadhesive character of the gels. For example, addition of 20% HEC increased T-1249 release to 135 $\mu$ g and the addition of 20% croscarmellose increased dapivirine release to 24 mg.

**CONCLUSIONS:** The studies have shown that non-aqueous silicone elastomer gels are capable of releasing microbicides having a wide range of physicochemical characteristics, including molecular weight and hydrophilicity, and that release may be modified through the incorporation of hydrophilic excipients.

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## Quick-Dissolve Films as Drug Delivery Systems for Combination Microbicides

A. Akil<sup>\*1,2</sup>, C. Dezzutti<sup>1,3</sup>, B. Moncla<sup>1,3</sup>,  
M. Parniak<sup>3</sup>, P. Graebing<sup>1</sup>, M. Cost<sup>1</sup>,  
I. Dumanli<sup>4</sup>, L.C. Rohan<sup>1,2,3</sup>

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**BACKGROUND:** Combinations of anti-HIV drugs are being evaluated as microbicides. Quick-dissolve films are one of several strategies for vaginal delivery of drug combinations. Films containing a combination of the entry inhibitor maraviroc (MVC) and a reverse transcriptase inhibitor (tenofovir(TFV) or dapivirine(DPV)) were developed and assessed for their physical, chemical, biocompatibility, and anti-HIV properties.

**METHODS:** Polyvinyl alcohol (PVA) based films were made using solvent casting techniques. Physical and chemical properties as well as toxicity and bioactivity were characterized. Specific properties studied were appearance, weight, thickness, tensile strength, water content, disintegration, dissolution, and drug content. Film toxicity and anti-HIV activity were assessed in cell based models (ie TZM-bl) and ectocervical explant cultures. Additionally, compatibility of films with resident microflora (*Lactobacillus*) was assessed.

**RESULTS:** The developed films were soft, flexible, and semi-transparent. Film mechanical properties were tested and the tensile strength of the films was acceptable at >1000 kg/m<sup>2</sup> for DPV/MVC combination film and >2000 kg/m<sup>2</sup> for TFV/MVC combination film. Both films had <0.1% (w/w) residual water. Film disintegration was achieved in <10 minutes. The USP dissolution test was performed on the DPV/MVC combination film and showed ~50% of both drugs were released in 10 minutes. No toxicity was observed upon DPV/MVC combination film exposure in the TZM-bl cell assay. Also, the films showed no toxicity against resident strains of *Lactobacillus*. *In vitro* bioactivity testing showed the DPV/MVC film to be active against HIV-1. Ongoing stability studies show the films maintain targeted specifications over three months.

**CONCLUSIONS:** These studies show that combinations of anti-HIV drugs can be successfully formulated into a quick-dissolve vaginal film with acceptable physiochemical characteristics, bioactivity, and safety profile.

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## Development and Evaluation of Novel Solid Dosage Forms of Tenofovir

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**BACKGROUND:** The conventional vaginal dosage forms are known to have poor user compliance because of problems of poor retention, leakage, and messiness. The present project was undertaken to develop novel bioadhesive vaginal tablets of Tenofovir, with the target product profile of releasing the drug over an extended period of time. Tenofovir, a Nucleotide Reverse Transcriptase Inhibitors (NtRTIs) is currently being developed as a Microbicide.

**METHODS:** The tablet formulation was designed to disintegrate rapidly in small volumes of vaginal fluid and prepared by direct compression tabletting method. The almond-shaped tablets were prepared from GRAS (generally regarded as safe) or compendial excipients for vaginal administration. Weight of the tablets was 1 gm with a target dose of 40 mg of Tenofovir. The optimized formulations were characterized for a set of pharmacopoeial and non pharmacopoeial parameters such as weight variation, disintegration time, hardness, thickness, moisture content, and assay. A reverse phase HPLC method was developed for assay of the drug substance and drug product and validated for linearity, specificity, accuracy, precision, system suitability, LOD and LOQ. Stability of the tablets was evaluated as per standard ICH conditions of 4°C, 35°C/60% RH and 40°C/75% RH.

**RESULTS:** The tablets were successfully prepared with targeted disintegration time of around one minute, and the required hardness, moisture content, and thickness. Because of small dose, optimizing the process for content uniformity was highly challenging. Tablet excipients did not interfere with quantitation of TFV. The formulation was stable for six months at 40°C/75% RH (assay = 98.4% of initial after 6 months) as assessed by physical stability and assay.

**CONCLUSION:** Novel, rapidly disintegrating bioadhesive vaginal tablets of TFV were developed, evaluated, and found to be stable under accelerated stability conditions for six months. The formulation can be taken up for scale up and further evaluation.

**229****Design of an Intravaginal Ring for the Simultaneous Delivery of IQP-0528 and Tenofovir**J.T. Clark, M.Kaur, P.F. Kiser  
*University of Utah, Salt Lake City, Utah 84112, USA*

**BACKGROUND:** Several recently published articles, along with ongoing research projects in our group have demonstrated the sustained release of various anti-HIV compounds from intravaginal rings (IVRs), alluding to the possibility of their eventual use as effective microbicide delivery vehicles. IQP-0528 is a pyrimidinedione analogue which has been shown to function as both a reverse transcriptase and cell-entry inhibitor for HIV-1. We have developed a polyether urethane IVR capable of the sustained delivery of IQP-0528 and tenofovir, a well-characterized reverse transcriptase inhibitor.

**METHODS:** We have developed two prototypes which incorporate both compounds into a polyurethane IVR. The first was a two-segment IVR. Tenofovir (PMPA) was loaded (13.9% w/w) into Tecophilic HP-60D-20 (a water-swellable variant of Tecoflex) and IQP-0528 (0528) was loaded (17.6% w/w) into Tecoflex EG-85A. Cylindrical rods (d~5 mm) of each formulation were formed by twin-screw, hot-melt extrusion. Two rod segments with a 7:1 length ratio (HP-60D-20/PMPA:EG-85A/0528), were joined to form a 55 mm (outer diameter) IVR. Sink condition release kinetics were modeled by incubation of IVRs in 25 mM sodium acetate buffer with 2% Solutol HS-15 (pH=4.2) for 30 days in an orbital shaker (37°C, 80 rpm). Aliquots of the incubation media were analyzed by high performance liquid chromatography (HPLC) to determine API content. The mechanical stabilities of the ring-welds were also evaluated by performing compression and extension tests on the IVRs before incubation. In the second prototype, we successfully fabricated rod segments of Tecophilic loaded with both PMPA and 0528 at similar loadings to those in their individual formulation (a combined drug loading approximately 30% w/w).

**RESULTS:** The two-segmented IVRs exhibited release kinetic profiles which mainly agree with those derived from previous data obtained using segments of the individual formulations. The minimum release rates over the 30-day experiments were approximately 3 mg/day (PMPA) and 400 µg/day (0528). The ring-welds showed no mechanical instability under the compression and extension tests.

**CONCLUSIONS:** We have developed an IVR system capable of the 30-day sustained delivery of tenofovir along with a promising new anti-viral compound, IQP-0528.

**241****Production and Preservation of Highly Viable *Lactobacillus* for Mucosal Delivery of Therapeutics *In Situ***Q. Xu\*, R. Yu, Q. Xia, W. Huang, L. Jia, X. Liu, P. Lee, Y. Liu  
*Osel, Inc., Santa Clara, CA, USA*

**BACKGROUND:** The human vaginal mucosa, a key target of sexually transmitted infections, is populated with commensal bacteria. The predominant H<sub>2</sub>O<sub>2</sub>-producing *Lactobacillus* species play a key role in the maintenance of reproductive health of women, and could be genetically engineered as a self-renewing vehicle for mucosal delivery of protein-based HIV inhibitors. The success of this approach will depend, in part, on the level and duration of *in situ* *Lactobacillus* colonization, a surrogate marker for efficacy. Equally important is production of a highly viable product that is stable over time. Thus, we undertook comprehensive investigations into the optimal fermentation and preservation conditions for *Lactobacillus* for mucosal delivery.

**METHODS:** Cell viability (ratio of live to total number of cells) of *L. jensenii* strains expressing an extracellular N-terminally modified cyanovirin-N or intracellular β-glucuronidase was evaluated during fermentation in a Microbial Fermentor and upon preservation by spray- or freeze-drying. An Olympus IX51 microscope was reconfigured with a dark field condenser and a Petroff-Hausser counting chamber for the determination of total cell count. The live cell count was determined by colony forming units (CFU) on MRS plates. In addition, cell recovery of dried *L. jensenii* powders was evaluated post-rehydration in MRS broth and simulated vaginal fluid at various pH.

**RESULTS:** Fermentation conditions affected *Lactobacillus* viability and survival of *Lactobacillus* during preservation. Maintenance of pH with ammonium hydroxide coupled with glucose supplementation supported the fermentation of *Lactobacillus* with up to 10<sup>10</sup> CFU per ml and >90% cell viability when bacterial growth reached early stationary phase. Addition of a polyol and sodium ascorbate along with another antioxidant to the preservation matrix (skim milk and trehalose) significantly increased cell viability of the dried *Lactobacillus* powders. As cell recovery of the dried preparations was sensitive to pH upon rehydration, a transiently buffered preservation matrix was further developed to produce *Lactobacillus* powder that was highly viable at 10<sup>11</sup> CFU per gram of powder.

**CONCLUSIONS:** Highly viable *Lactobacillus* powders can be produced and formulated by optimizing cell viability during fermentation, preservation, and rehydration *in vitro*. Work is in progress to formulate *Lactobacillus* for improved heterologous protein expression and product stability, and to select the appropriate dosage form for mucosal delivery *in situ*.

# POSTER SESSIONS

## SESSION 29

### Poster Discussion (PD7): *In Vitro/In Vivo Evaluations—An Essential Bridge for Product Development*

**Moderators:** Charlene Dezzutti, Betsy Herold

Monday, May 24, 3:00 pm–4:00 pm

Rooms 319–321

# 238

### Safety Testing of Silicone Elastomer Matrix Vaginal Rings Containing UC781 and Tween 80 using Epivaginal (VEC-606) Tissues

C. McConville<sup>\*1</sup>, D. Friend<sup>2</sup>, D. Woolfson<sup>1</sup>, K. Malcolm<sup>1</sup>

<sup>1</sup>School of Pharmacy, Queen's University Belfast, Belfast, UK; <sup>2</sup>CONRAD, Arlington, VA, USA

**BACKGROUND:** The microbicidal agent UC781 has very limited aqueous solubility. A recent study in pig-tailed macaques showed poor pharmacokinetic levels, suggesting that, unlike *in vitro* release testing which uses relatively large volumes of a hydroalcoholic release medium, the vaginal environment does not provide sufficient solubilizing power to dissolve UC781 that has permeated to the ring surface. To overcome this solubility obstacle, the non-ionic surfactant Tween 80 was coformulated with UC781 in silicone elastomer rings. This study reports *in vitro* release into simulated vaginal fluid (SVF), and safety testing of the rings using the EpiVaginal tissue model.

**METHODS:** UC781+Tween 80 macaque matrix silicone elastomer vaginal rings were prepared and placed onto the apical surface of pre-wet (175µl PBS) EpiVaginal (VEC-606) tissues. After 24hr, 72hr and 7d, half of the culture medium from each tissue was collected and tested for inflammatory cytokine levels by ELISA. Barrier function of the vaginal tissue was tested on D1, D3 and D7 using transepithelial electrical resistance (TEER). Tissue viability was tested on D7 of the study using an MTT assay. *In vitro* release was performed in 5mL SVF and UC781 release analyzed by HPLC.

**RESULTS:** A 100mg UC781 ring released 1.7mg UC781 in 5mL SVF over 14d, compared to 10.2mg from the same ring containing 10% Tween 80. Placebo, UC781 and UC781+Tween rings induced a 1.7, 1.7 and a 4.0 increase respectively in IL-8 levels on D1. However, levels were similar to PBS and SVF controls on D3, and on D7 IL-8 values for all rings dropped significantly below control values. No significant changes in IL-6 were observed for tissues exposed to rings in the presence of SVF. Increases in IL-1 $\alpha$  were observed on D1 and D3 for all rings but values were similar to D7 control values. No significant changes in IL-1 $\beta$  were noted. TEER values for PBS, placebo, UC781 and UC781+Tween rings were 412, 610, 598 and 518 Ohm cm $^2$  respectively. The tissue viability for the placebo, UC781 and UC781+Tween rings were 86, 90 and 83% compared with PBS control having a tissue viability of 101%.

**CONCLUSIONS:** The formulation of Tween 80 in a 100mg UC781 matrix vaginal ring increases UC781 release by ten fold over 14d into SVF. The combination of UC781 and Tween 80 in the rings did not compromise measured tissue properties. Tissue viability on D7 for all test materials was >83%. Therefore, all test materials should be minimally to non-irritating *in vivo*.

# 245

### MRI Evaluation of the Distribution and Retention of Multiparticulates in the Human Vagina

S. Mehta<sup>\*1</sup>, G. Villeirs<sup>2</sup>, H. Verstraelen<sup>3</sup>, E. Mehuy<sup>1</sup>, C. Vervae<sup>1</sup>, J.P. Remon<sup>1</sup>

<sup>1</sup>Laboratory of Pharmaceutical Technology, Ghent University, Ghent, Belgium; <sup>2</sup>Department of Radiology, Ghent University Hospital, Belgium; <sup>3</sup>Department of Obstetrics and Gynecology, Ghent University Hospital, Belgium

**BACKGROUND:** Conventional vaginal formulations are associated with several disadvantages, such as leakage, messiness and low retention (for semi-solids) and rapid loss from the vagina (for tablets). We developed a multi-particulate pellet system (pellets are spherical particles with diameter varying from 400–1500 µm), as a novel vaginal drug delivery system. It was anticipated that due to their small particle size, pellets will evenly distribute in the vaginal cavity and will be less sensitive to gravity than tablets, resulting in longer retention.

The objective of this pilot study was to evaluate the *in vivo* distribution and retention of this novel vaginal drug delivery system in human vagina, using Magnetic Resonance Imaging (MRI).

**METHODS:** The starch-based pellets were produced by extrusion-spheronisation. A high-amyllose starch with sorbitol and Hydroxy propyl methyl cellulose mixture was granulated with water containing Gadolinium, as the MRI contrast agent. This wet mixture was extruded, spheronised and dried to obtain pellets (d = 500 µm). The pellets were filled into a hard gelatin capsule (size 00) and administered intravaginally to the study patient (n=1), with a suitable applicator. The T1-weighted MRI was performed immediately after administration, after 6h, 8 h, 12 h and 24 h post-administration.

**RESULTS:** Immediately after administration, no Gadolinium was observed as the capsule was still intact. The scan taken after 6 h indicated the disintegration of the capsule and clustering of the pellets into the cervix and proximal vagina. At 8 h post-administration, the pellets were spread further down the vagina. After 12 h, the anterior portion of cervix and the entire vagina was covered by the pellets. After 24 h, the pellets were still present covering the complete vagina, but in a smaller amount. The study patient reported some pellet loss between 12h and 24h after administration during urination. The transverse images clearly indicated the W-shape of the vagina, confirming the complete coverage of the vaginal mucosa.

**CONCLUSIONS:** The multi-particulate pellet system seems a promising new vaginal drug delivery system, resulting in complete coverage of the vaginal mucosa (similar to gel) and acceptable retention time. These preliminary data should be confirmed for inter and intra subject variation, on a large number of volunteers. A drawback of this formulation was the slow initial disintegration time of the capsule. To overcome this problem further research will be focused on compression of the pellets into fast disintegrating tablets.

# POSTER SESSIONS

SESSION 29  
Monday, May 24, 2010

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## In Vitro Release of Tenofovir and Acyclovir from a Silicone Vaginal Ring Platform

J. Moss<sup>1</sup>, M. Baum<sup>1</sup>, A. Malone<sup>2</sup>, D. Friend<sup>3</sup>, E. Kopin<sup>1</sup>, S. Kennedy<sup>1</sup>, C. Nguyen<sup>1</sup>, J. Smith<sup>3</sup>, C. Cortez<sup>1</sup>, T. Smith<sup>1,2</sup>

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<sup>2</sup>Auritec Pharmaceuticals, Santa Monica, CA, USA;

<sup>3</sup>CONRAD, Arlington, VA, USA

**BACKGROUND:** Intravaginal ring delivery of combinations of microbicides is a promising approach for prevention of sexually-transmitted disease, but requires a method of providing simultaneous, independent release of multiple agents. We report the development of an intravaginal ring (IVR) delivery platform for 30-day delivery of the reverse transcriptase inhibitor tenofovir (TFV) and the guanosine analogue antiviral acyclovir (ACV), and evaluation of its in vitro release characteristics.

**METHODS:** A pellet of ~15 mg TFV or ACV is prepared by first compressing a core followed by coating with semi-permeable polymer, resulting in a "pod." The pod is then incorporated into a silicone ring or segment during an injection molding process. The release rate for each pod is controlled independently, determined by the size of a delivery window that is mechanically formed in the silicone during molding. Segments contain 1–2 pods, and rings up to 10 pods. Within a ring, each pod can be identical or contain different drugs; in this study, TFV and ACV were formulated in separate pods. *In vitro* release studies of the rings were carried out in water and simulated vaginal fluid. The concentrations of TFV and ACV released into solution were determined by UV-vis and HPLC.

**RESULTS:** *In vitro* release from segments containing a single pod was measured for delivery window diameters from 0.35–2.0 mm, and was in the range 2–430 µg/day (TFV) and 3–81 µg/day (ACV). For TFV, a 30-day study showed release rates of  $43.5 \pm 5$  µg/day from single pod segments ( $n=10$ ) and  $385 \pm 57$  µg/day release from 10-pod rings ( $n=10$ ), both with 1.0 mm delivery windows. For single ACV pod segments, a 1.2 mm delivery window resulted in 14 µg/day release (30 days,  $n=4$ ). Segments containing one TFV and one ACV pod (0.5 mm delivery windows), showed simultaneous release rates of 11 µg/day TFV and 6 µg/day for ACV ( $n=3$ ).

**CONCLUSIONS:** An IVR delivery platform for simultaneous, independently-controlled release of multiple microbicides has been developed and evaluated for *in vitro* release characteristics of TFV and ACV. Release rates for each drug can be controlled independently, and tailored to specific dose requirements by varying the delivery window size during manufacture.

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## Assessing HIV-1 Cross-Resistance *In Vitro* against RTI-based Microbicides Currently Under Development

P. Selhorst<sup>1</sup>, K. Terrazas-Aranda<sup>1</sup>, J. Michiels<sup>1</sup>, L. Heyndrickx<sup>1</sup>, A. Vazquez<sup>2</sup>, M. Quinones-Mateu<sup>2</sup>, K. Ariën<sup>1</sup>, G. Vanham<sup>1,3</sup>

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<sup>2</sup>Diagnostic Hybrids, Cleveland, OH, USA;

<sup>3</sup>University of Antwerp, Antwerp, Belgium

**BACKGROUND:** The use of microbicides based on reverse transcriptase inhibitors (RTI) by undiagnosed, HIV+ woman could potentially induce viral resistance compromising subsequent therapeutic options. Moreover, RTI-based microbicides that are inactive against resistant strains might promote the selective transmission of these viruses. Therefore, we investigated the resistance profile of four RTI currently under development as a microbicide (TMC120, UC781, MIV160, TNF).

**METHODS:** Resistance against these four microbicides and two first-line therapeutics (NVP and EFV) was induced by serial passage of three HIV-1 isolates (subtype B, C, CRF02\_AG) in PBMC with increasing compound concentrations. Inhibition of these resistant viruses was evaluated (i) in TZM-bl cells for the four RTI microbicides and the therapeutics AZT, NVP, DLV, EFV and (ii) using a HIV-1 Phenotyping Assay (VIRALARTSTM/HIV) to test all FDA-approved PI, RTI and INI. A full resistance matrix was obtained for each candidate RTI-based microbicide. Resistance associated mutations (RAMs) were identified by sequencing the viral pol gene and resistance was expressed as intermediate (FC<100) or complete (FC>100). Replicative fitness of p2/p7/p1/p6/PR/RT/INT-recombinant viruses was determined using viral growth kinetics and growth competition experiments.

**RESULTS:** HIV-1 resistance to the NNRTI UC781 was induced *in vitro* within five weeks while resistance to the NNRTI TMC120 and MIV160 only occurred after twelve weeks. Virus resistant to the NtRTI TNF could not be generated under these conditions. As expected, viruses resistant to the NNRTI TMC120, UC781 or MIV160 remained fully sensitive to the NRTI AZT, but lost all sensitivity to NVP and DLV. Although EFV lost all activity against MIV160 resistant virus, it retained some activity against TMC120 and UC781 resistant strains. Viruses resistant to EFV were no longer suppressed by TMC120, UC781, MIV160. NNRTI-resistant viruses showed a decrease in viral replicative fitness. Cross-resistance was not observed with the NtRTI candidate microbicide TNF.

**CONCLUSIONS:** The large scale introduction of mono-RTI-based microbicides should therefore be considered with caution.

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## A Two-Compartment *In Vitro* Release Model for the Testing of HIV Microbicide Formulations

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**BACKGROUND:** *In vitro* release testing of vaginal formulations is usually performed in a one-compartment model (OCM) where the release medium, usually comprising pH-adjusted water, an aqueous surfactant solution or a solvent-water solution, provides sink conditions throughout the release experiment. Although this model is useful in evaluating the effect of formulation parameters upon release, it rarely reflects *in vivo* conditions. Here we report use of a two-compartment diffusion cell model (TCM, comprising a small volume donor, a large volume receptor, and separated by a model epithelial membrane) to more closely mimic *in vivo* vaginal release and tissue absorption following administration of a UC781 vaginal ring.

**METHODS:** Macaque-sized matrix silicone elastomer vaginal rings containing 100mg UC781 were prepared by injection molding, and *in vitro* release testing performed using both OCM (20mL simulated vaginal fluid, SVF) and TCM (5mL SVF in donor cell and variable quantities of Tween 80; silicone elastomer membrane; 100mL 3:2 ethanol/water in receptor cell). In the TCM, drug levels were measured by HPLC in both donor and receptor cells, representing fluid and tissue levels respectively. Rings containing 100mg UC781 and 10% w/w Tween 80 were also manufactured and tested

**RESULTS:** The amount of UC781 released from rings was significantly influenced by the choice of release model. Greatest release (56mg/14 days) was measured in the ethanol/water OCM, compared with no measurable release into SVF only. Increasing the concentration of Tween 80 in the SVF medium (1, 3 and 5% w/w) led to increased UC781 release (11, 16 and 18mg, respectively), demonstrating that vaginal fluid solubility of UC781 may be rate-determining *in vivo*. In the TCM, UC781 accumulates in the receptor cell medium over time, despite not being measured in the donor medium containing the ring device. Incorporation of Tween 80 directly into the ring provided enhanced release in both donor and receptor cells.

**CONCLUSIONS:** Release of UC781 was influenced by the choice of release medium and the inclusion of Tween 80 in the ring. Although use of SVF-only in the OCM indicated no measurable UC781 release from rings, data from the TCM confirms that UC781 is not only released but is also capable of penetrating across the model epithelial membrane. The TCM may therefore provide a more representative *in vitro* release model for mimicking *in vivo* absorption.

# POSTER SESSIONS

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## Modulation of Epithelial Innate Immune Function by *T. vaginalis*

R. Fichorova<sup>\*1</sup>, V. Tang<sup>1</sup>, R. Trifonova<sup>1</sup>, G. Hayes<sup>2</sup>, D. Beach<sup>2</sup>, B. Singh<sup>2</sup>

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**BACKGROUND:** Worldwide the extracellular parasitic protozoan *Trichomonas vaginalis* (TV) infects over 180 million people annually and causes trichomoniasis, the most common non-viral sexually transmitted disease. Trichomoniasis has been linked to increased HIV-1 transmission, and other serious public health problems e.g. preterm birth, cervical cancer and bacterial vaginosis. The predominant cell surface glycoconjugate of the parasite, the TV lipophosphoglycan (LPG) plays a critical role in the parasite adhesion and mediates host inflammatory responses to infection. The molecular mechanisms of parasite-host interactions and related risks of viral infection and complications have not been fully understood to date.

**METHODS:** Immortalized and primary cervical and vaginal epithelial cells, TLR- and galectin-deficient cell lines were exposed to multiple strains of *T. vaginalis* or purified *T. vaginalis* LPG and bacterial pathogenic determinants. These exposures were also tested in the presence of physiologic concentrations of steroid hormones. Innate immune responses were measured by multiplex protein, phosphoprotein and mRNA analysis. ANOVA was used to analyse differences between the treatment groups.

**RESULTS:** *T. vaginalis* and LPG induced TLR-4 independent and prostaglandin-enhanced production of proinflammatory mediators e.g. IL-8 and prostaglandin PGE<sub>2</sub> and upregulated the expression of several members of the galectin family of carbohydrate recognizing proteins known to be involved in the HIV pathogenesis. Purified LPG and *T. vaginalis* showed high affinity binding to galectins modulating the vaginal inflammatory responses and HIV replication e.g. gal-1 and gal-3. Galectin-deficient cell lines failed to respond to LPG.

**CONCLUSIONS:** These results suggest pathogenic mechanisms underlying the epidemiologic association between trichomoniasis and HIV infection risk.

## SESSION 30

### Poster Discussion (PD8): Clinical Trial Adherence, Acceptability, and Retention

**Moderators:** Ariane Van der Straten, Yasmin Halima

Monday, May 24, 3:00 pm–4:00 pm

Rooms 403–405

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## Biophysical & Behavioral Acceptability of Microbicides

S. Verguet<sup>\*1</sup>, B. Young-Holt<sup>2,3</sup>, A.J. Szeri<sup>4</sup>

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**BACKGROUND:** First generation microbicide candidates are delivered via gel vehicles, intended to coat the vaginal epithelium before protected sexual intercourse for the user. The waiting time before protection depends on biophysical properties of the gel, which restricts the potential choices for an effective product. In other words, the gel vehicle first must be physically synthesizable, then acceptable to the user, and finally applied in a manner that promotes adequate coating by the time it is to function. We explore here women's preferences about microbicide attributes when biophysical constraints are imposed upon their choices.

**METHODS:** Microbicide acceptability was assessed using structured questionnaires (n=70) with socio-economically diverse women aged 18–55 from California. Participants were asked to provide their preferences about different microbicide attributes, namely the wait time between application and intercourse, and the gel texture. The trade-off between wait time and gel texture was assessed by a mathematical model constraining coating rates upon changes of the gel's physical attributes. Preferences were ranked on a scale from 1 to 7. We asked independently about the first two attributes and then about the trade-off item when the biophysical constraints necessary for the product effectiveness were applied.

**RESULTS:** Results showed that women in steady relationships are more likely than women in casual relationships to prefer a microbicide gel that spreads very fast ( $p < 0.000001$ ) and that is thick ( $p < 0.001$ ), when they were asked the questions independently. However, when they were asked the same question 'constrained' by the biophysical reality, we observed that a quasi-uniform preference for gels of different thicknesses (and so waiting times) stood out ( $p > 0.05$ ).

**CONCLUSIONS:** Biophysical constraints do matter and women's preferences regarding microbicide attributes change when biophysical constraints restrict the range of options. In the case of gel vehicle microbicides, we observe no distinct preference for each of the gels available after controlling for physical reality. In that sense, developers should offer a range of formulations in order to address the preference of all users.

# POSTER SESSIONS

SESSION 30  
Monday, May 24, 2010

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## Socio-Behavioral Approaches to Maximize Adherence in a Phase 3 PrEP Trial

A. Corneli<sup>\*1</sup>, J. Murphy<sup>1</sup>, V. Mtimkulu<sup>3</sup>, K Ahmed<sup>3</sup>, K. Agot<sup>2</sup>, J. Odhiambo<sup>2</sup>, C. Wong<sup>1</sup>, M. Lanham<sup>1</sup>, L. Johnson<sup>1</sup>, L. Van Damme<sup>1</sup>

<sup>1</sup>Family Health International, Research Triangle Park, NC, USA; <sup>2</sup>IMPACT Research & Development Organization, Bondo, Kenya; <sup>3</sup>Setshaba Research Centre, Soshanguve, South Africa

**BACKGROUND:** HIV pre-exposure prophylaxis (PrEP) will probably face the same challenge in adherence to study product as microbicide clinical trials. FEM-PrEP—a phase 3, oral PrEP clinical trial for HIV prevention in women—uses socio-behavioral formative research and behavioral monitoring to try to maximize adherence.

**METHODS:** Prior to the clinical trial, 45 in-depth interviews (IDIs) and 12 focus group discussions (FGDs) were conducted with potential trial participants in Bondo, Kenya, and Pretoria, South Africa, to identify potential barriers and facilitators to study product adherence. All IDIs and FGDs were recorded and transcribed/translated into English for content analysis. Data were used to inform adherence counseling messages, procedures, and tools.

During the trial, monitoring of adherence data is conducted regularly to identify common reasons for missing study pills and adherence trends.

**RESULTS:** A key finding from the formative research was that 66% of women in Bondo and 79% in Pretoria said it would be difficult not to share or sell the study product. Reasons included wanting to help an HIV-positive friend or another participant, needing money, or believing that the pill is effective. In response, adherence counseling messages were tailored at each site to include site-specific reasons for not sharing/selling study pills.

Monitoring of the trial adherence data found that the main reasons for missing the study pill included participants being away from home, simply forgetting to take the pill, and having a change in normal daily activities. In response, counselors assess these issues with each participant during adherence counseling.

**CONCLUSIONS:** Formative data inform adherence messages, counseling, and tools by tailoring them to include issues pertinent to the study population at each site. Counselors use trial adherence data to provide additional counseling on adherence barriers commonly found among trial participants.

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## Vaginal Practices Among Nigerian Women and Its Implication for Microbicide Development and Use

O. Ezechi<sup>\*1</sup>, J.C. Enemuoh<sup>2</sup>, K.S. Oyedele<sup>1</sup>

<sup>1</sup>Nigerian Institute of Medical Research, Lagos Nigeria, <sup>2</sup>Sister of Love, Okota, Lagos Nigeria

**BACKGROUND:** The urgency and need to quickly deploy a safe and effective microbicide requires simultaneous research into factors that may impact on their effectiveness at community level. This study assessed the types and pattern of vaginal practices among Nigerian women.

**METHODS:** Questionnaire based cross-sectional survey of women seen within a predefined strata to determine the type and pattern of vaginal practices and use of vaginal products. Data analysis with SPSS.

**RESULTS:** Majority were married (53.4%) and above 30 years of age (53.7%). Multiple sexual partnerships rate were 18.0%. Contraceptive uses rate was low with consistent condom use rate of 29.4%. Partner refusal (59.7%), and reduction of sexual pleasure (32.9%) were the reasons for non use of condom. 94.5% of the women have used a vaginal product for hygiene, enhancement of sexual pleasure, to please my partner, medication and prevention of pregnancy. Vaginal products used were petroleum jelly, commercial douching preparation, pessaries, gel, herbs and alum. Majority (91.7%) reported washing the vagina after sex with water, soap or antiseptic solutions. 30.3% have heard about microbicide, however majority will use it if found to protect against STI and HIV infection (89.7%) and prefers gel form (97.2%) to a suppository because gels are less messy (76.6%) and serves as lubricant (36.8%). Non availability and cost will be limitation.

**CONCLUSIONS:** Various forms of vaginal practices exists among Nigerian women that might impact on the use and effectiveness of microbicide. It is recommended that future microbicide trial should take this finding into consideration.

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## Acceptability of Optical Coherence Tomography During a Clinical Study

C.R. Breitkopf<sup>\*1</sup>, M.L. Loza<sup>1</sup>, K.L. Vincent<sup>1</sup>, L.R. Stanberry<sup>2</sup>, S.L. Rosenthal<sup>2</sup>

<sup>1</sup>University of Texas Medical Branch, Galveston, TX, USA; <sup>2</sup>Columbia University Medical Center, New York, NY, USA

**BACKGROUND:** Optical coherence tomography (OCT) has shown promise in animal studies for non-invasive evaluation of microbicide safety. Translating OCT into clinical trials requires participants to tolerate the procedure. Research on colposcopy experiences has focused on the context of diagnostic evaluations. Understanding women's experiences will allow research staff to provide useful information to participants.

**METHODS:** As part of a clinical trial, 30 U.S. women have a pelvic exam and pap smear at a screening visit, and 3 visits with colposcopy and OCT (baseline, 6 hours post-use of 5.5 days of gel use, 1 week later). In qualitative interviews, they were queried about experiences of the gynecologic exam. Participants are reimbursed \$475.

**RESULTS:** Data regarding the experience of OCT and colposcopy have been analyzed for 15 of 26 enrolled women. Data analysis will be completed prior to the meeting. The women are 29 years with 10 being Hispanic, 10 white, 4 African-American and 2 Asian. Women found the process acceptable, were intrigued by having images taken, and appreciated developing better ways to look at the vagina. During the exam, findings verbally reported for documentation had the potential to cause worry since the women heard unfamiliar words (e.g. erythema); one searched the words on the Internet. Some women found that the speculum rotation of the OCT exam caused mild discomfort. The post-product use exam was experienced by some as uncomfortable, believed to be related to irritation from the gel. Others believed the exams got easier as the gynecologist gained experience with their anatomy. With regards to frequency of exams, some believed that the week between exams allowed their vagina to recover and tolerate another exam.

**CONCLUSIONS:** Among women willing to be in a study requiring multiple pelvic exams, colposcopy/OCT were acceptable and intriguing. Women experienced but tolerated mild discomfort from the procedure, and some found the ancillary conversations worrisome. Research staff should provide participants with information about the range of women's experiences, and be sensitive to what they may overhear.

# POSTER SESSIONS

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## Innovative Strategies in a Phase IIB Microbicide Trial Results in High Retention—Experiences from CAPRISA 004

J. Frohlich, F. Ntombela, S. Maarschalk, N. Yende, S. Abdoor Karim, Q. Abdoor Karim, and the CAPRISA Vulindlela Recruitment and Retention Team

Centre for AIDS Programme of Research in South Africa. CAPRISA

**BACKGROUND:** Microbicide trials need to include effective retention strategies to retain study participants in order to ensure scientific integrity and adequate statistical power. Maintaining a retention rate of 90% per annum is especially challenging in rural, poorly-resourced and hard to reach communities. Vulindlela, a rural community in KwaZulu-Natal does not have street maps, street names, house numbers or reliable telephone contact information. The purpose of this study was to assess the implementation of newly developed CAPRISA 004 retention strategies to attain the protocol required retention target of 90% per annum.

**METHODS:** HIV negative sexually active 18–40 year-old women (N=614) were enrolled over 18 months. Follow-up assessments were conducted monthly. The retention strategies were developed through ongoing feedback and dialogue with the resident members, the CAPRISA Community Research Support Group (CRSG) and study participants in Vulindlela. The strategy includes: community mapping with transect walks, a local resource inventory, descriptive maps drawn by participants to illustrate the route from their houses to the CAPRISA rural site; daily information sessions; ongoing information sessions to ensure better understanding of compliance to visit schedule; encouraging male involvement; participant recognition and annual certificate award; communication and relationship skills-building; daily monitoring of retention rates by dedicated retention team with remedial action and flexible clinic hours. Challenges incurred during implementation and regular feedback from participants continually informed the ongoing adjustments to the site retention strategy. Retention rates were calculated by dividing the number attending the clinic by the number expected to attend.

**RESULTS:** Vulindlela is one of two sites in the CAP 004 study. Only the Vulindlela data are reported here. Volunteers were followed for a minimum of 12 months and a maximum of 30 months. With 520 participants in active follow-up, the overall study retention rate was 95.9% at study exit. The average monthly and quarterly retention rate was 90% and 93.8% respectively. Loss to follow-up (n=21), deaths due to natural causes (n=2), inappropriate enrolment (n=1), voluntary withdrawal (n=3) impacted on retention rates in this highly challenging rural environment.

**CONCLUSION:** In this rural community a retention rate of greater than 90% was achieved through involvement of community members, the CAPRISA CRSG, and iterative participant feedback. Retention monitoring is a demanding ongoing process requiring extensive partnership with community members, teamwork from site staff. Adequate resources are critical in achieving high retention rates in rural settings.

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## The Use of Quality Control Panels in Clinical Trials

T. Crucitti\*, K. Fransen<sup>1</sup>, G. Beelaert<sup>1</sup>, S. Abdellati<sup>1</sup>, V. Cuylaerts<sup>1</sup>, T. Vermoesen<sup>1</sup>, L. Van Damme<sup>2</sup>

<sup>1</sup>HIV/STI Reference Laboratory, Institute of Tropical Medicine, Antwerp, Belgium; <sup>2</sup>Family Health International, Research Triangle Park, NC, USA

**BACKGROUND:** The laboratories collaborating in the FEM-PrEP (Truvada®) phase III clinical trial receive at regular intervals quality control panels for analysis. The aim of the quality control is to assess the performance of the laboratory and to take corrective actions if needed. Quality control panels should cover all analysis as requested by the study protocol and be as much as possible similar to the specimens collected from study participants. Commercially available quality control panels usually do not meet those criteria.

**METHODS:** The following quality control (QC) panels are distributed across the FEM-PrEP collaborating laboratories

ANALYTE	NATURE OF SPECIMEN	STORAGE CONDITION	PREPARED BY
Urine rapid pregnancy test	Urine from pregnant woman	Frozen	Home-made
Urine dipstick	Urine, spiked	Frozen	Home-made
HIV rapid tests	Plasma	Frozen	Home-made
HbsAg rapid test	Plasma	Frozen	Home-made
HbsAb	Plasma	Frozen	Home-made
Syphilis	Serum	Frozen	Home-made
<i>C. trachomatis/</i> <i>N. gonorrhoeae</i> PCR	Diluted PBS, spiked	Frozen	Home-made
Chemistry	Freeze dried	Room temperature	Commercial
Nugent score	Vaginal smear or photograph	Room temperature	Home-made
CD4 count	Fresh blood in stabilization tube	Room temperature	Home-made

The chemistry panels are left-over panels from the Belgian national external quality assessment (EQA) scheme. All study laboratories receive the same panels except for the Nugent score panels.

The freeze-thaw and temperature stability of the panels consisting of frozen specimens was validated. The shipment of the frozen panels to the collaborating laboratories is performed using a dry shipper with temperature registration.

**RESULTS:** Quality control panels are tested by the collaborating study laboratories every 4 months, the HIV rapid testing QC panels are tested every 2 months. Back-up panels are stored at the ITM and tested when discordant results are obtained. The results of the chemistry panel are evaluated by comparing the results with the consensus results obtained by the Belgian laboratories using the same method and analyzer. In the event of a discordant result, the potential reason is investigated in collaboration with the local laboratory, and remediation is sought. The impact on the study results is evaluated.

**CONCLUSIONS:** The inclusion of quality control panels is part of a quality assurance program. It is uttermost important that the quality of the control panels itself is guaranteed. Therefore panels should be characterized and validated.

# ORAL SESSIONS

## SESSION 31

Monday, May 24, 2010

## SESSION 31

### Oral Abstracts (OA16): Biomarkers in the Female Genital Tract

**Moderators:** Jules O'Rear, Jeanna Piper

Monday, May 24, 4:30 pm–6:15 pm

Ballroom

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### Gene Expression Profile of Vaginal Epithelial Cells Exposed to Pro-Immunoinflammatory Agents—Identification of Novel Biomarkers of Mucosal Safety

I. Zalenskaya\*, N. Yousefieh, T. Joseph, S. Schriver, L. Cote, G. Doncel

CONRAD, Eastern Virginia Medical School, Norfolk, VA, USA

**BACKGROUND:** Susceptibility to HIV infection may be increased by cervicovaginal mucosal inflammation and immune activation. Therefore, it is important to develop biomarkers that identify these conditions and can be used in the selection of safe and efficacious anti-HIV microbicide candidates. The purpose of this study was to analyze gene expression profiles of human vaginal cells exposed to proinflammatory and noninflammatory compounds in order to identify these biomarkers.

**METHODS:** VK2/E6E7 cells were treated with non-inflammatory (NIC) and pro-inflammatory (PIC) compounds. NIC (class 1) included hydroxyethyl and carboxymethyl cellulose. PIC included cytotoxic surfactant compounds, nonoxynol-9, C31G and benzalkonium chloride (class 2), TLR ligands, PAM3CSK4, MALP-2 and imiquimod, and the pro-inflammatory cytokine, TNF-alpha (class 3). Gene expression was evaluated by cDNA microarray technique using Affymetrix oligonucleotide GeneChip Human Genome U133 Plus 2.0. Genes showing statistically different expression between groups were identified using ANOVA or t-test with false discovery rate set to 0.05.

**RESULTS:** Comparison between PIC and NIC groups identified around 500 differentially expressed genes in class 2 and about 100 genes in class 3 with at least 2-fold upregulation compared to class 1. Among them, about 80 genes were upregulated in both PIC groups reflecting common cellular responses. Gene set enrichment analysis revealed that the Hinata NFkB pathway (genes activated by NFkB) was upregulated in most PIC treatments. Eleven genes were selected for further analysis. Most of these genes are involved in immune and/or inflammatory responses. A custom-made heatmap allowed for easy identification of PIC and NIC. Microarray results for these genes were confirmed by qPCR. Protein expression of four selected genes, CCL20, PTGS2, IL8 and IL13RA2 was detected by immunoblot, flow cytometry, or ELISA.

**CONCLUSIONS:** Gene expression profiling revealed a group of genes that are significantly upregulated in human vaginal cells in response to immunoinflammatory stimuli, and therefore, can serve as biomarkers of genital mucosal inflammation and immune activation.

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### Comparison of Swabs, Lavage and Diluents to Quantify Candidate Biomarkers for Microbicide Safety and Genital Tract Mucosal Immunity

C.S. Dezzuti<sup>1\*</sup>, C.W. Hendrix<sup>2</sup>, J. Marrazzo<sup>3</sup>, F. Gai<sup>4</sup>, L. Wang<sup>4</sup>, F. Hladik<sup>3</sup>, U. Parikh<sup>1</sup>, J. Mellors<sup>1</sup>, S. Hillier<sup>1</sup>, B.C. Herold<sup>5</sup>

<sup>1</sup>For the Microbicide Trials Network Biomedical Sciences Working Group University of Pittsburgh, Pittsburgh, PA, USA; <sup>2</sup>Johns Hopkins University, Baltimore, MD, USA; <sup>3</sup>University of Washington, Seattle, WA; <sup>4</sup>SCHARP Statistical Center, Seattle, WA, USA; <sup>5</sup>Albert Einstein College of Medicine, Bronx, NY, USA

**BACKGROUND:** Microbicides could induce changes in the genital tract that augment or interfere with product efficacy. Collection of biological samples to measure changes in the genital tract is a critical adjunct to clinical studies. However, the optimal methods of sample collection and storage are not known.

**METHODS:** Genital tract fluid was collected from healthy women (n=24) and from women with bacterial vaginosis (BV) (Nugent score  $\geq 7$ ) (n=23) by Dacron (Puritan 25-806) or flocked (Copan) vaginal and cervical swab (collected from each subject at each site diluted into 1 mL of saline), and by cervicovaginal lavage (CVL) with 10 mL of saline (n=11), Normosol (n=22) or water (n=14). Samples were centrifuged and supernatants were divided into aliquots and stored for analysis of the levels of endogenous anti-HIV (TZM-bl assay) and anti-*E. coli* (reduction of *E. coli* growth) activity and the concentration of cytokines, chemokines and antimicrobial proteins (Luminex or ELISA). Mixed-effect models that take into account correlations among repeat measures were used to compare differences in the levels of cytokines, chemokines, and anti-microbial activities among different diluents, sample type, and BV groups. Kruskal-Wallis test was used to compare differences of defensin concentrations between CVL diluents.

**RESULTS:** Significantly greater anti-HIV activity was recovered from genital tract fluid collected by CVL in saline (median [range]; 71% [12, 110]) compared to CVL in Normosol (43% [-7, 63]) (mean difference: 41%, p-value: 0.02) or from Dacron vaginal (49% [-118, 91]) (35%, 0.01) or cervical (27% [-112, 100]) (51%, <0.0001) swabs. Flocked swabs (62% [-42, 117]) had greater anti-HIV activity than Dacron swabs (42% [-118, 100]) (26%, <0.0001). CVL collected in saline (65% [48, 100]) or Normosol (79% [20, 200]) inhibited *E. coli* growth equally well while vaginal swabs had no significant reduction in endogenous *E. coli* activity. In general, flocked swabs had greater anti-*E. coli* activity than Dacron swabs (18%, 0.01). Overall, cervical swabs had approximately 0.5 log<sub>10</sub> higher levels of cytokines, chemokines, and antimicrobial proteins as compared to vaginal swabs. Likewise flocked swabs had approximately 0.5 log<sub>10</sub> higher levels of most mediators compared to Dacron swabs. BV was associated with loss of anti-*E. coli* activity (p-value: 0.06).

**CONCLUSIONS:** CVL collected with saline and flocked swabs may provide the best samples for use in biomarker studies.

# ORAL SESSIONS

## 210

### Anti-HIV Activity in Cervical-Vaginal Secretions from HIV-Positive and HIV-Negative Women Correlates with Innate Antimicrobial Factors and Disease Progression

M. Ghosh<sup>\*1</sup>, J. Fahey<sup>1</sup>, Z. Shen<sup>1</sup>, T. Lahey<sup>1</sup>, S. Cu-Uvin<sup>2</sup>, K. Mayer<sup>1</sup>, C. Wira<sup>1</sup>

<sup>1</sup>Dartmouth Medical School, Lebanon, NH, USA;

<sup>2</sup>Brown University, Providence, RI, USA

**BACKGROUND:** Previous studies from our laboratory have shown that secretions from isolated human female reproductive tract epithelial cells contain a spectrum of endogenous antimicrobials, some with anti-HIV-1 activity. Recognizing that the rate of heterosexual transmission per coital act is very low (1:122-1:1000), we hypothesized that cervical-vaginal lavages (CVL) of women contain endogenous antimicrobials that are protective against pathogens.

**METHODS:** CVL were collected from 15 HIV(-) and 57 HIV(+) women by washing the cervical-vaginal area with 10cc neutral PH saline solution. HIV(+) women were categorized according to peripheral CD4 counts with Group 1 cut-off values set at >350, Group 2 at 200-350, and Group 3 at <100. None were not on ARV. To measure anti-HIV activity, CVL were pre-incubated with R5/M-tropic, X4/T-tropic, and transmitted/founder HIV-1 viruses and added to indicator cell line TZM-bl to measure infection. Antimicrobials and IgG levels were quantified using ELISA assays.

**RESULTS:** In both the HIV(+) and HIV(-) CVL, we found a range of anti-HIV-1 activity against all viral strains, including the transmitted/founder virus. A particular CVL sample showed differential activity against different strains of viruses ranging from zero to 100%. The overall anti-HIV activity decreased with disease progression (as defined by low CD4 counts) and no activity was observed in samples with CD4<100. ICVL HIV viral load was positively correlated with levels of HDB2, Elafin, and SLPI, MIP3 $\alpha$ , IL8, IL1 $\alpha$ , and IL1RA suggesting the involvement of local immune drivers. Although HIV(-) CVL showed similar levels of HIV neutralizing activity, we did not observe significant correlations with the antimicrobials tested so far indicating other innate and adaptive factors might be involved.

**CONCLUSIONS:** Our data demonstrate that both HIV(+) and (-) CVL contain endogenous antimicrobials that are capable of inhibiting HIV-1 infection but this intrinsic activity might be lost as a result of general failure of the immune system due to development of AIDS. The identification of some of the antimicrobials responsible for this activity suggests that the presence of these factors may, in part, explain why the frequency of infectious virus in CVL is low, and also the relatively low rate of heterosexual transmission per coital act.

## 211

### Vaginal Distribution of Cell-Free and Cell-Associated HIV Surrogates Following Simulated Intercourse

N.A. Louissaint<sup>\*1</sup>, S. Nimmagadda<sup>2,3</sup>, R.P. Bakshi<sup>1</sup>, P. Rahul<sup>1</sup>, J. Anderson<sup>1</sup>, E.J. Fuchs, J. Edward<sup>1</sup>, K. King<sup>1</sup>, K. Macura<sup>1,3</sup>, C.W. Hendrix<sup>1,2</sup>

<sup>1</sup>Departments of Pharmacology and Molecular Sciences; <sup>2</sup>Medicine; <sup>3</sup>Radiology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA, 21287

**BACKGROUND:** To inform development of topical HIV microbicide formulations designed to prevent sexual HIV infection, we developed methods to quantify HIV distribution within the lumen and tissues of the FGT. The goal of this feasibility study was to explore the luminal distribution of cell-associated and cell-free HIV surrogates in the female genital tract (FGT) following simulated vaginal intercourse.

**METHODS:** Apheresis-derived autologous lymphocyte-rich cells were radiolabeled with 100 $\mu$ Ci <sup>111</sup>Indium-oxine (cell-associated HIV surrogate). Along with 500 $\mu$ Ci <sup>99m</sup>Tc-sulfur colloid (HIV-sized 100nm particle, cell-free HIV surrogate), labeled cells were resuspended in 3mL hydroxyethylcellulose gel semen simulant. An artificial phallus with urethra was used for simulated coitus and dosing of HIV surrogates via ejaculation. Post-dosing, dual isotope SPECT/CT† and MRI‡ images were acquired at 1 $^{\circ}$ , 2 $^{\circ}$ , 4 $^{\circ}$ , and 5 $^{\circ}$  hours with energy windows discriminating <sup>111</sup>In from <sup>99m</sup>Tc signal. 7 hours post-dosing, tissue biopsies were collected, and direct sampling (using endoscopic brushes) was carried out in 4 locations in the FGT. Enzymatic digestion was performed on the tissue biopsies to obtain total and CD4 cells (obtained via magnetic cell sorting). Radioactivity was measured using a  $\gamma$ -counter.

**RESULTS:** SPECT/CT and MR analysis shows distribution in the vagina with the highest signal intensity in the fornices. There was no detectable signal in the endometrial space. The T1-weighted MRI shows gadolinium chelate signal distributed throughout the vagina. SPECT/CT image analysis revealed an average of 63% coincidental distribution of the cell-free and cell-associated HIV surrogates. The dose was retained up to 4 hours post-dosing, with approximately 1/3 of the radioactivity present. Direct assessments with endoscopic brushes correlate with SPECT/CT. SPECT/CT and MR distributions coincide.

**CONCLUSIONS:** There is no evidence of distribution of HIV surrogates (autologous lymphocytes and HIV-sized particles) into the uterine cavity up to five hours after simulated intercourse. SPECT/CT and MRI were useful to determine distribution and the retention of the surrogates over time. Surrogate distribution suggests that topical microbicides do not need to migrate to the uterus for efficacy. This feasibility study demonstrates the use noninvasive dual-isotope and MR imaging, combined with simulated intercourse to analyze HIV surrogate distribution.

## 212

### A Phase I Study to Assess Genital Tract Mucosal Immunity Following Repeated Vaginal Application of 1% Tenovir Gel

M.J. Keller<sup>\*1</sup>, R.P. Madan<sup>1</sup>, N.M. Torres<sup>1</sup>, S. Cho<sup>1</sup>, S. Eisinger<sup>2</sup>, J. Chen<sup>2</sup>, E. Carroll<sup>1</sup>, A. Lee<sup>1</sup>, C.W. Hendrix<sup>2</sup>, B.C. Herold<sup>1</sup>

<sup>1</sup>Albert Einstein College of Medicine, Bronx, NY, USA; <sup>2</sup>Johns Hopkins University, Baltimore, MD, USA

**BACKGROUND:** Previous studies highlight the need to expand biomarkers of efficacy & safety in Phase I studies. The objective of this study was to examine the effect of 14 daily vaginal applications of 1% tenofovir (TFV) (cases) or placebo gel (controls) on mucosal immune mediators & endogenous antimicrobial activity. A secondary aim was to evaluate pharmacokinetics (PK) & pharmacodynamics (PD) by examining the anti-HIV activity in cervicovaginal lavage (CVL).

**METHODS:** A prospective, randomized, double-blind, placebo-controlled study was conducted among 30 healthy, sexually abstinent women. Vaginal pH, total protein, concentrations of cytokines, chemokines, antimicrobial proteins & endogenous activity (anti-HSV & anti-*E. coli*) were quantified in CVL collected on days (D) 0, 3, 7, 14 & 21. Peripheral blood mononuclear cells (PBMCs) & endocervical cells (via cytobrush on D14) were obtained for analysis of TFV diphosphate (TFV-DP) concentrations by validated methods. PD were assessed by comparing the anti-HIV activity in CVL. Continuous variables were compared by t test. Changes within groups for D0-21 were compared by one-way repeated measures ANOVA.

**RESULTS:** 56 women were screened, 30 enrolled & 26 completed the study (12 TFV, 14 placebo). The gels were well tolerated and adverse events were similar in the 2 groups. There was a small, but significant increase in vaginal pH on day 14 for cases compared to controls (pH 4.93 vs 4.65, p< 0.01). TFV or placebo use was not associated with significant changes in inflammatory cytokines, chemokines or antimicrobial proteins. There was no loss in endogenous anti-*E. coli* or anti-HSV activity. Anti-HIV activity (mean % inhibition  $\pm$ SD) in CVL from cases was significantly higher on D3 (81 $\pm$ 22), 7 (79 $\pm$ 27) & 14 (76 $\pm$ 36) compared to D0 (33 $\pm$ 45) (p<0.001) & the activity was greater than that observed in CVL from controls on D3 (p=0.001), 7 (p=0.07) & 14 (p=0.03). The concentration of TFV-DP in PBMCs on D3-21 was below the lower limit of quantitation for all subjects except one. In the cytobrushes, TFV-DP was detectable in all subjects & ranged from 41.5-4460 fmol/sample.

**CONCLUSIONS:** Repeated vaginal application of 1% TFV gel was not associated with a reduction in endogenous antimicrobial activity, loss of protective mediators or a proinflammatory response. CVL from women who applied TFV had significantly increased anti-HIV activity, which may reflect the activity of residual drug in genital tract secretions.

**213****Colposcopy: Still Useful in Microbicide Safety Trials?**C. Mauck<sup>\*1</sup>, D.H. Weiner<sup>2</sup>, J. Lai<sup>2</sup><sup>1</sup>CONRAD, Arlington, VA, USA; <sup>2</sup>Family Health International, Research Triangle Park, NC, USA

**BACKGROUND:** Colposcopy has been standardized for microbicide research. Epithelial disruption is assumed to be of most concern, since grossly observed disruption was associated with HIV infection in one trial. However, colposcopically observed disruption has not been correlated in a Phase III trial with risk of HIV, nor has any other colposcopic finding. The justification for continuing colposcopy has recently been questioned. This secondary analysis assessed what would have been missed in 9 microbicide safety trials if colposcopy had not been done.

**METHODS:** 9 phase I microbicide safety trials conducted between 2002 and 2008 with data on whether findings were seen by both naked eye exam and colposcopy or by colposcopy alone were reanalyzed. Findings were counted each time observed.

**RESULTS:** 693 colposcopic findings were observed. Of these, 317 (45.7%) were seen by colposcopy alone and would have been missed if colposcopy had not been done. 26 findings of deep epithelial disruption were observed, 7 (26.9%) by colposcopy alone. Analogous figures for peeling, petechiae/echymoses and erythema were 49/94 (52.1%), 112/195 (57.4%), and 97/254 (38.2%), respectively. The 7 deep findings were < 5mm and involved 1 woman in each of 4 studies. Six were lacerations and 1 an abrasion. Six were on the cervix and 1 on the vaginal wall. 331/693 findings were small (<0.5cm): of these, 199 (60.1%) were seen only by colposcopy. 209 large (>1 cm) findings were seen, 51 (24.4%) of which were seen only by colposcopy. The anatomical site with the greatest proportion of findings that would have been missed was the cervix: 196/378 (51.9%).

**CONCLUSIONS:** Almost half of findings observed in these 9 trials would have been missed without colposcopy. A quarter of the deep disruptions would not have been seen, consistent with an earlier small study designed to look at this question, although such findings are rare. The clinical significance of more common findings, e.g. erythema, is unknown. Somewhat surprisingly, about a quarter of large findings would have been missed, as would about half of findings on the relatively visible cervix. While colposcopic findings have not been validated as an indicator of HIV risk, neither has any other safety marker. Colposcopy is subjective and dependent on examiner effort, but discontinuing colposcopy in safety trials would be premature until we have a more objective, validated replacement. Efforts are underway to identify such a marker.

**214****Natural Variability of Phosphate Complicates Safety Monitoring in a Phase 2 Microbicide Trial**K. Bunge<sup>\*1</sup>, M. Swenson<sup>2</sup>, A. Pather<sup>3</sup>, D. Peters<sup>2</sup>, K. Gomez<sup>4</sup>, S. Gandham<sup>2</sup>, and the MTN001 Study Team<sup>1</sup>Magee-Womens Hospital, Pittsburgh, PA, USA; <sup>2</sup>SCHARP, Seattle, USA; <sup>3</sup>South African Medical Research Council, Durban, KwaZulu-Natal, South Africa; <sup>4</sup>Family Health International, Durham, NC, USA

**BACKGROUND:** Concerns over potential renal toxicity of tenofovir have necessitated careful monitoring of renal function in microbicide trial participants exposed to this drug. In particular, decreases in serum phosphate levels may represent drug induced proximal tubular dysfunction.

**METHODS:** A Phase 2, six-sequence, three-period, open-label crossover study of tenofovir vaginal gel and oral tablets is being conducted at four U.S. and three Sub-Saharan African sites. The primary objective is to compare the acceptability, adherence and pharmacokinetics of oral, vaginal and dual product use. Safety labs, including phosphate, are checked at screening, enrollment and follow-up. Potential participants with a phosphate level lower than the local laboratory's lower limit of normal at screening are excluded. Enrolled participants with a Grade 3 or 4 phosphate while on oral product have study product held.

**RESULTS:** To date, 153 women have been enrolled across all sites. At one South African site where screening data are available, 19% (17 of 90) of screened participants had a Grade 2–4 phosphate level according to the Division of AIDS Toxicity Table. Across all sites, the mean change in phosphate levels between screening and enrollment was -0.034 mg/dL (STD 0.556); the median change was -0.1mg/dL. The min, max for phosphate change was (-1.9, +1.5 mg/dL) and the IQR (-0.4, 0.3). In follow-up to date, there have been 54 Grade 2 phosphate levels and seven Grade 3 phosphate levels reported.

**CONCLUSIONS:** Site specific data from at least one site demonstrate a high baseline prevalence of hypophosphatemia in the recruited population. While the small median and mean changes in phosphate levels for the entire cohort suggest consistency in serum phosphate levels over time, the large range in changes prior to study intervention suggest large natural variability. Decreases in serum phosphate detected in follow-up may represent natural variation rather than drug induced toxicity. Instituting product hold for transient hypophosphatemia may be unnecessary.

# ORAL SESSIONS

## SESSION 32

### Oral Abstracts (OA17): Communication of and Advocacy for HIV Prevention Clinical Trials

**Moderators:** Gita Ramjee, Mitchell Warren

Monday, May 24, 4:30 pm–6:00 pm

Rooms 301–304

# 215

### Preparing Participants for Results of HIV Prevention Trials: An Example from MDP 301 in Northern KwaZulu-Natal, South Africa

M. Mzimela\*, H. Ndlovu, N. Mhlongo, M. Gafos, and the MDP team

Africa Centre for Health and Population Studies, University of KwaZulu-Natal, South Africa

**BACKGROUND:** The Africa Centre for Health and Population Studies in northern KwaZulu-Natal, South Africa was one of six sites participating in the Microbicides Development Programme MDP 301 clinical trial. After enrolment commenced in April 2006, the site used annual participant events to ensure ongoing contact with participants beyond the duration of their 12-month follow-up period. This analysis assesses the effectiveness of these events in preparing participants for result timelines and potential result scenarios.

**METHODS:** Over 50% of the 1177 women enrolled in MDP301 at the site attended the second participant event in November 2008. A variety of communication techniques were used to reinforce a series of key messages, including oral presentations, visual aids, theatrical plays and songs. After the event 4 focus group discussions (FGD) were conducted with a total of 37 event attendees. The FGDs were recorded, transcribed in isiZulu, translated into English and imported into NVivo 2 for analysis. A grounded theory approach was used to assess participants' understanding of three key messages: result timelines of HPTN035 and MDP301 (both evaluating PRO2000); possible result scenarios; implications of each scenario.

**RESULTS:** All participants in the FGDs included the three event messages of interest when asked to name the top ten messages of the event. Participants accurately recalled the timelines and result scenarios of HPTN035, result scenarios of MDP301, and the possible impact of HPTN035 results on the MDP301 trial. All participants knew that PRO2000 may be found not to reduce the risk of infection and over 50% believed this would be the case. A few participants confused the messages regarding access to PRO2000 thinking that they would be able to access PRO2000 gel as soon as MDP301 released the results, if it was found to be safe and effective.

**CONCLUSIONS:** Participant information sheets provide an overview of the timelines and possible trial outcomes. However, timelines often change, results from other trials can impact on the trial, and the result messages are rarely finalized until the final year of the trial. The participant events provided an excellent platform to present the result scenarios to over 50% of participants ever enrolled in the trial, over half of whom had already completed follow up; it allowed time to repeat key messages and facilitated the evaluation of comprehension of messaging in advance of final result dissemination.

# 216

### Communications Challenges: How the Internet and 24-Hour Media Affect Local Dissemination of Trial Results

D. Baron<sup>\*1</sup>, S. Harlan<sup>2</sup>, L. Heise<sup>3</sup>, J. Moffett<sup>2</sup>, E. Robinson<sup>2</sup>

<sup>1</sup>Global Campaign for Microbicides, PATH, Johannesburg, South Africa; <sup>2</sup>Family Health International, Research Triangle Park, NC, USA;

<sup>3</sup>The London School of Hygiene & Tropical Medicine, London, UK

**BACKGROUND:** As clinical trial sites disseminate the results of complex studies, they are increasingly called on to manage communications and interact with news media. Rapid online coverage has quickened the pace and broadened the circulation of results announcements. Yet local stakeholders (e.g., governments) require formal, in-person notification—a time-consuming process. The conflicting pressures between the immediacy of the Internet and slower, traditional modes of communications present new challenges to dissemination of results by sites.

**METHODS:** Under the Microbicides Media and Communications Initiative (MMCI), Family Health International and the Global Campaign for Microbicides have developed a practical guide, *Communications Handbook for Clinical Trials*. As background research, we reviewed extensive case studies from the field of prevention research, monitored media, and conducted over 88 interviews with network-based communications experts, trial site staff, sponsors and advocates across Africa, Asia, Latin America, Europe and the US.

This presentation examines how the rise of interest by online media in large-scale trials is affecting local dissemination strategies and stakeholder communications. We analyzed the recent announcements of the Thai vaccine and MDP 301 microbicide studies, highlighting how embargo restrictions, negative coverage, and the fast-paced, global media enterprise compete with local stakeholders' needs for interpersonal and formal communication. Through these cases, we share lessons learned and creative approaches for sites dealing with such challenges.

**RESULTS:** Despite an improvement in communications and dissemination planning, most sites still lack the resources to respond in "real-time" to inaccurate and inflammatory media. Many sites lose staff by the time of local results dissemination, leaving them vulnerable and often ill equipped to respond effectively to controversy.

**CONCLUSIONS:** Media coverage of trial results can influence the perceptions of funders, policy makers and ethics review committees. Online media interest in trials continues to expand. As trials become more complex, the field needs to employ creative, effective communications techniques to share results with local and global audiences virtually and in-person.

**217**

### **Preparing Communities for Results of HIV Prevention Trials: The Case of Communities Participating in MDP 301 in Masaka, Uganda**

R. Hasunira<sup>1</sup>, D. Kibira<sup>1</sup>, M. Chatani<sup>2</sup>, A. Kaggwa<sup>2</sup>, E. Bass<sup>2</sup>

<sup>1</sup>HEPS: Coalition for Health Promotion and Social Development; <sup>2</sup>AVAC: Global Advocacy for HIV Prevention

**BACKGROUND:** HIV prevention research clinical trials often have elaborate mechanisms of disseminating results with trial participants and trial communities. This may not always be the case with other stakeholders, including volunteers' spouses/partners, relatives, local leaders, civil society and the broader community. And yet, the broader community's support has substantial impact on the outcome of any trial. How communities are engaged before, during and after trials determines their contribution and support for ongoing and future trials. An AVAC-GCM HIV Prevention Research Advocacy Fellowship in partnership with HEPS-Uganda was designed to document experiences and opinions related to the release of results of the MDP 301 clinical trial of PRO 2000 candidate microbicide on the communities in Masaka district in Uganda as a case-study on community engagement.

**METHODS:** Qualitative data was collected using in-depth personal interviews with investigators and staff of MDP 301 and of other trials in Uganda; members of the Community Advisory Board (CAB); former participants; regulators; and civil society leaders. A survey of preparation materials was also done. Information sharing meetings were held with community members. The release and dissemination of results was monitored in meetings and media. The information gathering and analysis process will be completed by April 2010.

**RESULTS:** Volunteers had a strong expectation that PRO 2000 would work and their reaction varied from calm disbelief to regret, and even shock. The results review and compare the MDP 301 Masaka site's dissemination plan to reach communities directly involved with the trial (CAB, volunteers) and the broader community with what actually occurred and how the results dissemination was perceived. The report also includes reactions to and interpretation of results across communities and individuals. Media monitoring demonstrates how key messages from the trial were actually understood and translated.

**CONCLUSIONS:** As community engagement plans and results dissemination plans are developed, it is important to evaluate the impact of the plans and how far their reach and scope is. This feedback supports the development of further research literacy programs but also assesses community ownership of the process, and willingness to support further research and possible rollout of new prevention options.

**218**

### **The Joy of Tech: IRMA as a Model for Maximizing Electronic Communications to Build and Nurture an Advocacy Network**

J. Pickett<sup>\*1,2</sup>, M.A. LeBlanc<sup>2</sup>

<sup>1</sup>International Rectal Microbicide Advocates, Chicago IL, USA; <sup>2</sup>AIDS Foundation of Chicago, Chicago IL, USA

**BACKGROUND:** Created in 2005 by four people in two countries, the International Rectal Microbicide Advocates—the first advocacy initiative dedicated to rectal microbicide research and development—faced the challenge of building a network that connected researchers, advocates, policy makers and funders globally. Facilitating communication with stakeholders across disciplines and time zones was, and remains, critical to building, nurturing, and maturing an advocacy movement.

**METHODS:** IRMA maintains a vigorous, highly utilized, moderated listserv in which information is regularly disseminated and members engage in multi-directional dialogue and debate. Regular global teleconferences feature speakers from around the world who are leading rectal microbicide research and advocacy efforts, providing an important opportunity for members who are not able to travel to conferences to learn the latest developments from experts in the field. Additionally, IRMA maintains a resource-rich website, an active blog which is available on Facebook and through a syndication service called Feedburner among other means of accessibility, and a dynamic presence on the social networking sites Facebook and Twitter. In addition to information dissemination, these tools personalize the movement by providing a platform to highlight the faces behind the work.

**RESULTS:** From very modest origins, IRMA has become a forceful advocacy network currently consisting of over 800 researchers, advocates, policy makers and funders in over 60 countries on six continents. This exponential growth, and the expanding awareness of and interest in rectal microbicides, is largely due to IRMA's effective utilization of electronic methods of communication. The blog received 14,244 visits from 9,240 unique visitors from January 1 to December 31, 2009 and the website received 6,906 visits from 5,228 unique visitors. IRMA's Facebook group currently has 309 members, and on Twitter, 782 followers (both as of January 22, 2010).

**CONCLUSIONS:** With few resources, a large, multinational advocacy network, that is dynamic, engaging, and informative can be developed and nurtured through the use of online technologies and telephonic communications. These methods support a central forum for exchange, debate, and networking, through which interested individuals from around the world can regularly share their diverse perspectives on improving understanding and action around rectal microbicide research and development.

**219**

### **Grassroots to Government: The Role of Community Advocates in Mobilizing Government Support for Microbicide and Prevention Research—Case Study of South Africa and Kenya**

S. Dube<sup>\*1</sup>, P. Irungu<sup>2</sup>, Y. Halima<sup>1</sup>

<sup>1</sup>Global Campaign for Microbicides, South Africa; <sup>2</sup>Global Campaign for Microbicides, Kenya;

<sup>3</sup>Global Campaign for Microbicides, USA

**BACKGROUND:** In Sub-Saharan Africa where the HIV epidemic continues to proliferate, innovative programming and collaboration between stakeholders is most needed. At its heart, government policies need to be informed by communities need and interventions that can effectively be integrated into the lives of those most at risk. With offices in South Africa and Kenya, the Global Campaign for Microbicides (GCM) works closely with both grassroots organizations and with government stakeholders, including the National AIDS Control Councils that play a pivotal role in determining policy on HIV. By engaging in district and national level dialogues, GCM is able to support an enabling environment for continued research on new prevention technologies, informed by local epidemics, national health priorities and the voices of those most affected.

**METHODS:** We present here a "grassroots to government" approach to advocacy and share our experiences of working closely with the National AIDS Control Council in Kenya and South African National AIDS Council in South Africa. Through membership of technical committees on prevention research and gender task teams, as well as participation in national events with policy makers, GCM has been able to both contribute to policy discussions and monitor shifts around HIV prevention. We outline protocols for entry, participation and negotiation that reflect country strategies and structures.

**RESULTS:** The grassroots to government model of engaging approach, we have organized HIV prevention summits, bringing together civil society and governments, thereby increasing the capacity of advocates and policy makers engaged in HIV prevention research. Such a model has positively affected national policy dialogues including national strategic planning on HIV.

**CONCLUSIONS:** National HIV policies and programs must, by necessity, be informed by the experiences of those most greatly affected. Advocates based within affected communities have the knowledge and skills to profile these needs to ensure that national AIDS strategies consider issues of access, affordability, vulnerability and sustainability of emerging interventions.

# ORAL SESSIONS

## SESSION 33

### Special Session (SS1): Bridging to the Future

**Moderators:** Kim Eva Dickson, Henry Gabelnick

Monday, May 24, 4:30pm–6:00pm

Rooms 319–321

# 220

### The Microbicide Development Strategy

Polly Harrison

AVAC: Global Advocacy for HIV Prevention, USA

There have been many changes in microbicide development and the environments in which that takes place, as well as many discussions about those changes, what they mean, and how the microbicide field could and should adapt accordingly. This presentation and the report on which it is based distills those conversations and builds on the series of strategy documents and exercises led by the Alliance Microbicide Development beginning in 2006: the *Microbicide Development Strategy/MDS* in 2006, *Mapping the Microbicide Effort* in 2007, and the *Scorecard* in early 2009. The *Scorecard* exercise surveyed 50 key researchers to, first, quantitatively and qualitatively assess advances toward realization of the “Priority Actions” in the *MDS* and flag what they thought needed focus now. Those conclusions were then analyzed by five Working Groups who presented their conclusions and led discussion at the October 2009 Annual Alliance Meeting, to which other presentations at the Alliance meeting and other pertinent meetings added richness and further introspection. The result, a report entitled *Microbicides—Ways Forward*, is summarized in this presentation.

# 221

### Comprehensive Resources for HIV Microbicides and Biomedical Prevention

Phil Markham<sup>\*1</sup>, James Cummins<sup>2</sup>

<sup>1</sup>Advanced BioScience Laboratories, Inc., <sup>2</sup>National Institutes of Health

This presentation will include an overview of NIAID contract resources that are available to support non-clinical development, production, testing, and characterization of candidate microbicides and other non-vaccine biomedical prevention strategies. The roles of NIH and the Contractor (Advanced Bioscience Laboratories, Inc.) will be described, including the request/approval process for obtaining contract support and examples of specific activities that are available to product developers.

# 222

### The CHAARM Program

Charles Lacey

University of York, UK

The CHAARM programme will develop new specific anti-retroviral microbicides that could reduce the transmission of HIV via sexual intercourse at the vaginal or rectal portals. CHAARM aims to develop new ARMs which can prime a pipeline of promising candidates and will also develop microbicide combinations. Combination microbicides may be much more potent and effective than single agent microbicides and, importantly, may reduce the likelihood of emergence of drug-resistant HIV.

The project is a €12,000,000 European Commission Framework 7 funded programme involving scientists with expertise in a wide range of different disciplines. The project runs from 1 Jan 2010–31 Dec 2014 and brings together 31 institutions (Universities, SMEs, Research Institutes, Pharmaceutical Companies and Microbicide Developers) in 12 countries, including 8 EU Member States, as well as Switzerland, South Africa, the United States of America and Ukraine. As well as identifying new microbicides and combinations, CHAARM will develop rigorous procedures for testing efficacy and safety using new model systems. It will also investigate formulation and potential scale-up of production of microbicides. The programme will include human studies to determine microbicide safety and will investigate biomarkers associated with health or damage at mucosal surfaces.

## SESSION 34

### Mini-Symposia (MS6): ART: Challenges, Pitfalls, and Opportunities

**Moderators:** Jim Rooney, Lut van Damme

Monday, May 24, 4:30pm–6:00pm

Rooms 403–405

## 223

### Is it Time for Test and Treat?

Ken Mayer

*Brown University, Boston, MA, USA*

The use of HAART to decrease HIV transmission and acquisition has gained increased attention recently, since several observational studies have found that patients on ART may be less likely to transmit HIV to their partners. However, for HAART to be optimally effective for prevention, patients will need to be adherent, have stable medical care, and will need to decrease risk taking behaviors, since residual genital secretion HIV may be detected in patients whose plasma viremia is suppressed, particularly in the setting of intercurrent STDs. Antiretrovirals may also protect uninfected persons from HIV acquisition, either administered prior to, or post, a high-risk exposure (PrEP or PEP). Animal and observational human studies have demonstrated the utility of PEP. Animal data suggest that topical or systemic antiretroviral PrEP may be effective. Studies are underway in multiple countries to evaluate PrEP efficacy in diverse populations. Although tenofovir plus/minus emtricitabine have been the most studied chemoprophylactic medications, newer trials are evaluating drugs with other mechanisms of action, and alternative formulations. If current studies demonstrate efficacy, longer term monitoring will be needed to evaluate potential long-term toxicities, risk compensation, and virologic resistance, in order to fully understand the risks and benefits of chemoprophylaxis.

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### When Health Care Priorities Collide—Using Drugs for Treatment and Prevention

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Treatment as prevention and pre-exposure prophylaxis are two important prevention modalities currently being studied to reduce new HIV infections in the developing world. However, programmatic obstacles in the roll-out of HIV therapy in resource-poor settings are often cited as reasons why these two prevention modalities will be difficult to implement outside of a controlled trial setting.

This presentation will review the current state of the ART roll-out in South Africa and discuss the issues that may arise to impede the implementation of programs that would use antiretrovirals in healthy populations in order to prevent new infections. In addition, models of optimal delivery of anti-retroviral therapy to HIV-negative patients will be explored and discussed.

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### Antiretrovirals for Treatment and Prevention—Two Trains on a Collision Course?

John Mellors

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Antiretroviral-based pre-exposure prophylaxis (PrEP) is a promising HIV-1 prevention strategy that is being studied in several clinical trials. There is concern, however, about the potential spread of HIV drug resistance arising from PrEP, particularly if the same antiretrovirals, such as tenofovir and FTC, are used for both PrEP and first-line antiretroviral therapy (ART) of HIV-infected individuals. This concern is amplified in resource-constrained settings where second- and third-line treatment options are limited. The biological and epidemiological basis for this tension between ART and PrEP will be illustrated, along with strategies to prevent a major collision.



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### Dual Protection Polyurethane Intravaginal Rings for the Simultaneous Delivery of Levonorgestrel and UC781

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**BACKGROUND:** Most vaginal microbicides in clinical investigation have been formulated as single-drug dosage forms designed for application to the vagina prior to intercourse. However, a compelling rationale exists for providing long-term, controlled release of vaginal microbicides to provide prolonged protection against heterosexually transmitted HIV infection and to improve user adherence. The goal of the present study was to develop a polyurethane intravaginal ring (IVR) capable of sustained delivery of both UC781, a potent anti-HIV agent and levonorgestrel (LNG), a potent contraceptive.

**METHODS:** Polyurethane rods containing 1 or 5%wt LNG and 1–10% UC781 were prepared by melt extrusion at 147°C using a twin-screw extruder to form transparent rods through a 4.2 mm die. Rods were cut into approximately 1.5 cm lengths and were weighed and dimensions measured with digital calipers. Rod segments (end-capped) were placed in 20-mL scintillation vials, and 5 mL of release media (2% Solutol or 0.05% Solutol in acetate buffer, pH 4, N=3) was added to each vial and were incubated at 37°C on a shaker (80 RPM). Release media were replaced daily and analyzed for drug content by HPLC.

**RESULTS:** To confirm the melt-extrusion processing stability of LNG in the polymers, we first evaluated drug-polymer extrusions under elevated stress conditions at 145°C via controlled mixing/cycling in the extruder chamber. The data obtained showed no evidence of drug loss, regardless of exposure time to elevated temperatures. LNG release was detected in all release media conditions tested. Cumulative release from the rod segments in 2% or 0.05% Solutol® (acetate buffer, pH 4.2) appeared to be linear with time and was proportional to the solubility of LNG and UC781 in the release media. LNG release for a full IVR after 30 days were in the following order: 2116 µg (5 wt% LNG in 2% Solutol) > 937 µg (1 wt% LNG in 2% Solutol) > 132 µg (5 wt% LNG in 0.05% Solutol) > 130 µg (1 wt% LNG in 0.05% Solutol).

**CONCLUSIONS:** The data from this study demonstrated controlled released LNG and UC781 from monolithic polyether polyurethane ring segments under *in vitro* conditions. More work on the optimization of drug loading in the formulation and evaluation of proto-type combo-ring formulation is in progress.

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### Development of Silicone Vaginal Rings for Delivery of MIV-150 as a Microbicide

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**BACKGROUND:** Vaginal rings are promising drug delivery devices for microbicides, since they might improve adherence and eliminate the need for daily application. To explore the utility of rings loaded with MIV-150, we investigated drug-loaded silicone matrix rings. Little has been done to correlate the release rates seen *in vitro* to actual *in vivo* release rates. We measured the release rates of MIV-150 from silicone rings in 50% aqueous ethanol and water sink for up to one month. Rings suitable for macaque studies were prepared; their release rates were monitored in both sinks, and total drug release was compared to that in macaques.

**METHODS:** Matrix rings were prepared from platinum catalyzed silicone elastomer. The thermal stability of MIV-150 was evaluated in neat and formulated forms. Sink conditions were determined by monitoring the release of MIV-150 loaded rings over the desired sampling period using HPLC. *In vitro* release rates of MIV-150 were monitored in water and 50% aqueous ethanol sinks. Aliquots were removed daily for 1 to 2 months, and released amounts analyzed by HPLC. Efficacy of the MIV-150 rings in macaques was monitored after SHIV-RT challenge. Total MIV-150 released was determined by extraction followed by HPLC analysis. MIV-150 released *in vivo* during the efficacy study was then compared to that released in the water and 50% ethanol sinks.

**RESULTS:** DSC showed no change between neat MIV-150 and silicone rings with MIV-150, suggesting stability under ring processing conditions. For a 200 mg MIV-150 vaginal ring, we saw an average daily release of 248 µg and 533 µg in water and 50% ethanol, respectively. In the *in vivo* study 2 of the 7 macaques fitted with the MIV-150 loaded rings became infected; 2 of the 5 macaques fitted with placebo rings became infected. Our data indicated *in vivo* release appears to be more closely correlated to release in a water sink than in an aqueous ethanol sink; 3.0, 2.8, and 6.8 mg of MIV-150 released from the rings in one month in macaques, water, and 50% ethanol, respectively.

**CONCLUSIONS:** MIV-150 tolerates the conditions required to prepare silicone vaginal rings. This data suggests a ring capable of releasing greater amounts of MIV-150 is needed. Based on a comparison of total amounts of MIV-150 released from the rings in one month, water appears to represent the *in vivo* release seen in macaques better compared to 50% ethanol.

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### Formulation Enhances Pyrimidinedione IQP-0528 Anti-HIV Activity in Polarized Cervical Explants

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**BACKGROUND:** Pyrimidinediones are highly potent small molecule inhibitors that have a dual mechanism of action against HIV infection: viral entry and reverse transcriptase inhibition. *In vitro* testing showed that IQP-0528 was the most potent. However, *ex vivo* testing using polarized cervical explant cultures showed IQP-0528 did not protect against HIV infection. We hypothesized that IQP-0528 required formulation to make it more soluble to better penetrate the tissue. Therefore we tested formulated IQP-0528 to ensure it was effective against HIV infection of polarized cervical explants.

**METHODS:** Unformulated and formulated IQP-0528 was tested. IQP-0528 was formulated at 0.25% in a hydroxyethylcellulose (HEC) or a carbopol base. Cervical explants were set-up in duplicate, placed in a transwell, and sealed to create a polarized system with the tissue at the air-liquid interface. The basolateral side received medium containing PHA-p and IL-2 for the initial 48 h. Unformulated IQP-0528, IQP-0528 gels, or placebo gels were mixed with HIV-1<sub>BaL</sub>, applied to the apical side of the explant overnight, and then washed off. Basolateral media was collected and replenished every 3 to 4 days for 21 days. HIV p24 was measured by ELISA and infection was confirmed by immunohistochemistry (IHC) for HIV p24-positive cells.

**RESULTS:** HIV growth in control cervical explants showed peak viral replication within days 10 to 18 of culture. Statistical analysis (Wilcoxon matched pairs test) was done on day 18 to determine reduction of p24 by unformulated and formulated IQP-0528. Unformulated IQP-0528 did not prevent HIV infection in the polarized cervical explants. However, 0.25% IQP-0528 HEC and carbopol gels significantly reduced p24 levels by 4 log<sub>10</sub> as compared to the control p24 levels ( $p < .05$ ). Neither placebo demonstrated a significant reduction of HIV p24 levels. Efficacy was confirmed by IHC showing IQP-0528 gel-treated tissues were negative for p24-positive cells while control- and placebo-treated tissues were positive.

**CONCLUSIONS:** While unformulated IQP-0528 was shown to be effective against HIV *in vitro*, it was not effective in *ex vivo* polarized cervical explants. Application of formulated IQP-0528 overcame this issue by solubilizing it and thus was effective at preventing HIV infection in the polarized cervical explants. These data illustrate the importance of formulation in the delivery of effective molecules and of polarized explants for their evaluation.

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### Design of an Intravaginal Ring for the Simultaneous Delivery of IQP-0528 and Tenofovir

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**BACKGROUND:** Several recently published articles, along with ongoing research projects in our group have demonstrated the sustained release of various anti-HIV compounds from intravaginal rings (IVRs), alluding to the possibility of their eventual use as effective microbicide delivery vehicles. IQP-0528 is a pyrimidinedione analogue which has been shown to function as both a reverse transcriptase and cell-entry inhibitor for HIV-1. We have developed a polyether urethane IVR capable of the sustained delivery of IQP-0528 and tenofovir, a well-characterized reverse transcriptase inhibitor.

**METHODS:** We have developed two prototypes which incorporate both compounds into a polyurethane IVR. The first was a two-segment IVR. Tenofovir (PMPA) was loaded (13.9% w/w) into Tecophilic HP-60D-20 (a water-swellable variant of Tecoflex) and IQP-0528 (0528) was loaded (17.6% w/w) into Tecoflex EG-85A. Cylindrical rods (d=5 mm) of each formulation were formed by twin-screw, hot-melt extrusion. Two rod segments with a 7:1 length ratio (HP-60D-20/PMPA:EG-85A/0528), were joined to form a 55 mm (outer diameter) IVR. Sink condition release kinetics were modeled by incubation of IVRs in 25 mM sodium acetate buffer with 2% Solutol HS-15 (pH=4.2) for 30 days in an orbital shaker (37°C, 80 rpm). Aliquots of the incubation media were analyzed by high performance liquid chromatography (HPLC) to determine API content. The mechanical stabilities of the ring-welds were also evaluated by performing compression and extension tests on the IVRs before incubation. In the second prototype, we successfully fabricated rod segments of Tecophilic loaded with both PMPA and 0528 at similar loadings to those in their individual formulation (a combined drug loading approximately 30% w/w).

**RESULTS:** The two-segmented IVRs exhibited release kinetic profiles which mainly agree with those derived from previous data obtained using segments of the individual formulations. The minimum release rates over the 30-day experiments were approximately 3 mg/day (PMPA) and 400 µg/day (0528). The ring-welds showed no mechanical instability under the compression and extension tests.

**CONCLUSIONS:** We have developed an IVR system capable of the 30-day sustained delivery of tenofovir along with a promising new anti-viral compound, IQP-0528.

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### A Two-Compartment *In Vitro* Release Model for the Testing of HIV Microbicide Formulations

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**BACKGROUND:** *In vitro* release testing of vaginal formulations is usually performed in a one-compartment model (OCM) where the release medium, usually comprising pH-adjusted water, an aqueous surfactant solution or a solvent-water solution, provides sink conditions throughout the release experiment. Although this model is useful in evaluating the effect of formulation parameters upon release, it rarely reflects *in vivo* conditions. Here we report use of a two-compartment diffusion cell model (TCM, comprising a small volume donor, a large volume receptor, and separated by a model epithelial membrane) to more closely mimic *in vivo* vaginal release and tissue absorption following administration of a UC781 vaginal ring.

**METHODS:** Macaque-sized matrix silicone elastomer vaginal rings containing 100mg UC781 were prepared by injection molding, and *in vitro* release testing performed using both OCM (20mL simulated vaginal fluid, SVF) and TCM (5mL SVF in donor cell and variable quantities of Tween 80; silicone elastomer membrane; 100mL 3:2 ethanol/water in receptor cell). In the TCM, drug levels were measured by HPLC in both donor and receptor cells, representing fluid and tissue levels respectively. Rings containing 100mg UC781 and 10% w/w Tween 80 were also manufactured and tested

**RESULTS:** The amount of UC781 released from rings was significantly influenced by the choice of release model. Greatest release (56mg/14 days) was measured in the ethanol/water OCM, compared with no measurable release into SVF only. Increasing the concentration of Tween 80 in the SVF medium (1, 3 and 5% w/w) led to increased UC781 release (11, 16 and 18mg, respectively), demonstrating that vaginal fluid solubility of UC781 may be rate-determining *in vivo*. In the TCM, UC781 accumulates in the receptor cell medium over time, despite not being measured in the donor medium containing the ring device. Incorporation of Tween 80 directly into the ring provided enhanced release in both donor and receptor cells.

**CONCLUSIONS:** Release of UC781 was influenced by the choice of release medium and the inclusion of Tween 80 in the ring. Although use of SVF-only in the OCM indicated no measurable UC781 release from rings, data from the TCM confirms that UC781 is not only released but is also capable of penetrating across the model epithelial membrane. The TCM may therefore provide a more representative *in vitro* release model for mimicking *in vivo* absorption.

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### Quick-Dissolve Films as Drug Delivery Systems for Combination Microbicides

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**BACKGROUND:** Combinations of anti-HIV drugs are being evaluated as microbicides. Quick-dissolve films are one of several strategies for vaginal delivery of drug combinations. Films containing a combination of the entry inhibitor maraviroc (MVC) and a reverse transcriptase inhibitor (tenofovir(TFV) or dapivirine(DPV)) were developed and assessed for their physical, chemical, biocompatibility, and anti-HIV properties.

**METHODS:** Polyvinyl alcohol (PVA) based films were made using solvent casting techniques. Physical and chemical properties as well as toxicity and bioactivity were characterized. Specific properties studied were appearance, weight, thickness, tensile strength, water content, disintegration, dissolution, and drug content. Film toxicity and anti-HIV activity were assessed in cell based models (ie TZM-bl) and ectocervical explant cultures. Additionally, compatibility of films with resident microflora (*Lactobacillus*) was assessed.

**RESULTS:** The developed films were soft, flexible, and semi-transparent. Film mechanical properties were tested and the tensile strength of the films was acceptable at >1000 kg/m<sup>2</sup> for DPV/MVC combination film and >2000 kg/m<sup>2</sup> for TFV/MVC combination film. Both films had <0.1% (w/w) residual water. Film disintegration was achieved in <10 minutes. The USP dissolution test was performed on the DPV/MVC combination film and showed ~50% of both drugs were released in 10 minutes. No toxicity was observed upon DPV/MVC combination film exposure in the TZM-bl cell assay. Also, the films showed no toxicity against resident strains of *Lactobacillus*. *In vitro* bioactivity testing showed the DPV/MVC film to be active against HIV-1. Ongoing stability studies show the films maintain targeted specifications over three months.

**CONCLUSIONS:** These studies show that combinations of anti-HIV drugs can be successfully formulated into a quick-dissolve vaginal film with acceptable physiochemical characteristics, bioactivity, and safety profile.

**232****Phenylboronate-Salicylhydroxamate Crosslinked Hydrogels as a pH Responsive Microbicide Vaginal Drug Delivery Vehicle**

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**BACKGROUND:** New functional materials capable of modulating their properties based on biological cues are a continued focus of study in advanced drug delivery vehicle design. Of particular interest are new materials that sense the presence of HIV-1 or HIV-1 containing fluids and rapidly modulate their transport properties in response to this stimulus thus creating a barrier that inhibits interaction of HIV-1 with susceptible tissues and cells. The reversibility of covalent crosslinks formed by phenylboronate (PBA) and salicylhydroxamate (SHA) has been exploited to provide a pH-responsive gel for application to the vaginal tract. We have examined the properties of both symmetric and asymmetric crosslinked gels to determine their performance under simulated conditions, including rheological properties, HIV-1 transport, and *ex vivo* toxicity.

**METHODS:** Dynamic rheology evaluated the frequency-dependent viscoelastic properties of the gel as a function of pH. This method was also employed to characterize the self-healing properties of the asymmetric covalently crosslinking gels. Nanoparticle tracking assessed the transport of both fluorescently labeled HIV-1 and 100 nm sulfate-modified latex particles in the PBA-SHA crosslinked gel as a function of pH and in the presence of vaginal and penile tissue. Gel toxicity was quantified by MTT and cytokine analysis as well as by exposure to cervical explant tissue.

**RESULTS:** The ensemble-averaged mean squared displacement at lag times greater than three seconds reveals that transport of the HIV-1 and 100 nm particles becomes impeded by the polymer matrix. The viability of vaginal explants exposed to this formulation was 81.18%±0.008. Comparison of tissue histology before and after exposure to the gel showed no evidence of significant morphological changes. The asymmetric crosslinked gel displays self-healing properties after repeated break cycles at both pH 4.5 and 7.5 demonstrating that increasing the ratio of PBA to SHA provides a material that can form a self-healing gel across the full vaginal pH range.

**CONCLUSIONS:** pH responsive gels based on the PBA-SHA crosslink thus display properties of a potentially effective microbicide: biologically safe, the potential to significantly reduce the transport of HIV-1 to susceptible tissues and thus prevent the first stage of male-to-female transmission of HIV-1, and the ability to restructure after exposure to shear.

**233****Delivery of a SAMT (NCp7 Inhibitor) from an Intravaginal Ring**

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**BACKGROUND:** Nucleocapsid (NCp7) plays a crucial role in the HIV replication cycle including reverse transcription, integration and RNA encapsidation. NCp7 inhibitors attack the coordinated zinc within the zinc finger binding domain of NCp7, ejecting the zinc and resulting in the production of defective virions. Zinc ejectors like 3-nitrosobenzamide, disulfide benzamide and azodicarbonamide exhibited cellular toxicity, non-selectivity and short- half lives, limiting formulation development. S-acyl 2-mercaptopbenzamide thioester derivatives (SAMT) have been shown to exhibit a broad range of action against wild type, drug resistant, and multi-drug resistant HIV clinical isolates through their ability to rapidly inactivate HIV. A study has indicated that NCp7 inhibitors impede trans infection in cellular and explants models and protect non-human primates from infection. However, the formulation aspects of SAMT derivatives have not been studied. This work embodies the IVR formulation development of the N-[2-(3, 4, 5-trimethoxybenzoylthio) benzoyl]-β-alanine amide (SAMT-10), an analogue selected as a lead from three SAMT analogues studied.

**METHODS:** 1% SAMT-10 (Imquest BioSciences) was loaded in Tecoflex EG-85A and extruded into 4.2 mm rods using mini Haake twin extruder at 145°C. The rod segments were endcapped and subjected to 30 days (d) release testing under sink conditions in acetate buffer at pH 4.2 containing 0.05 and 2.0% solutol. The release media was replaced daily with fresh medium for 30d. The release media for 30d as well as the extracted SAMT-10 from IVR segments at the end of 30d was analyzed using a reversed phase liquid chromatography using a water-methanol gradient at 285 nm and by mass spectrometry. SAMT-10 IVR is currently evaluated on human epivaginal tissue (MatTek corp) for tissue toxicity, drug permeability, cell viability, and cytokine and drug release. The anti-viral activity of SAMT-10 has been measured.

**RESULTS:** The 30d cumulative data for SAMT-10 IVR indicated 55.7 ± 6.8% (n=3) release of SAMT-10. The extracted SAMT-10 from the IVR segments at the end of 30d showed no degradation products but the media samples containing released SAMT-10 showed products of hydrolysis as confirmed by mass spectrometric analysis. The IC<sub>50</sub> is 3.8μM.

**CONCLUSIONS:** ~ 50% SAMT-10 release by the end of 30d makes SAMT-10 IVR formulation promising for a long acting microbicide. The resultant products of degradation of the SAMT derivatives exhibit anti-viral activity *in-vivo* (study published) making SAMT-10 delivery promising for topical microbicide.

**234****UC781 Polymeric Thin Films—Optimization and Stability Assessment**

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**BACKGROUND:** Initially, a UC781 prototype film was developed using a hand pouring technique. The films had acceptable physicochemical properties. These non-optimized films showed safety in the non-human primate model. Efforts were taken to optimize the film formulation for scalable manufacture. Film formulation optimization is required to develop a formulation which can be manufactured in a GMP setting using a solvent casting method in anticipation of clinical trial initiation.

**METHODS:** Film formulation optimization efforts utilized a thin film applicator. Sedimentation studies were conducted to ensure content uniformity in the polymer solution. The dispersion of UC781 through out the polymer solution was optimized by evaluating 1) different impeller types for improved mixing, 2) manufacturing procedure changes, 3) use of dispersing agents, and 4) the impact of increasing viscosity of the solutions used for film preparation. Films were evaluated for: appearance, thickness, mass, disintegration time, tensile strength, water content, drug content, dissolution, bioactivity, toxicity, and lactobacilli toxicity. Stability assessments were conducted.

**RESULTS:** Films containing either micronized or nonmicronized UC781 were made using the thin film applicator. Comparisons between handpoured and film applicator prepared films showed that films with acceptable mechanical strength, more rapid disintegration time and lower water content were obtained using the film applicator. Further optimization was conducted to achieve drug content uniformity. An acceptable optimized film formulation was developed which used PEG 600 as the dispersing agent. The modified dispersed UC781 film formulation (1mg/film) resulted in films weighing ~100 mg and were ~114μm in thickness. Water content was <1% and disintegration time was <2 minute. The films were of adequate mechanical strength (tensile strength ~400 kg/m<sup>2</sup>).

**CONCLUSIONS:** UC781 was successfully dispersed within a vaginal thin film dosage form to achieve acceptable attributes such as tensile strength, dissolution, and safety.

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**235****Microbicide Placebo Gels Hinder Diffusion of HIV Virions**B.E. Lai<sup>\*1</sup>, A.T. Tutt<sup>1</sup>, J.W. Bess Jr.<sup>2</sup>, J.D. Lifson<sup>2</sup>, D.F. Katz<sup>1</sup><sup>1</sup>Duke University, Durham, NC, USA; <sup>2</sup>National Cancer Institute at Frederick, Frederick, MD, USA

**BACKGROUND:** The extent to which vaginal gels act as physical barriers to HIV during transmission, independent of any antiviral properties, is not well understood. This could affect functioning of microbicide gels and placebos. To better understand HIV transport in vaginal gels, we quantified diffusion coefficients of HIV virions in 2 placebo gels used in microbicide clinical trials.

**METHODS:** We measured diffusion coefficients of HIV in the hydroxyethyl cellulose (HEC) and methylcellulose (MC) clinical placebos, and in 20 and 50% (v/v) dilutions of those gels in PBS, using particle tracking of fluorescently-labeled HIV-1 virions.

**RESULTS:** Diffusion coefficients of HIV virions in undiluted HEC and MC were  $4 \pm 2 \times 10^{-12} \text{ cm}^2/\text{s}$  and  $7 \pm 2 \times 10^{-12} \text{ cm}^2/\text{s}$  ( $n = 3$ ), respectively, approximately 4 orders of magnitude lower than the diffusion coefficient of HIV virions in PBS. Diffusion coefficients in HEC and MC were not significantly different statistically, but there was a trend for higher values in MC. Diffusion coefficients increased with extent of gel dilution but were still reduced in 20% and 50% diluted gels by approximately 2 and 3 orders of magnitude, respectively, vs. PBS.

**CONCLUSIONS:** Placebo gels used in microbicide clinical trials may act as barriers to diffusion of HIV virions. Based on values of diffusion coefficients measured here, over 48 hours would be required for HIV virions to diffuse across an undiluted layer of gel of thickness typically observed for vaginal gels *in vivo* (100  $\mu\text{m}$ ). Diffusion coefficients here for HIV virions in placebo gels are similar to those reported previously for HIV virions in human cervicovaginal mucus ( $<10^{-11} \text{ cm}^2/\text{s}$ ) and in a crosslinked hydrogel engineered to act as a barrier to HIV ( $2 \times 10^{-12} \text{ cm}^2/\text{s}$ ). The ability of a gel to function as a physical barrier to HIV transport from semen to tissue will also depend upon its distribution over the epithelium and effects of dilution by vaginal fluids or semen. Results here can serve as a baseline for future design of gels that act as barriers to HIV transmission. Presumably, a gel engineered to function as a barrier to HIV should out-perform these placebo gels, particularly in response to dilution. The possible barrier function of placebo gels should also be considered in the design and interpretation of microbicide clinical trials.

**236****Formulation of Tenofovir Containing a Quick-Dissolve Film Product**L. Wang<sup>\*1</sup>, C. Dezzutti<sup>2</sup>, B. Moncla<sup>2</sup>, M. Parniak<sup>2</sup>, R. Mallipeddi<sup>3</sup>, D. Friend<sup>4</sup>, L.C. Rohan<sup>1,2,3</sup><sup>1</sup>Magee Womens Research Institute, Pittsburgh, PA, USA; <sup>2</sup>University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; <sup>3</sup>University of Pittsburgh School of Pharmacy, Pittsburgh, PA, USA; <sup>4</sup>CONRAD, Arlington VA, USA

**BACKGROUND:** Tenofovir (TFV) is being evaluated as a microbicide drug candidate. Currently, semisolid and oral formulations (administered as TFV-disoproxil fumarate) are being evaluated in the clinic. To expand the dosage forms available for delivery of TFV, a quick-dissolve film was developed. Films are discreet, convenient dosage forms with advantages such as no applicator requirement, no mess, and inexpensive manufacture.

**METHODS:** Initial target dosing level was set at 40 mg/film equivalent to that of a single dose of a 4 ml 1% TFV gel product. Three polymers, PVA, HPMC, and Sodium carboxymethylcellulose (Na CMC) were evaluated for development of a TFV-containing film. TFV incorporation was by dispersion and solubilization methods. The effect of pH on TFV solubility was studied using the pH adjusting excipients sodium hydroxide, triethanolamine (TEA), and phosphate buffer. Films were prepared using solvent casting techniques. Physicochemical characterization of the film included appearance, thickness, disintegration time, tensile strength, drug content, content uniformity, dissolution, and short-term stability.

**RESULTS:** PVA- and HPMC-based film formulations required pH adjustment for TFV solubilization. However, drug crystallization occurred in these formulations. Na CMC-based film solutions were able to solubilize TFV without pH adjustment (10 mg/film). This film formulation had acceptable properties and short-term stability. Modification of the Na CMC-based film formulation by addition of TEA resulted in film drug loading levels of 20 mg/film. This film formulation had acceptable properties and short-term stability. Dissolution studies show that TFV is rapidly released (>75%) in the first 6 minutes.

**CONCLUSIONS:** A new quick-dissolve Na CMC-based film platform containing TFV was developed. Achievable drug loading level was dependent on inclusion of pH adjusters. Developed films showed acceptable physical characteristics and short-term stability. Formulation efforts to combine TFV and UC781 in a single quick-dissolve film are ongoing.

**237****Preformulation Studies for a UC781 Rectal Microbicide Product**L. Wang<sup>\*1</sup>, R.L. Schnaare, P. Anton<sup>2</sup>, L.C. Rohan<sup>1,3</sup><sup>1</sup>Magee Womens Research Institute; <sup>2</sup>UCLA; <sup>3</sup>University of Pittsburgh School of Pharmacy

**BACKGROUND:** Preclinical and clinical studies have identified UC781 as a potential anti-HIV microbicide. However, incorporation of UC781 in typical aqueous-based formulations is challenging due to its hydrophobicity. To overcome this limitation, lipid solvents were assessed for their capacity to enhance UC781 solubility and utilization in a rectal-specific formulation.

**METHODS:** In this study supported by DAIDS IPCP #AI060614, Caprylic/capric triglyceride (GTCC) and isopropyl myristate (IM) as lipid solvents were evaluated in three sets of studies assuming that UC781 decomposes via an oxidative mechanism. 1) The impact of primary factors, light, acid, oxygen, H<sub>2</sub>O, and BHA (butylated hydroxyanisole), were evaluated via a factorial design experiment. 2) Singlet oxygen quenchers including lycopene, carotene, ascorbyl palmitate, bixin, BHA, BHT and cholesterol were evaluated. 3) UC781 stability was evaluated in lipid solvents preconditioned with nitrogen purging, heat, BHA, or water.

**RESULTS:** UC781 showed significantly enhanced solubility in GTCC and IM (3400  $\mu\text{g}/\text{ml}$  and 376  $\mu\text{g}/\text{ml}$  respectively) but was not stable in either lipid solvent. This degradation was found to be light sensitive. BHA provided some protection from degradation but was insufficient for long term stabilization. Among the singlet oxygen quenchers evaluated, 1% and 10% cholesterol and 0.1% lipoic acid were most promising. Surprisingly, ascorbyl palmitate was found to increase UC781 degradation. In the preconditioned experiments, nitrogen purging protected UC781 from degradation in GTCC providing a 90% shelf-life at 25°C and 8°C of 1.2 years and 2.2 years respectively and 3.1 years and 19.5 years respectively at 25°C and 8°C in IM.

**CONCLUSIONS:** Although the solubility of UC781 can be enhanced using GTCC and IM, decomposition of UC781 occurs over time. Several promising stabilizing strategies for UC781 in the GTCC lipid solvent were identified such as incorporation of cholesterol or lipoic acid and nitrogen purging. BHA and nitrogen purging was effective in stabilizing UC781 in IM.

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### Safety Testing of Silicone Elastomer Matrix Vaginal Rings Containing UC781 and Tween 80 using Epivaginal (VEC-606) Tissues

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**BACKGROUND:** The microbicidal agent UC781 has very limited aqueous solubility. A recent study in pig-tailed macaques showed poor pharmacokinetic levels, suggesting that, unlike *in vitro* release testing which uses relatively large volumes of a hydroalcoholic release medium, the vaginal environment does not provide sufficient solubilizing power to dissolve UC781 that has permeated to the ring surface. To overcome this solubility obstacle, the non-ionic surfactant Tween 80 was coformulated with UC781 in silicone elastomer rings. This study reports *in vitro* release into simulated vaginal fluid (SVF), and safety testing of the rings using the EpiVaginal tissue model.

**METHODS:** UC781+Tween 80 macaque matrix silicone elastomer vaginal rings were prepared and placed onto the apical surface of pre-wet (175µl PBS) EpiVaginal (VEC-606) tissues. After 24hr, 72hr and 7d, half of the culture medium from each tissue was collected and tested for inflammatory cytokine levels by ELISA. Barrier function of the vaginal tissue was tested on D1, D3 and D7 using transepithelial electrical resistance (TEER). Tissue viability was tested on D7 of the study using an MTT assay. *In vitro* release was performed in 5mL SVF and UC781 release analyzed by HPLC.

**RESULTS:** A 100mg UC781 ring released 1.7mg UC781 in 5mL SVF over 14d, compared to 10.2mg from the same ring containing 10% Tween 80. Placebo, UC781 and UC781+Tween rings induced a 1.7, 1.7 and a 4.0 increase respectively in IL-8 levels on D1. However, levels were similar to PBS and SVF controls on D3, and on D7 IL-8 values for all rings dropped significantly below control values. No significant changes in IL-6 were observed for tissues exposed to rings in the presence of SVF. Increases in IL-1 $\alpha$  were observed on D1 and D3 for all rings but values were similar to D7 control values. No significant changes in IL-1 $\beta$  were noted. TEER values for PBS, placebo, UC781 and UC781+Tween rings were 412, 610, 598 and 518 Ohm cm<sup>2</sup> respectively. The tissue viability for the placebo, UC781 and UC781+Tween rings were 86, 90 and 83% compared with PBS control having a tissue viability of 101%.

**CONCLUSIONS:** The formulation of Tween 80 in a 100mg UC781 matrix vaginal ring increases UC781 release by ten fold over 14d into SVF. The combination of UC781 and Tween 80 in the rings did not compromise measured tissue properties. Tissue viability on D7 for all test materials was >83%. Therefore, all test materials should be minimally to non-irritating *in vivo*.

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### Development and Evaluation of Novel Solid Dosage Forms of Tenofovir

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**BACKGROUND:** The conventional vaginal dosage forms are known to have poor user compliance because of problems of poor retention, leakage, and messiness. The present project was undertaken to develop novel bioadhesive vaginal tablets of Tenofovir, with the target product profile of releasing the drug over an extended period of time. Tenofovir, a Nucleotide Reverse Transcriptase Inhibitors (NtRTIs) is currently being developed as a Microbicide.

**METHODS:** The tablet formulation was designed to disintegrate rapidly in small volumes of vaginal fluid and prepared by direct compression tabletting method. The almond-shaped tablets were prepared from GRAS (generally regarded as safe) or compendial excipients for vaginal administration. Weight of the tablets was 1 gm with a target dose of 40 mg of Tenofovir. The optimized formulations were characterized for a set of pharmacopoeial and non pharmacopoeial parameters such as weight variation, disintegration time, hardness, thickness, moisture content, and assay. A reverse phase HPLC method was developed for assay of the drug substance and drug product and validated for linearity, specificity, accuracy, precision, system suitability, LOD and LOQ. Stability of the tablets was evaluated as per standard ICH conditions of 4°C, 35°C/60% RH and 40°C/75% RH.

**RESULTS:** The tablets were successfully prepared with targeted disintegration time of around one minute, and the required hardness, moisture content, and thickness. Because of small dose, optimizing the process for content uniformity was highly challenging. Tablet excipients did not interfere with quantitation of TFV. The formulation was stable for six months at 40°C/75% RH (assay = 98.4% of initial after 6 months) as assessed by physical stability and assay.

**CONCLUSION:** Novel, rapidly disintegrating bioadhesive vaginal tablets of TFV were developed, evaluated, and found to be stable under accelerated stability conditions for six months. The formulation can be taken up for scale up and further evaluation.

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### A Polyurethane Intravaginal Ring for the Delivery of UC781

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**BACKGROUND:** UC781 is a non-nucleoside reverse transcriptase inhibitor currently under investigation in clinical trials for the prevention of sexual transmission of HIV. Our research group has developed a polyurethane intravaginal ring (IVR) formulation for the sustained delivery of UC781.

**METHODS:** IVRs were fabricated using a melt-extrusion method in which the hydrophobic polyurethane Tecoflex® EG-85A was co-extruded with various drug loadings of UC781. Differential scanning calorimetry (DSC) was employed to study phase transition. *In vitro* release studies were performed in 0.05% and 2.0% Solutol HS 15 at pH 4.2. In addition, the EpiVaginal™ *in vitro* tissue (VEC-606) irritation model was used to study biocompatibility of the IVR with and without UC781. Statistical analyses were performed either with the Student t-Test or ANOVA.

**RESULTS:** DSC analysis of UC781 alone and UC781 co-extruded with Tecoflex® EG-85A revealed an exothermic peak at 237°C; this temperature is well above the extrusion processing temperature of 145°C. 28-day release studies from a 2 wt% UC781-loaded IVR demonstrated a total cumulative release of 1.86 ± 0.73 mg (2.91 ± 1.14 % of total loading) in 0.05% Solutol and a cumulative release of 7.28 ± 2.73 mg (11.82 ± 5.44 % of total loading) in 2.0% Solutol. Moreover, a 5 wt% UC781 loaded IVR demonstrated a total cumulative release of 7.64 ± 0.87 mg (4.51 ± 0.56 % of total loading) in 0.05% Solutol and a cumulative release of 56.07 ± 15.97 mg (34.51 ± 9.50 % of total loading) in 2.0% Solutol. The EpiVaginal™ *in vitro* tissue irritation model indicated both placebo and drug-containing IVRs (2 wt% and 5 wt% UC781) had no significant impact on cell viability (MTT assay), tissue integrity (histology) and barrier function (TEER) after 7 days of exposure in comparison to controls. In addition, no significant increases in pro-inflammatory cytokine (IL-1 $\alpha$ , IL-6, TNF- $\alpha$ , and IFN- $\gamma$ ) expression were observed. Transport of UC781 across EpiVaginal™ tissues appears to be loading dependent with fluxes of approximately 0.06 and 0.2 pmol/cm<sup>2</sup>/min for 2 and 5 wt% UC781 loadings, respectively. The amount of UC781 retained in tissues also appear to be dose dependent and ranged from 5.34 µg (2 wt% UC781 IVR) to 14.4 µg (5 wt% UC781 IVR) on day 7.

**CONCLUSIONS:** Overall, we demonstrated that polyurethane-based IVRs are biocompatible *in vitro* and can provide sustained release of UC781. Based on these results, further development of an IVR for UC781 is justified.

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### Production and Preservation of Highly Viable *Lactobacillus* for Mucosal Delivery of Therapeutics *In Situ*

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**BACKGROUND:** The human vaginal mucosa, a key target of sexually transmitted infections, is populated with commensal bacteria. The predominant H<sub>2</sub>O<sub>2</sub>-producing *Lactobacillus* species play a key role in the maintenance of reproductive health of women, and could be genetically engineered as a self-renewing vehicle for mucosal delivery of protein-based HIV inhibitors. The success of this approach will depend, in part, on the level and duration of *in situ* *Lactobacillus* colonization, a surrogate marker for efficacy. Equally important is production of a highly viable product that is stable over time. Thus, we undertook comprehensive investigations into the optimal fermentation and preservation conditions for *Lactobacillus* for mucosal delivery.

**METHODS:** Cell viability (ratio of live to total number of cells) of *L. jensenii* strains expressing an extracellular N-terminally modified cyanovirin-N or intracellular β-glucuronidase was evaluated during fermentation in a Microbial Fermentor and upon preservation by spray- or freeze-drying. An Olympus IX51 microscope was reconfigured with a dark field condenser and a Petroff-Hauser counting chamber for the determination of total cell count. The live cell count was determined by colony forming units (CFU) on MRS plates. In addition, cell recovery of dried *L. jensenii* powders was evaluated post-rehydration in MRS broth and simulated vaginal fluid at various pH.

**RESULTS:** Fermentation conditions affected *Lactobacillus* viability and survival of *Lactobacillus* during preservation. Maintenance of pH with ammonium hydroxide coupled with glucose supplementation supported the fermentation of *Lactobacillus* with up to 10<sup>10</sup> CFU per ml and >90% cell viability when bacterial growth reached early stationary phase. Addition of a polyol and sodium ascorbate along with another antioxidant to the preservation matrix (skim milk and trehalose) significantly increased cell viability of the dried *Lactobacillus* powders. As cell recovery of the dried preparations was sensitive to pH upon rehydration, a transiently buffered preservation matrix was further developed to produce *Lactobacillus* powder that was highly viable at 10<sup>11</sup> CFU per gram of powder.

**CONCLUSIONS:** Highly viable *Lactobacillus* powders can be produced and formulated by optimizing cell viability during fermentation, preservation, and rehydration *in vitro*. Work is in progress to formulate *Lactobacillus* for improved heterologous protein expression and product stability, and to select the appropriate dosage form for mucosal delivery *in situ*.

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### Sustained Delivery of Tenofovir from Polyurethane Intravaginal Rings

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**BACKGROUND:** Polyurethane intravaginal rings (IVRs) are promising vehicles for the delivery of microbicides to prevent the heterosexual transmission of HIV. Tenofovir, or 9-R-2-phosphonomethoxypropyl adenine (TFV), is a nucleotide analogue reverse transcriptase inhibitor. Since it is a hydrophilic molecule matrix IVR devices composed of water-swellable polyether urethane matrices were investigated for the delivery of multi-milligram quantities of TFV from an IVR with dimensions similar to Nuvaring.

**METHODS:** Swellable polyurethane (Tecophilic HP-60D-20) rods were fabricated with varying concentrations of TFV (2-25% w/w) using cryo-pulverization and melt-extrusion technologies. Incorporation of TFV into the polymer matrix was completed without the use of solvents. One experiment also evaluated 1% titanium dioxide as an excipient. *In vitro* release profiles were observed by incubating rod segments in 25 mM sodium acetate buffer (pH=4.2) for up to 60 days. Release media were changed daily and 24-hour incubation samples were stored and analyzed by high-performance liquid chromatography for TFV content.

**RESULTS:** Release of TFV from this system was sustained for 60 days, although release was not zero-order. For an IVR with 5 mm cross-sectional diameter and 50 mm OD, this system yielded a burst release of 8 mg during the first 24 hours and a steady state release rate of greater than 2 mg tenofovir per day after 60 days incubation in the release media. Rods prepared with titanium dioxide showed little or no burst release, but appear to have similar steady state release kinetics to those without titanium dioxide. At thirty days incubation, the percentage of TFV released per day does not vary drastically across the entire TFV loading domain (2-25% w/w) regardless of the presence or absence of titanium dioxide.

**CONCLUSIONS:** Using a water-swellable polyether urethane matrix (Tecophilic HP-60D-20), we have achieved sustained, loading-dependent release of tenofovir from an IVR for up to 60 days. Addition of 1% titanium dioxide eliminates the burst-release effect normally seen with TFV from this polymer and therefore increases the duration of release.

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### Development of Microbicide-Releasing SILCS Diaphragm

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**BACKGROUND:** There is a need for HIV microbicides that simultaneously provide contraception. The SILCS diaphragm is a single-size contraceptive diaphragm developed by PATH and CONRAD. Its polymeric construction (nylon spring core and overmolded silicone elastomer membrane) potentially allows for incorporation and sustained release of HIV microbicides. However, the high processing temperature and poor permeation characteristics of nylon are major obstacles to further development. This study describes the feasibility and preliminary development of a microbicide-releasing SILCS diaphragm.

**METHODS:** Various model diaphragm devices were constructed comprising microbicide-loaded thermoplastic rods inserted into silicone elastomer tubing, and continuous and discontinuous *in vitro* release evaluated over 14 days. Mechanical testing on a range of alternative thermoplastic materials was also performed. Candidate diaphragm devices, prepared by injection molding of POM spring cores and overmolded with silicone elastomer, were assessed for mechanical characteristics and *in vitro* release. User acceptability studies are ongoing.

**RESULTS:** Thermoplastic rods in silicone tubing showed sustained release of UC781 (PEVA and PCL provided cumulative release of 325 and 95 mcg over 14 days into isopropanol:water mixture, respectively). POM copolymer was selected for spring core fabrication, owing to its low processing temperature for compounding and injection molding (140°C), good release characteristics (1, 5 and 10% w/w loadings provided cumulative release over 14 days of 1.1, 6.4 and 30.8mg, respectively) and similar mechanical properties to nylon (flexural modulus 2.9GPa, core compression force 4.4N). Addition of UC781 did not have significantly effect the mechanical performance of spring cores.

**CONCLUSIONS:** The nylon spring core of the current SILCS diaphragms has been successfully replaced with an alternative lower melting POM thermoplastic. Mechanical performance matches the current SILCS device and sustained release of candidate microbicides has been demonstrated.

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**Novel Non-Aqueous Silicone Elastomer Gels for Practical Formulation of HIV Microbicides**

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**BACKGROUND:** Semi-solid aqueous gels (e.g. hydroxyethylcellulose (HEC) and Carbopol gels) are used routinely for the formulation of HIV microbicides. Such gels are inexpensive, easy to manufacture, provide solubilisation of water-soluble actives, and may be prepared in mucoadhesive forms for enhanced mucosal retention. However, they also require preservation, do not dissolve water-insoluble actives resulting in dispersed gel systems which may provide suboptimal absorption and activity, and are diluted by vaginal fluids leading to decreased retention. It is widely accepted that a more diverse range of formulation options is needed in order to accommodate the varying physicochemical characteristics of microbicide candidates. A particular focus should be directed to the formulation of HIV microbicides with limited water solubility (e.g. dapivirine, UC781). Here we report the potential of non-aqueous, hydrophobic silicone elastomer gels for the formulation of microbicides.

**METHODS:** Silicone elastomer gels were prepared containing different lead candidate microbicide compounds (T-1249 (4995Da), AZT (269Da, logP +0.6), dapivirine (326Da, logP +4.6), CMPD167 (575Da, logP +6.2), UC781 (336, logP +5.4) and various excipients to modify release. *In vitro* release studies were performed in simulated vaginal fluid (SVF) using both single and dual-compartment models.

**RESULTS:** The silicone elastomer gels were not dissolved or diluted by SVF during release testing. All the gels tested provided measurable release of each microbicide candidate in quantities reflecting the relative hydrophilic/hydrophobic character of the microbicide. For example T-1249 released up to 32 $\mu$ g, AZT released 9.5 mg and dapivirine released 9.8 mg, CMPD167 released 38 mg over 8 hours. The incorporation of hydrophilic excipients into the silicone elastomer gels modified the release rates, and also increased the mucoadhesive character of the gels. For example, addition of 20% HEC increased T-1249 release to 135 $\mu$ g and the addition of 20% croscarmellose increased dapivirine release to 24 mg.

**CONCLUSIONS:** The studies have shown that non-aqueous silicone elastomer gels are capable of releasing microbicides having a wide range of physicochemical characteristics, including molecular weight and hydrophilicity, and that release may be modified through the incorporation of hydrophilic excipients.

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**MRI Evaluation of the Distribution and Retention of Multiparticulates in the Human Vagina**

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**BACKGROUND:** Conventional vaginal formulations are associated with several disadvantages, such as leakage, messiness and low retention (for semi-solids) and rapid loss from the vagina (for tablets). We developed a multi-particulate pellet system (pellets are spherical particles with diameter varying from 400–1500  $\mu$ m), as a novel vaginal drug delivery system. It was anticipated that due to their small particle size, pellets will evenly distribute in the vaginal cavity and will be less sensitive to gravity than tablets, resulting in longer retention.

The objective of this pilot study was to evaluate the *in vivo* distribution and retention of this novel vaginal drug delivery system in human vagina, using Magnetic Resonance Imaging (MRI).

**METHODS:** The starch-based pellets were produced by extrusion-spherisation. A high-amylose starch with sorbitol and Hydroxy propyl methyl cellulose mixture was granulated with water containing Gadolinium, as the MRI contrast agent. This wet mixture was extruded, spherised and dried to obtain pellets ( $d = 500 \mu$ m). The pellets were filled into a hard gelatin capsule (size 00) and administered intravaginally to the study patient ( $n=1$ ), with a suitable applicator. The T1-weighted MRI was performed immediately after administration, after 6h, 8 h, 12 h and 24 h post-administration.

**RESULTS:** Immediately after administration, no Gadolinium was observed as the capsule was still intact. The scan taken after 6 h indicated the disintegration of the capsule and clustering of the pellets into the cervix and proximal vagina. At 8 h post-administration, the pellets were spread further down the vagina. After 12 h, the anterior portion of cervix and the entire vagina was covered by the pellets. After 24 h, the pellets were still present covering the complete vagina, but in a smaller amount. The study patient reported some pellet loss between 12h and 24h after administration during urination. The transverse images clearly indicated the W-shape of the vagina, confirming the complete coverage of the vaginal mucosa.

**CONCLUSIONS:** The multi-particulate pellet system seems a promising new vaginal drug delivery system, resulting in complete coverage of the vaginal mucosa (similar to gel) and acceptable retention time. These preliminary data should be confirmed for inter and intra subject variation, on a large number of volunteers. A drawback of this formulation was the slow initial disintegration time of the capsule. To overcome this problem further research will be focused on compression of the pellets into fast disintegrating tablets.

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**RC101 Formulated as a Vaginal Film: Safety Studies in the Macaque Model**

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**BACKGROUND:** RC101, a retrocyclin analog, was formulated as a quick-dissolving vaginal film and assessed for safety in the pigtailed macaque model.

**METHODS:** The cervicovaginal environments of five pigtailed macaques were assessed prior to, thirty minutes after and 24-hours after each of four daily exposures to RC101 vaginal films. Eighteen months later, this experiment was repeated in six macaques (the original five, plus one new animal) with slightly different measures. Product safety assessments in both experiments included colposcopy, vaginal flora and pH. One experiment or the other also assessed vaginal cytology, drug location and bioavailability; though these studies are not discussed here. In both experiments, animals controlled for themselves by completing both the test and the placebo arm (cross-over design), separated by five to six weeks.

**RESULTS:** None of the animals in these studies developed abnormal colposcopic findings attributable to repeated applications of RC101 film or placebo film. No difference in vaginal pH measurements was noted in placebo versus test animals. Populations of H<sub>2</sub>O<sub>2</sub>-producing lactobacilli fluctuated slightly in all animals in which these organisms were detected in both study arms. *Viridans streptococci* remained stable throughout. No increase in *E.coli* or enterococcus was detected.

**CONCLUSIONS:** In summary, the RC101 film formulation was well tolerated after 4 daily applications in this primate model. The RC101 film formulation was indistinguishable from placebo film by colposcopy, vaginal pH and microflora assessments.

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### Effects of B-cyclodextrin and Its Derivatives on the Apparent Aqueous Solubility of MIV-150

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**BACKGROUND:** MIV-150 is a potent NNRTI with exceedingly low solubility in water (ca. 25 ng/mL). The aim of these pre-formulation studies was to enhance the solubility of MIV-150 using cyclodextrin to develop a useful vaginal microbicide dosage form to block HIV infection. In these studies, cyclodextrins ( $\beta$ CD, Me $\beta$ CD, HP $\beta$ CD, and SBE7- $\beta$ CD) and methods of complexation on solubility of MIV-150 were assessed.

**METHODS:** The phase solubility of MIV-150 in the four cyclodextrin solutions was determined in this study. MIV-150 was dispersed into solutions containing cyclodextrins ( $\beta$ CD, Me $\beta$ CD, HP $\beta$ CD, and SBE7- $\beta$ CD) followed by shaking for 72 hrs in vials. Mixtures were then filtered through a 0.2  $\mu$ m Nylon filter for HPLC analysis to determine the amount of MIV-150 dissolved in water through complexation with cyclodextrins. Different methods of complexation, autoclaving, lyophilization, kneading, and shaking, were evaluated to optimize the complexation efficiency of MIV-150. The solubility of MIV-150 in water was analyzed using HPLC.

**RESULTS:** MIV-150 is a poorly water-soluble base with a pKa of 11.2. The aqueous solubility is pH dependent, but < 0.1  $\mu$ g/ml at pH 7.0. Cyclodextrins can greatly improve the aqueous solubility of MIV-150. The apparent solubility of MIV-150 was increased to 1.3  $\mu$ g/ml ( $\beta$ -CD 1.5% w/v), to 17.9  $\mu$ g/ml (SBE7- $\beta$ CD 20% w/v), to 18.0  $\mu$ g/ml (HP $\beta$ CD 20% w/v), and to 70  $\mu$ g/ml (Me $\beta$ CD 20% w/v). The solubility-enhancing ability of cyclodextrins is also depending on different methods used for complexation. Within four different methods, autoclaving showed the best solubility enhancing ability on MIV-150. This method increased the apparent solubility of MIV-150 to 5.6  $\mu$ g/ml ( $\beta\beta$ -CD 1.5% w/v), 22  $\mu$ g/ml (SBE7- $\beta$ CD 10% w/v), 46  $\mu$ g/ml (HP $\beta$ CD 10% w/v), and 60  $\mu$ g/ml (Me $\beta$ CD 10% w/v).

**CONCLUSIONS:** Cyclodextrins provide formulation advantages by enhancing the aqueous solubility of MIV-150. The phase solubility showed a linear relationship of MIV-150 and cyclodextrins for the four cyclodextrins tested. The autoclave method offered an easy and quick way to prepare complexes of MIV-150 and cyclodextrins. In addition, HP $\beta$ CD and SBE7- $\beta$ CD were found to be more useful solubilizing agents for MIV-150 for their low toxicity and comparatively high solubility enhancing effects.

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### Vaginal Delivery of UC781 in Woman's Condom Capsules

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**BACKGROUND:** The female condom provides protection against pregnancy, HIV, and other sexually transmitted diseases. PATH, in collaboration with CONRAD, has developed a new female condom that has been shown to be highly acceptable to a range of users. One modification involves improving insertion with the addition of a rounded polyvinyl alcohol based capsule at the end of the condom, which gathers the condom pouch together. Once the condom is inserted, the capsule quickly disintegrates and the condom unfolds inside the vagina. To further refine the female condom and add a second layer of protection the microbicide drug candidate UC781, a non-nucleoside reverse transcriptase inhibitor, was incorporated into the condom capsule adopting aspects of vaginal thin film technology.

**METHODS:** An appropriate polymer platform for the addition of UC781 to the condom capsules was identified through evaluations of 1) manufacturing process changes [dip-ability, curing, and peel-ability from mandrel]; 2) puncture strength; 3) thickness; and 4) disintegration time. UC781 was incorporated into polymer capsule platforms implementing strategies learned from previous vaginal film formulation efforts with drug candidate. Developed film capsule prototypes were characterized evaluating physical and mechanical properties, drug content uniformity, water content, disintegration time, dissolution, toxicity, and bioactivity.

**RESULTS:** Two condom capsule prototype formulations were identified that retained acceptable manufacturing attributes with respect to product appearance, capsule length, and capsule thickness. Rapid disintegration time was achieved. However, several aspects of the formulation require optimization such as drug content uniformity and mechanical strength.

**CONCLUSIONS:** UC781 can be incorporated into a modified film capsule formulation platform. However, the capsule formulation must undergo further optimization to achieve better drug content uniformity, establish long term stability, and product scalability.

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### Preclinical Stability and Excipient Compatibility Leading to the Development of Combination Gel Formulations of UC781 and Tenofovir

F.R. Damian<sup>\*1</sup>, J. Fabian<sup>1</sup>, M.R. Clark<sup>2</sup>, H. Elgendi<sup>2</sup>, G.F. Doncel<sup>2</sup>, D.R. Friend<sup>2</sup>, T. McCormick<sup>2</sup>, P.F. Kiser<sup>2</sup>

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**BACKGROUND:** Microbicide formulations of the potent HIV-1 inhibitors UC781 and tenofovir (TFV) are in clinical trials, and have demonstrated efficacy in viral challenged animal models. The objective of this work was to study the compatibility of UC781 and TFV with excipients that might be used in the formulation of gels and for development of prototype combination gels.

**METHODS:** Compatibility studies of UC781 dispersions were evaluated in the presence of TFV, hydroxyethylcellulose (HEC), propylene glycol, and EDTA/citric acid. Also, compatibility studies of TFV were performed in the presence of UC781, Carbopol 974P, methylcellulose and propylene glycol. The excipients, TFV and UC781 were placed in acetate buffer (20 mM, pH 5.2). The studies were conducted under four different conditions: 40°C/75% RH in the dark, 50°C in the dark, room temperature (RT) in the dark and RT in ambient light. Samples were collected at various time points and tested for drug content using HPLC. From these results prototype formulations of UC781 and TFV were made with HEC. These gels alone and in combination were subjected to preclinical cytotoxicity and antiviral testing against their matching placebos.

**RESULTS:** After 6 months of storage, UC781 showed equal to or better recovery in the presence of all excipients investigated compared with itself under dark conditions (UC781 recovery ranged from 90-103%), except with propylene glycol which showed a recovery of 89%. Extensive degradation of UC781 under light conditions was observed. TFV was stable in the presence of all excipients except methyl cellulose. It was observed that UC781 was compatible with TFV under all storage conditions. Likewise, tenofovir did not affect recovery of UC781. Based on these results prototype gels were developed containing 3% HEC. These gels showed acceptable cytotoxicity in vaginal cell lines and antiviral activity (EC 50 of 0.05  $\mu$ g/mL and 0.07  $\mu$ g/mL) against HIV-1 IIIB and BaL respectively.

**CONCLUSIONS:** Based on these results we conclude that UC781 and TFV can be formulated together in a gel that contains Carbopol, HEC, or EDTA/Citric acid and are stable for 6 months at 50°C when protected from light. We have identified the compositions with favorable physical properties and formulated prototype gels with both drugs that exhibit acceptable antiviral activity.

**250****Preclinical Stability Evaluation of Vaginal Rings Containing Dapivirine and Maraviroc**

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<sup>1</sup>Queen's University Belfast, Belfast, Northern Ireland, UK; <sup>2</sup>International Partnership for Microbicides, Silver Spring, MD, USA

**BACKGROUND:** A 28-day silicone elastomer vaginal ring containing dapivirine, a non-nucleoside reverse transcriptase inhibitor, is currently being evaluated in clinical trials. These rings have been reported safe and well tolerated in healthy HIV-negative women. Rings are now being developed to provide sustained release of microbicide combinations to increase efficacy. In this study, the pharmaceutical stability of a prototype silicone elastomer matrix ring containing a combination of dapivirine and maraviroc (a chemokine receptor antagonist) was assessed.

**METHODS:** Silicone elastomer matrix vaginal rings containing dapivirine (25 mg) and maraviroc (100 mg) were manufactured by injection molding at 140°C and stored in semi-permeable pouches in a temperature and humidity-controlled environment (30°C/65%RH and 40°C/75%RH). Ring samples were pulled at t=0,1,2,3M and tested for *in vitro* release (29 days), drug content, related substances and mechanical properties (tensile and compression).

**RESULTS:** *In vitro* release profiles for the rings did not vary significantly either with storage time or storage condition, each compound displaying matrix-type kinetics. Mean Day 1 dapivirine release was 1942 µg ( $\pm 473$  µg) declining to 197 µg ( $\pm 5.0$  µg) on Day 29, while mean Day 1 maraviroc release was 4516 µg ( $\pm 807$  µg) declining to 137 µg ( $\pm 8.9$  µg). The mean cumulative amount of each microbicide released over 29 days, for each timepoint, at either stability condition, was similar (11.9 mg dapivirine  $\pm 0.4$  mg and 11.9 mg maraviroc  $\pm 0.9$  mg). There were no significant differences ( $p < 0.05$ ) in compression between rings stored at 30°C/65%RH and 40°C/75%RH over 3 months. Drug content was maintained within specification and no related substances greater than 0.1% were detected.

**CONCLUSIONS:** This study demonstrates that silicone elastomer vaginal rings containing dapivirine and maraviroc are stable when stored at 30°C/65%RH and 40°C/75%RH over three months in respect of release, drug content, related substances and mechanical properties. T=6M data will be available March 2010.

**251****Vaginal Microbicide Formulation Using a New Drug Delivery**

B. Hao, Q. Wang, Z. Wu  
Nanjing University, Nanjing, 210093, China

**BACKGROUND:** Sexual transmission of HIV-1 has become a major route of the epidemic in China, accounting for more than 70% of the new infection in 2009. A female-controlled, low-cost preventative microbicide is urgently needed to contain the spread of the disease. Our candidate microbicide Nifeviroc (TD0232) is a small molecule CCR5 antagonist that exhibits potent inhibitory activity against CCR5 virus with low toxicity. However, Nifeviroc has poor solubility in neutral pH. We developed the inclusion complex of TD0232-β-cyclodextrin to improve its solubility and stability, and to provide sustained and controlled release. The inclusion complex was formulated in methylcellulose and investigated for drug stability, release kinetics and cellular toxicity.

**METHODS:** The inclusion complex of TD0232-β-cyclodextrin was prepared by freeze-drying technique. Two optimal inclusion complex preparations were tested for their *in vitro* stability and solution in aqueous medium. *In vitro* release and stability studies of the inclusion incorporated in these gels were tested, too. Rheological evaluation of gels containing the complex was investigated.

**RESULTS:** The solubility of TD0232 in the inclusion complex was increased in aqueous neutral medium by more than 200 times, and the inclusion complex was stable under various conditions. The inclusion complex of TD0232-β-cyclodextrin is fairly compatible with 2.5% γ-carrageenan and methylcellulose, and formulation of the inclusion complex in the gels appeared to further increase the solubility of TD0232. *In vitro* analyses were conducted to evaluate the cytotoxicity of the inclusion complex in a number of cell lines, including genital epithelial cells, and the results showed that the inclusion complex was well tolerated by the cells. *In vivo* studies were carried out in rabbits in a vaginal irritation model to investigate inflammatory reaction, degradation and immune stimulation resulted from the application of the inclusion complex on the vagina. Results demonstrated that the inclusion complex induced no observable local inflammation, and systemic immune stimulation. We also failed to detect any degradation of the carrier and the infiltration into the blood.

The inclusion complex of TD0232-β-cyclodextrin carrier system in gel provided sustained and controlled vaginal delivery of anti-HIV-1 agent without observable adverse effects.

**CONCLUSIONS:** The formulation of carrageenan and methylcellulose gels containing the inclusion complex provided a compatible and stable formulation for the delivery of the lipophilic Nifeviroc without adverse effect. The controlled release properties of the inclusion complex could be a beneficial property for a microbicide and facilitate the efficacy of the anti-HIV agent.

**252****A CCR5 Inhibitor Microbicide Formulated as an Inclusion Complex**

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**BACKGROUND:** Sexual transmission of HIV-1 has become a major route of the epidemic in China, accounting for more than 70% of the new infection in 2009. A female-controlled, low-cost preventative microbicide is urgently needed to contain the spread of the disease. Our candidate microbicide Nifeviroc (TD0232) is a small molecule CCR5 antagonist that exhibits potent inhibitory activity against CCR5 virus with low toxicity. However, Nifeviroc has poor solubility in neutral pH. We developed the inclusion complex of TD0232-β-cyclodextrin to improve its solubility and stability, and to provide sustained and controlled release. The inclusion complex was formulated in methylcellulose and investigated for drug stability, release kinetics and cellular toxicity.

**METHODS:** The inclusion complex of TD0232-β-cyclodextrin was prepared by freeze-drying technique. Two optimal inclusion complex preparations were tested for their *in vitro* stability and solution in aqueous medium. *In vitro* release and stability studies of the inclusion incorporated in these gels were tested, too. Rheological evaluation of gels containing the complex was investigated.

**RESULTS:** The solubility of TD0232 in the inclusion complex was increased in aqueous neutral medium by more than 200 times, and the inclusion complex was stable under various conditions. The inclusion complex of TD0232-β-cyclodextrin is fairly compatible with 2.5% γ-carrageenan and methylcellulose, and formulation of the inclusion complex in the gels appeared to further increase the solubility of TD0232. *In vitro* analyses were conducted to evaluate the cytotoxicity of the inclusion complex in a number of cell lines, including genital epithelial cells, and the results showed that the inclusion complex was well tolerated by the cells. *In vivo* studies were carried out in rabbits in a vaginal irritation model to investigate inflammatory reaction, degradation and immune stimulation resulted from the application of the inclusion complex on the vagina. Results demonstrated that the inclusion complex induced no observable local inflammation, and systemic immune stimulation. We also failed to detect any degradation of the carrier and the infiltration into the blood.

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**CONCLUSIONS:** The formulation of carrageenan and methylcellulose gels containing the inclusion complex provided a compatible and stable formulation for the delivery of the lipophilic Nifeviroc without adverse effect. The controlled release properties of the inclusion complex could be a beneficial property for a microbicide and facilitate the efficacy of the anti-HIV agent.

# MONDAY POSTERS

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### Acceptability of a Novel Device for Vaginal Drug Delivery

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**BACKGROUND:** Current methods for vaginal delivery of microbicides have significant limitations with respect to uniform gel retention, leakage, tissue coverage, and user acceptance. FHI has developed a novel device for vaginal drug delivery made from soft, medical-grade non-woven textile materials. Each packaged device was saturated with 10 mL of an FDA-approved vaginal lubricant, and prepared by an FDA-registered manufacturer.

**METHODS:** We recruited 40 monogamous, non-pregnant women: 20 family planning clients and 20 students. We asked women to insert and remove two devices, and be interviewed about their experience and opinions regarding the device. Women inserted and removed the first device at the clinic and the second device at home. For the device insertion at home, we asked women to discuss the study with their male partner and wear the device during intercourse if their partner agreed. We also asked women if their partner would agree to be interviewed about his experience, with a goal of interviewing ten men. Female participants' pelvic and outer genital areas were examined at clinic visits.

**RESULTS:** Female participants were aged 18 to 51, and had experience with a vaginal product: tampons (28 women), vaginal suppositories (11), or female condoms (3). For ease of insertion, 21 women said "very easy" and 19 said "easy." For leakage, only 6 said "yes," but without any problems or discomfort. For ease of removal, 32 said "very easy" and 8 said "easy." Thirty-four women wore the device overnight and had sexual intercourse while wearing the device. Five women did not wear the device during intercourse because their partners did not agree. One woman was lost to follow-up. None displayed any evidence of irritation. We interviewed ten male partners. Analyses of the qualitative data are underway.

**CONCLUSIONS:** Preliminary data are promising for further development of this device for vaginal delivery of microbicides. Its perceived similarity to tampons may have contributed to acceptability.

## 254

### Biophysical Computation of Vaginal Dilution and Distribution of Microbicide Gels

A. Szeri<sup>\*1</sup>, S. Tasoglu<sup>1</sup>, S. Park<sup>1</sup>, Y. Gao<sup>2</sup>, D. Katz<sup>2</sup>

<sup>1</sup>University of California, Berkeley, CA, USA; <sup>2</sup>Duke University, Durham, NC, USA

**BACKGROUND:** Successful microbicides require potent APIs that achieve targeted pharmacokinetics (PK). Drug delivery/PK by microbicide gels relates to vaginal distribution (epithelial surface area coated; local gel thickness) coupled to drug release rate. Predicting vaginal distribution is invaluable. This derives from biophysical mechanisms of vaginal gel spreading/retention: those incorporate gel properties, as influenced by the time course of gel dilution, with vaginal forces against the gel, and the anatomy and surface properties of the vaginal canal.

**METHODS:** We created an advanced biophysical model of pre-coital vaginal gel spreading. It incorporates the dynamics of gel dilution and their effect on local gel rheological properties. Experimental rheological measurements of test gels (undiluted and serial dilutions with simulants of vaginal fluids) are incorporated into a sophisticated gel rheological constitutive equation, that includes yield stress. This is combined with forces due to vaginal elasticity and gravity, and gel volume, in applying biomechanical equations of gel motion. Outputs are gel distribution, local gel dilution and velocity, as functions of time after gel insertion. The model is being applied to current microbicide gels and placebos.

**RESULTS:** Vaginal elasticity is generally more important than gravity in initiating gel spreading. Yield stress can significantly contain a gel in the fornix and limit leakage from the introitus; it is biomechanically distinct from viscosity, halting flow at low shear rates. Carbopol creates gel yield stress, but cellulose does not. Gel volume significantly influences details of spreading and leakage, as do variations in vaginal elasticity. Gel dilution by vaginal fluids is slow and non-uniform, creating heterogeneous gel properties. Pre-coital extent of gel dilution overall is predicted to be low for as much as an hour, even for gels that mix readily with vaginal fluids.

**CONCLUSIONS:** Our method improves previous models of vaginal gel spreading, incorporating the kinetics of gel dilution – this is a key factor in vaginal gel distribution as well as in delivery of APIs and transport of HIV virions to tissue surfaces. Gel volume is also significant. Measurements of gel properties – even sophisticated, non-Newtonian rheology – cannot alone predict vaginal distribution and retention. Biomechanical modeling is needed. Our methodology can be incorporated into schemas for rational gel design, based on optimizing gel compositions and properties.

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### Compatibility of HIV/AIDS-Killing Microbicide with Condoms

J. Owusu-Boateng, Ph.D., L.B. Kassman, E. Carter—Condax Medical Products LLC (Condax), Rockville Center, NY

*Family Health International, Quality Diagnostic Laboratories, Research Triangle Park, MD, USA*

**BACKGROUND:** The subject evaluated the physical effects of two prototype condoms – the first is identified as MK2T, a Natural Rubber Latex (NRL) condom coated with an aqueous lubricant containing 1% sodium dodecyl sulfate, and the second is identified as MK2T-1, a NRL male condom coated with a hydrogel solution containing 1% sodium dodecyl sulfate. The objective was to compare airburst and freedom from holes test results on samples tested before and after application of the lubricants. A ten percent negative change (-10%) in airburst values and/or a significant increase in the number of pinholes would be considered a degradation in strength properties.

**METHODS:** Samples were coated with 500 mgs of lubricant, sealed in aluminum foil, and conditioned for 7 days at room temperature. Following conditioning, the samples were subjected to airburst and freedom from holes testing. Separate sets of samples without lubricant were sealed in aluminum foil, conditioned and subjected to the same test regimen to establish a baseline. Mineral oil was used as a negative control.

**RESULTS:** The study showed no significant change in airburst properties or a significant increase in pinholes for the MK2T and MK2T-1 phenotypes.

**CONCLUSIONS:** Treated condoms were tested for performance and stability, and based on the initial assessment of compatibility, the SDS had no adverse effects on the integrity of natural rubber latex condoms as the compound did not degrade the performance of the condoms.

**256****Evaluation of RC-101 Stability in the Presence of Human Vaginal Fluid**

L.C. Rohan<sup>1,2</sup>, A. Sassi<sup>1,2</sup>, K. Bunge<sup>1</sup>, T. Conrads<sup>1,2</sup>, B. Hood<sup>1,2</sup>, L. Rabe<sup>2</sup>, A.M. Cole<sup>3</sup>, D. Patton<sup>4</sup>, B. Ramratnam<sup>5</sup>, P. Gupta<sup>1</sup>

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<sup>2</sup>Magee Womens Research Institute, Pittsburgh, PA, USA; <sup>3</sup>University of Central Florida, Orlando, FL, USA;

<sup>4</sup>University of Washington, Seattle, WA, USA;

<sup>5</sup>Brown University, Providence, RI, USA

**BACKGROUND:** RC-101 is a cationic retrocyclin analog which has *in vitro* activity against X4 and R5 strains of HIV-1. Delivery of peptide and protein drugs to the vagina is challenging due to its dynamic environment. It is essential to have adequate bimolecular drug stability *in vivo*. A major barrier to *in vivo* stability is the biological fluid present. The purpose of this study was to investigate the stability of RC-101 in several conditions including the presence of human vaginal fluids (HVF).

**METHODS:** HVF was collected from healthy premenopausal female volunteers. Demographics of the subject population were collected by questionnaire. All subjects were tested for Chlamydia trachomatis, Neisseria gonorrhoeae, and bacterial vaginosis. HVF samples were pooled and fluid used in exposure studies. RC-101 was incubated either alone or in its formulated state with collected fluid at 37 °C for time periods up to 72 hours. The abundance of RC-101 before and after exposure was monitored by liquid chromatography coupled online with tandem mass spectrometry. A single pooled fluid sample from individuals with bacterial vaginosis (BV) was also evaluated for its impact on RC-101 stability.

**RESULTS:** The fluid collected represented individuals with a mean age of 31 + 8 years. Average pH for normal fluid samples collected was 4.5 ± 0.6. RC-101 was detected by LC-MS/MS assay at least 48 hours after incubation with HVF. In one of the pooled samples studied RC-101 was detected up to 72 hours after HVF incubation. No difference was observed between formulated and unformulated drug in these studies. Interestingly, RC-101 was not detected following exposure to HVF from BV patients.

**CONCLUSIONS:** The results obtained in this study will promote a better understanding of the impact of HVF on microbicide product development and efficacy. RC-101 was shown to have adequate stability in normal HVF for at least 48 hours. However, this stability may be compromised in individuals with BV.

**257****Silicone Elastomer Rings for Sustained Delivery of HIV Microbicides: Correlating Historical *In Vitro* and *In Vivo* Release Data**

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Queens University Belfast, Belfast, UK

**BACKGROUND:** There is considerable interest in vaginal ring devices for the sustained delivery of HIV microbicides. In recent years, a wealth of *in vitro* release data has been produced for various microbicide candidates from silicone elastomer rings. However, comparative release and pharmacokinetic (PK) data from animal and human clinical studies is limited. In this study, we have reviewed historical *in vitro* release and *in vivo* release/PK data (published and unpublished) in a bid to better understand the factors contributing to meaningful *in vitro*-*in vivo* correlations and to modify *in vitro* release models to more closely mimic *in vivo* release.

**METHODS:** Matrix-type silicone elastomer rings comprising a single antiretroviral compound, (5% w/w loading; dapivirine, maraviroc, DS004, UC781, lopinavir, ritonavir, saquinavir, CMPD167) were manufactured and evaluated for *in vitro* release using a standard sink condition model. The trends in *in vitro* release behaviour were interpreted according to the compounds' physicochemical characteristics, and, where data was available, correlated with PK and residual drug extraction data obtained from animal and human studies.

**RESULTS:** Significant differences in *in vitro* release rates and kinetics were observed for the various antiretroviral-loaded rings, which generally correlated with the compounds' measured solubilities in silicone and their respective octanol-water partition coefficients. The following trend of increasing *in vitro* cumulative release (28 days) was observed: SAQ < RIT < LOP < MAR < 167 < DAP < DS004 < UC781. However, *in vitro* release was not necessarily predictive of *in vivo* performance. For example, PK levels of SAQ and UC781 were very low following ring application in macaques, while DAP rings with significantly lower drug loadings produced higher and more reproducible PK levels in humans.

**Conclusions:** The study underscores the difficulties in predicting *in vivo* microbicide levels based on *in vitro* data. For effective *in vivo* release from silicone rings, microbicides must possess an appreciable degree of both aqueous and silicone elastomer solubility. Furthermore, current sink condition *in vitro* release models, which employ water-solvent mixtures to maintain diffusion-controlled rather than dissolution-controlled release, may erroneously predict that compounds displaying high *in vitro* release will perform best *in vivo*. New *in vitro* release models that more closely mimic *in vivo* conditions are needed.

**258****Family Health International (FHI) Condom Testing Comparison of SDS (MK2T)**

J. Owusu-Boateng, L.B. Kaszman  
Condax Medical Products LLC, Rockville Center, NY, USA

**BACKGROUND:** The subject evaluated the physical effects of two prototype condoms – the first is identified as MK2T, a Natural Rubber Latex (NRL) condom coated with an aqueous lubricant containing 1% sodium dodecyl sulfate, and the second is identified as MK2T-1, a NRL male condom coated with a hydrogel solution containing 1% sodium dodecyl sulfate. The objective was to compare airburst and freedom from holes test results on samples tested before and after application of the lubricants. A ten percent negative change (-10%) in airburst values and/or a significant increase in the number of pinholes would be considered a degradation in strength properties.

**METHODS:** Samples were coated with 500 mgs of lubricant, sealed in aluminum foil, and conditioned for 7 days at room temperature. Following conditioning, the samples were subjected to airburst and freedom from holes testing. Separate sets of samples without lubricant were sealed in aluminum foil, conditioned and subjected to the same test regimen to establish a baseline. Mineral oil was used as a negative control.

**RESULTS:** The study showed no significant change in airburst properties or a significant increase in pinholes for the MK2T and MK2T-1 phenotypes.

**CONCLUSIONS:** Treated condoms were tested for performance and stability, and based on the initial assessment of compatibility, the SDS had no adverse effects on the integrity of natural rubber latex condoms as the compound did not degrade the performance of the condoms.

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### **In Vitro Release of Tenofovir and Acyclovir from a Silicone Vaginal Ring Platform**

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<sup>2</sup>Aurited Pharmaceuticals, Santa Monica, CA, USA;

<sup>3</sup>CONRAD, Arlington, VA, USA

**BACKGROUND:** Intravaginal ring delivery of combinations of microbicides is a promising approach for prevention of sexually-transmitted disease, but requires a method of providing simultaneous, independent release of multiple agents. We report the development of an intravaginal ring (IVR) delivery platform for 30-day delivery of the reverse transcriptase inhibitor tenofovir (TFV) and the guanosine analogue antiviral acyclovir (ACV), and evaluation of its *in vitro* release characteristics.

**METHODS:** A pellet of ~15 mg TFV or ACV is prepared by first compressing a core followed by coating with semi-permeable polymer, resulting in a "pod." The pod is then incorporated into a silicone ring or segment during an injection molding process. The release rate for each pod is controlled independently, determined by the size of a delivery window that is mechanically formed in the silicone during molding. Segments contain 1–2 pods, and rings up to 10 pods. Within a ring, each pod can be identical or contain different drugs; in this study, TFV and ACV were formulated in separate pods. *In vitro* release studies of the rings were carried out in water and simulated vaginal fluid. The concentrations of TFV and ACV released into solution were determined by UV-vis and HPLC.

**RESULTS:** *In vitro* release from segments containing a single pod was measured for delivery window diameters from 0.35–2.0 mm, and was in the range 2–430 µg/day (TFV) and 3–81 µg/day (ACV). For TFV, a 30-day study showed release rates of  $43.5 \pm 5$  µg/day from single pod segments ( $n=10$ ) and  $385 \pm 57$  µg/day release from 10-pod rings ( $n=10$ ), both with 1.0 mm delivery windows. For single ACV pod segments, a 1.2 mm delivery window resulted in 14 µg/day release (30 days,  $n=4$ ). Segments containing one TFV and one ACV pod (0.5 mm delivery windows), showed simultaneous release rates of 11 µg/day TFV and 6 µg/day for ACV ( $n=3$ ).

**CONCLUSIONS:** An IVR delivery platform for simultaneous, independently-controlled release of multiple microbicides has been developed and evaluated for *in vitro* release characteristics of TFV and ACV. Release rates for each drug can be controlled independently, and tailored to specific dose requirements by varying the delivery window size during manufacture.

## 260

### **Measuring Adherence in the CAPRISA 004 Tenofovir Microbicide Gel Trial**

L.E. Mansoor<sup>\*1</sup>, Q. Abdoool Karim<sup>1,2</sup>, N. Yende<sup>1</sup>, S. Abdoool Karim<sup>1,2</sup>, on behalf of the CAPRISA 004 study team

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<sup>2</sup>Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, USA

**BACKGROUND:** The effectiveness of a microbicide in a clinical trial is dependent on both the efficacy of the product as well as the participants' willingness and ability to use the product as instructed. In contrast to previously tested microbicide candidates, adherence to product in CAPRISA 004 does not have a one-to-one correspondence to coital activity; two applicators are used for every sex day, irrespective of the number of sex acts within the day. A structured theory-based adherence support programme (ASP) was designed to enhance correct product use in CAPRISA 004.

**METHODS:** Adherence counselling was provided to study participants upon enrolment and additionally at each study visit. The Protocol Team monitored adherence rates over time, and individualized adherence counseling methods were updated and implemented systematically if needed to address lower-than-expected rates. At each monthly study visit, data on adherence to the product use regimen was collected via self report using structured behavioral assessment questionnaires. These were interviewer-administered instruments which ascertained participants' frequency of coitus, condom use, product use and timing thereof in relation to coitus.

**RESULTS:** Data from the first 6 months of trial participation showed that 34869 sex acts were reported in 890 women resulting in a mean of 7 sex acts per women per month. The 7-day recall showed a mean adherence level of 96%. The 24-hour recall showed 4546 last sex acts, 76.6% of which had gel and a condom being used and 20.3% had gel use only. On average 96% of sex acts reported in the last 24 hours had correct gel use, with 1.5% having gel use after sex only and 2.3% having gel use before sex only. The majority of participants used the gel within 4 hours before sex and 3 hours after sex.

**CONCLUSIONS:** The structured ASP and one-on-one sessions empowered participants to pre-empt situations that lead to non-adherence and make plans for situations in which they are less likely to adhere. This resulted in high levels of adherence being reached in CAPRISA 004 based on self-report. This technique, however, is not without limitations as some participants may experience inadequate recall, misunderstanding of questions and social desirability bias. Future clinical trials may consider a combination of self report and other more direct measures of adherence.

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### **Accounting for Used and Unused Applicators in a Phase III Microbicide Trial in Durban, South Africa**

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Medical Research Council, HIV Prevention Research Unit, South Africa

**BACKGROUND:** Assessing gel applicator returns plays an important role in determining adherence in microbicide trials. In many trials this is performed on unused gel applicators alone. The Microbicides Development Program (MDP) 301 study was a randomised, double-blind, placebo-controlled trial to evaluate the efficacy and safety of PRO 2000 gel for the prevention of vaginally acquired HIV infection. Of the total 9385 women enrolled in the trial, 2391 participants were enrolled at three recruitment centres in Durban, South Africa. Participants were followed up for a period of one year and an assessment of both returned used and unused applicators was done at every visit.

**METHODS:** Participants were counseled on study product adherence and asked to bring back all used and unused gel applicators at every visit. They were issued transparent resealable polypropylene bags in which to store and return their used applicators. Study staff documented the number of applicators issued and returned on gel accountability case record forms. Staff addressed any gel count discrepancies with participants.

**RESULTS:** 66% ( $n = 1567$ ) of all participants returned 100% of the applicators dispensed to them during their 12-month follow up. 21% ( $n=502$ ) participants returned more than 90% of their applicators while 8% ( $n=198$ ) returned between 70% and 90% of their applicators. Only 5% ( $n=126$ ) of participants returned under 70% of issued applicators. 98% of all dispensed applicators were returned. The high proportion of returns indicated good adherence to protocol and supported self-reported adherence data. Usage trends gained from returned applicators assisted appropriate prescribing of the coitally dependent product. Discrepancies in returns also prompted staff to probe for missing applicators and provided opportunities for appropriate counseling. Requesting used applicators to be returned enabled the study to take responsibility for the safe disposal of these applicators. 23 participants returned extra applicators that alerted staff to possible gel sharing or gel mixing. 22 faulty applicators were also returned alerting sponsors to the type and extent of defects experienced with study product.

**CONCLUSIONS:** Accounting for both used and unused applicators has a valuable contribution to study data and is recommended in future microbicide trials.

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### Daily Monitored Adherence in a Phase I/II Dapivirine Gel Study in Madibeng, South Africa

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**BACKGROUND:** Adherence to microbicide gel use is important for determining efficacy in Phase III trials. To date, an objective method for monitoring adherence to gel use has not been established. IPM is testing daily monitored adherence (DMA) in two safety trials of dapivirine microbicide gels at research centers in Africa. Madibeng Centre for Research (MCR) is one of the research centres participating in these safety trials.

**METHODS:** Participants enrolled in the safety trials chose either daily home visits by an outreach worker (OW) or daily contact at a drop-off centre. An OW was assigned to each participant based on her choice of DMA. A database was used to track OW visits, and tracking sheets were provided to the OW on a daily and weekly basis to prepare for and monitor visits with participants. Daily contacts were maintained barring public holidays and weekends. During contact visits, OWs collected used and unused gel applicators and completed gel applicator collection forms which included information on adverse events. Re-education of participants on gel use occurred as needed. Daily review of gel applicator collection forms ensured good tracking of self-reported adverse events.

**RESULTS:** MCR enrolled 17 participants who were followed for 6 weeks using DMA. Follow-up with DMA was successful at MCR; no participants were lost to follow-up. Participants reported a high level of adherence to gel use. No confusion arose if a different OW replaced the assigned OW. One participant requested a modification to her DMA; she complied with all contact visits and the gel regimen. One participant misunderstood the frequency of gel use and used 2 applicators on her first day; she was adherent upon re-education. Participants and OWs built a good rapport enabling timely reporting of adverse events.

**CONCLUSIONS:** DMA was successfully implemented and supported monitoring of adherence to the gel regimen in a Phase I/II microbicide safety trial. It also created opportunities to inform research centre personnel of adverse events and aided in early identification of misunderstandings regarding gel use enabling rectification. "Lost to follow-up" rates were minimised as daily contact visits allowed staff to familiarize themselves with participants' routines. If needed, smooth transfer of participants to replacement staff is essential for successful follow-up.

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### Socio-Behavioral Approaches to Maximize Adherence in a Phase 3 PrEP Trial

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**BACKGROUND:** HIV pre-exposure prophylaxis (PrEP) will probably face the same challenge in adherence to study product as microbicide clinical trials. FEM-PrEP—a phase 3, oral PrEP clinical trial for HIV prevention in women—uses socio-behavioral formative research and behavioral monitoring to try to maximize adherence.

**METHODS:** Prior to the clinical trial, 45 in-depth interviews (IDIs) and 12 focus group discussions (FGDs) were conducted with potential trial participants in Bondo, Kenya, and Pretoria, South Africa, to identify potential barriers and facilitators to study product adherence. All IDIs and FGDs were recorded and transcribed/translated into English for content analysis. Data were used to inform adherence counseling messages, procedures, and tools.

During the trial, monitoring of adherence data is conducted regularly to identify common reasons for missing study pills and adherence trends.

**RESULTS:** A key finding from the formative research was that 66% of women in Bondo and 79% in Pretoria said it would be difficult not to share or sell the study product. Reasons included wanting to help an HIV-positive friend or another participant, needing money, or believing that the pill is effective. In response, adherence counseling messages were tailored at each site to include site-specific reasons for not sharing/selling study pills.

Monitoring of the trial adherence data found that the main reasons for missing the study pill included participants being away from home, simply forgetting to take the pill, and having a change in normal daily activities. In response, counselors assess these issues with each participant during adherence counseling.

**CONCLUSIONS:** Formative data inform adherence messages, counseling, and tools by tailoring them to include issues pertinent to the study population at each site. Counselors use trial adherence data to provide additional counseling on adherence barriers commonly found among trial participants.

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### Factors Associated with Non-Adherence to Product Use at the Durban MDP 301 Centres.

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**BACKGROUND:** Successful completion of a clinical trial is dependent on maintaining a high level of product adherence. Poor adherence during a trial can decrease the power to detect an effect of the product on trial. Recognizing indicators and risk factors for non-adherence can help determine which participants may be potential non-adherers and subsequently address adherence issues at the onset of the trial. The aim of this paper is to identify factors associated with product non-adherence in the MDP 301 Durban centres.

**METHODS:** Consistent gel use was defined by 3 criteria which included attending at least 7 of the maximum 13 visits (unless pregnant or an HIV sero-converter), reporting gel use at the most recent sex act at 92% or more of attended visits, and returning at least one used applicator at all visits when gel use was reported at the most recent sex act. Chi-square tests were performed to test for association between consistent gel use and demographic variables, clinical characteristics at enrolment, STIs, other genital conditions, number of sex acts in the last week and number of sexual partners at enrolment.

**RESULTS:** There was no evidence of differences between adherers ( $n=992$ ) and non-adherers ( $n=720$ ) in number of sex acts, sexual partners, contraception use and STIs. Age, education level and presence of unusual genital discharge were the only baseline factors associated with non-adherence, although there was also strong evidence that inconsistent gel users were also less likely to report using a condom at last sex act throughout the trial. Participants under the age of 35 years had strong evidence of lower adherence ( $p < .0001$ ) than those aged 35–45+ years. Participants with no or little education had better adherence ( $p < .0001$ ) than those who completed primary or tertiary education. Non-adherers reported higher unusual genital discharge ( $p = 0.001$ ) than adherent participants. Reasons for product non-adherence included forgetting to insert the product, lost products, partner unaware of product use and ran out of product due to missed clinic visits.

**CONCLUSIONS:** It is recommended that special education efforts and adherence enhancement strategies be implemented to reinforce product adherence and condom use for younger participants, and those with more education. In order to facilitate long term adherence in the new Prep trials, additional novel approaches must be also be considered for women who have not used daily or oral methods.

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## Biophysical & Behavioral Acceptability of Microbicides

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**BACKGROUND:** First generation microbicide candidates are delivered via gel vehicles, intended to coat the vaginal epithelium before protected sexual intercourse for the user. The waiting time before protection depends on biophysical properties of the gel, which restricts the potential choices for an effective product. In other words, the gel vehicle first must be physically synthesizable, then acceptable to the user, and finally applied in a manner that promotes adequate coating by the time it is to function. We explore here women's preferences about microbicide attributes when biophysical constraints are imposed upon their choices.

**METHODS:** Microbicide acceptability was assessed using structured questionnaires (n=70) with socio-economically diverse women aged 18–55 from California. Participants were asked to provide their preferences about different microbicide attributes, namely the wait time between application and intercourse, and the gel texture. The trade-off between wait time and gel texture was assessed by a mathematical model constraining coating rates upon changes of the gel's physical attributes. Preferences were ranked on a scale from 1 to 7. We asked independently about the first two attributes and then about the trade-off item when the biophysical constraints necessary for the product effectiveness were applied.

**RESULTS:** Results showed that women in steady relationships are more likely than women in casual relationships to prefer a microbicide gel that spreads very fast ( $p < 0.000001$ ) and that is thick ( $p < 0.001$ ), when they were asked the questions independently. However, when they were asked the same question 'constrained' by the biophysical reality, we observed that a quasi-uniform preference for gels of different thicknesses (and so waiting times) stood out ( $p > 0.05$ ).

**CONCLUSIONS:** Biophysical constraints do matter and women's preferences regarding microbicide attributes change when biophysical constraints restrict the range of options. In the case of gel vehicle microbicides, we observe no distinct preference for each of the gels available after controlling for physical reality. In that sense, developers should offer a range of formulations in order to address the preference of all users.

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## Self-Reported Adherence to Daily Oral Truvada in the FEM-PrEP Trial

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**BACKGROUND:** Achieving high study product adherence in HIV prevention trials has posed significant challenges. FEM-PrEP, a randomized placebo-controlled PrEP trial, is faced with similar challenges.

**METHODS:** A multivariate analysis of FEM-PrEP baseline and follow-up data examined self-reported adherence to the daily study pill and associations between self-reported adherence, use of adherence aids and demographics at two study sites. Participants receive intense adherence counseling at monthly visits and are offered adherence aids.

**RESULTS:** This analysis included 833 monthly follow-up visits: 701 in Bondo, Kenya and 132 in Pretoria, South Africa (visits with missing adherence data were dropped from the analyses). Among 306 participants completing their week 4 visit, 82% reported taking their pill "always" (A) in the past 4 weeks, 17% "usually/almost always" (U/AA) and <2% "sometimes", "rarely" or "never." Self-reported adherence remained high at the week 8 (87% A, 13% U/AA, n=231), week 12 (91% A, 7% U/AA, n=156) and week 16 (93% A, 7% U/AA, n=98) visits. No significant associations were observed between participant baseline demographics and self-reported adherence in the last 4 weeks. At 809 follow-up visits, substantial proportions of participants reported using adherence aids promoted during study counseling sessions including: a calendar, 54%; cell alarms, 47% and a weekly pill container, 85%. All associations between adherence aid usage and self-reported adherence in the last 4 weeks were non-significant ( $p=0.05$ ).

**CONCLUSIONS:** Participant self-reported study pill adherence is high in FEM-PrEP. No significant associations have yet been observed between use of the any of the adherence aids provided in the trial and self-reported adherence. The validity of self-reported adherence data is unknown. Laboratory methods for measuring study drug adherence are currently limited, but will continue to be explored by the FEM-PrEP trial.

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## Vaginal Practices Among Nigerian Women and Its Implication for Microbicide Development and Use

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**BACKGROUND:** The urgency and need to quickly deploy a safe and effective microbicide requires simultaneous research into factors that may impact on their effectiveness at community level. This study assessed the types and pattern of vaginal practices among Nigerian women.

**METHODS:** Questionnaire based cross-sectional survey of women seen within a predefined strata to determine the type and pattern of vaginal practices and use of vaginal products. Data analysis with SPSS.

**RESULTS:** Majority were married (53.4%) and above 30 years of age (53.7%). Multiple sexual partnerships rate were 18.0%. Contraceptive uses rate was low with consistent condom use rate of 29.4%. Partner refusal (59.7%), and reduction of sexual pleasure (32.9%) were the reasons for non use of condom. 94.5% of the women have used a vaginal product for hygiene, enhancement of sexual pleasure, to please my partner, medication and prevention of pregnancy. Vaginal products used were petroleum jelly, commercial douching preparation, pessaries, gel, herbs and alum. Majority (91.7%) reported washing the vagina after sex with water, soap or antiseptic solutions. 30.3% have heard about microbicide, however majority will use it if found to protect against STI and HIV infection (89.7%) and prefers gel form (97.2%) to a suppository because gels are less messy (76.6%) and serves as lubricant (36.8%). Non availability and cost will be limitation.

**CONCLUSIONS:** Various forms of vaginal practices exists among Nigerian women that might impact on the use and effectiveness of microbicide. It is recommended that future microbicide trial should take this finding into consideration.

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## A Survey on Determinants of Acceptance of Microbicides Among Gambian Women

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**BACKGROUND:** With the possibility of launch of effective microbicides into the world market by 2010 and growing recognition of the potential value of microbicides for HIV/STI prevention, there is a need to assess the preparedness and determinants of the acceptability of this brand-new technology by the end users in the West African state of The Gambia. This is because failure to understand the key factors associated with microbicides' acceptability is likely to hinder the adoption and continued use of products that are effective in preventing HIV infection.

**METHODS:** We employed qualitative and quantitative tools to assess the knowledge, perception and factors that may influence adoption and use of microbicides among women attending health facilities in the coastal (urban) and provincial (rural) areas of western division of The Gambia. Focus group discussion and Key Informant Interviews were held to further elicit contextual information from the participants. Quantitative data was analysed by generating simple inferential statistics while content analysis and ethnographic summary of proceedings of Focus Group Discussions and Key Informant interviews were done.

**RESULTS:** Three hundred and nineteen women were approached while 294 (92.2%) agreed to participate in the survey. Majority (66.9%) were in the reproductive age group of 15-49 years. About 72% belonged to socio-economic class III and IV and 17.8 % had formal education. Eighty-five percent were multiparous (more than 2 children) and about 60% were in polygynous relationship. Knowledge and awareness about microbicides were poor with only 12.8% of participants have heard about microbicides and its purpose. Only 7% of participants could mention other forms of new HIV prevention technologies.

During the FGD, a brief pictorial talk on microbicides was given by MA and the participants were requested to say if they would be willing to adopt and use this new technology. Majority responded in affirmative but stressed that cost of microbicides and its endorsement by their male partners would go a long way in fully embracing microbicides.

**CONCLUSIONS:** Despite the fact that microbicides is female-dependent and female-controlled, male involvement is still an important factor that may shape its acceptance among Gambian women. There is also a great need to create awareness on the usefulness of microbicides among Gambian men and women.

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## Microbicide Acceptability Among Coitally Associated and Daily Users of Tenofovir Gel

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**BACKGROUND:** In a parallel behavioral study we assessed the differences in gel acceptability between participants randomized to coital use or daily use arms in the randomized, double-blinded, placebo-controlled clinical trial of 1% Tenofovir vaginal microbicide (HPTN 059 study).

**METHODS:** Data was collected at baseline and during three follow-up evaluations at two monthly intervals from 100 consenting, sexually active, married women in Pune, India. Information on behavioral domains collected on 6 point Likert scale was converted into high and low acceptability scores. Random intercept logistic modeling was performed to examine the simultaneous effects of study arms, follow-up time, socio-demographic factors and behavioral domains on gel acceptability.

**RESULTS:** The mean age of participants was 32.7 years; similar for those in coital and daily use arms. Nearly half of the study participants had completed less than ten years of schooling and were working. Women in both the study arms were similar in their socio demographic characteristics except for monthly income which was significantly higher in the coital arm.

Women liked the easy use of the gel as well as its potentially protective effect against HIV and while such perceptions increased during subsequent visits in both the arms they were more marked in the coital arm. Messiness was disliked by women in both arms. Similar to other microbicides trials, mixed responses were observed for appearance and smell of the product, possibility of covert use, women's control over the method and lack of need for interruption in sex.

Acceptability of gel was almost three times higher in participants of the coital use arm. [AOR=2.71, 95%CI:1.23–5.93, p=0.013]. Acceptability differed significantly at 2 and 6 month visits between the 2 study arms (68% vs 40%, p=0.006, and 64% vs. 46%, p=0.07 respectively). Acceptability was significantly lower among participants who reported "messiness" as the most disliked feature (AOR = 2.42, 95% CI:1.02-5.72, P=0.045) compared to those who did not, at the fourth month visit. None of the socio-demographic and behavioral domains predicted gel acceptability significantly.

**CONCLUSIONS:** Tenofovir gel was found more acceptable in a coitally-dependant setting as compared to its daily use. Efforts to empower women for better control over their sexual matters and addressing the issue of leaking during product manufacturing may help to improve microbicide acceptability.

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## Assessing HIV-1 Cross-Resistance *In Vitro* against RTI-based Microbicides Currently Under Development

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**BACKGROUND:** The use of microbicides based on reverse transcriptase inhibitors (RTI) by undiagnosed, HIV+ woman could potentially induce viral resistance compromising subsequent therapeutic options. Moreover, RTI-based microbicides that are inactive against resistant strains might promote the selective transmission of these viruses. Therefore, we investigated the resistance profile of four RTI currently under development as a microbicide (TMC120, UC781, MIV160, TNF).

**METHODS:** Resistance against these four microbicides and two first-line therapeutics (NVP and EFV) was induced by serial passage of three HIV-1 isolates (subtype B, C, CRF02\_AG) in PBMC with increasing compound concentrations. Inhibition of these resistant viruses was evaluated (i) in TZM-bl cells for the four RTI microbicides and the therapeutics AZT, NVP, DLV, EFV and (ii) using a HIV-1 Phenotyping Assay (VIRALARTSTM/HIV) to test all FDA-approved PI, RTI and INI. A full resistance matrix was obtained for each candidate RTI-based microbicide. Resistance associated mutations (RAMs) were identified by sequencing the viral pol gene and resistance was expressed as intermediate (FC<100) or complete (FC>100). Replicative fitness of p2/p7/p1/p6/PR/RT/INT-recombinant viruses was determined using viral growth kinetics and growth competition experiments.

**RESULTS:** HIV-1 resistance to the NNRTI UC781 was induced *in vitro* within five weeks while resistance to the NNRTI TMC120 and MIV160 only occurred after twelve weeks. Virus resistant to the NtRTI TNF could not be generated under these conditions. As expected, viruses resistant to the NNRTI TMC120, UC781 or MIV160 remained fully sensitive to the NRTI AZT, but lost all sensitivity to NVP and DLV. Although EFV lost all activity against MIV160 resistant virus, it retained some activity against TMC120 and UC781 resistant strains. Viruses resistant to EFV were no longer suppressed by TMC120, UC781, MIV160. NNRTI-resistant viruses showed a decrease in viral replicative fitness. Cross-resistance was not observed with the NtRTI candidate microbicide TNF.

**CONCLUSIONS:** The large scale introduction of mono-RTI-based microbicides should therefore be considered with caution.

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### Genetic Diversity and Drug Resistance of HIV-1 in a Cohort of Women in Nairobi, Kenya

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**BACKGROUND:** The success of antiretroviral therapy in treating HIV infection is hampered by the emergence of drug-resistant genetic variants. In this study, we have genotyped the reverse transcriptase region of the pol gene of HIV-1 virus isolated from HIV-1 positive patients in Nairobi cohort to determine HIV genetic diversity and anti-retroviral drug resistance mutations. The objective was to document HIV genetic diversity and drug resistance in a cohort of HIV-infected women in Nairobi Kenya.

**METHODS:** Plasma samples were collected for viral RNA extraction from 23 drug naïve patients. Out of the 23 drug naïve patients, initial point zero on follow up samples were collected for analysis. RT region of the pol gene of HIV-1 was amplified from the viral RNA genome by reverse transcription followed by nested PCR using specific primers and directly sequenced. RT sequence was used for phylogenetic analysis and determination of drug resistance mutations.

**RESULTS:** Phylogenetic analysis revealed that among the subjects, we found subtypes 1A (43.5%, n=10), C (26.1%, n=6), D (17.39%, n=4), A1D (13.04%, n=3). Based on the small size, the 13.04% intersubtype recombinants detected suggested, a higher circulating recombinant forms levels in rest part of Nairobi city, Kenya. In addition, from all the 23 subjects analysed no single mutation was detected that is linked to any antiretroviral drug resistance. This was based samples collected sampling point 0 on follow up.

**CONCLUSIONS:** This is the first follow up study in Kenya to determine the status of drug resistance mutations in HIV positive patients on follow up on drug resistance evolution within a cohort. No drug resistance mutations were detected sampling point zero.

It is still increasing confirmed that HIV subtype 1A is still predominant in Kenya. Additionally, the detection of high prevalence of recombinant forms indicates viral mixing in among the population, possibly as a result of dual infections.

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### The Use of a Topical Male Microbicide on Condom Migration Among Fishermen on the Beaches of Lake Victoria in Kisumu District, Kenya

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**BACKGROUND:** Condoms provide the best known protection from most STIs. The introduction microbicides is postulated to possibly lead to condom migration. We assessed the extent to which the use of a topical male microbicide by fishermen during a Phase I safety trial resulted in condom migration.

**METHODS:** In an 8-week randomized double-blind placebo-controlled crossover trial among 30 fishermen to assess the safety of 62% and 15% ethanol in emollient gel, participants were randomized to the initial use of either product for two weeks, washout period of 2 weeks then a crossover of product use for 2 weeks and followed up for final 2 weeks. The fishermen were consistently counseled on adherence to and use of the study product after every sexual act, safer sex practices and provided with unlimited male condoms. We collected information on condom use at baseline and at follow up visits information on product and condom adherence. We analyzed the data using paired t-test to test the difference between proportions of condom protected sexual acts reported at days 7 and 14 with those reported during the first washout period at day 28 and those reported at days 35 and 42 with those reported during the final 2 weeks at day 56.

**RESULTS:** Of the 30 fishermen who completed the follow up, half had sexual debut at age 14 (range: 11–25) with a mean of 2 (1–4) sexual partners in the month preceding the interview. The median number of life time sexual partners was 8 (2–50). There were a total of 798 reported sexual acts over the 8-week period and the study product was reported to have been used in 99.7% of the 702 acts when the participants had the product. At baseline, overall condom use was 15% that doubled to 32% during the 8-week follow up period. The mean proportion of sexual acts protected by condoms was .32, .35, .39, .28, .24 and .31 at days 7, 14, 28, 35, 42 and 56, respectively. Paired t-test showed no significant difference between proportion of sexual acts protected by condoms at days 7 and 14 relative to those reported at day 28 during the first washout period ( $p>.05$ ) and similarly showed no significant difference between proportion of sexual acts protected by condoms at days 35 and 42 relative to those reported during the final 2 weeks at day 56.

**CONCLUSION:** There is no evidence of condom migration with the use of a topical male microbicide in this population with low condom use. This population could benefit from a topical male microbicide.

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### Phase 2 Clinical Trial Testing Colonization Efficiency, Safety and Acceptability of *Lactobacillus crispatus* CTV-05 (LACTIN-V) in Women for the Prevention of Recurrent Bacterial Vaginosis

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**BACKGROUND:** Bacterial vaginosis (BV) is a common vaginal infection. The lack of endogenous lactobacilli and overgrowth of pathogens facilitate numerous gynecological complications including an increased risk of HIV acquisition. Approximately, 30% of BV recurs after antibiotic treatment. The bacterial therapeutic *Lactobacillus crispatus* CTV-05 (LACTIN-V) is intended to repopulate the normal vaginal flora. This completed trial measured the colonization efficiency, safety and acceptability of LACTIN-V following standard antibiotic treatment of BV.

**METHODS:** Twenty-four women diagnosed with BV were randomized in a 3:1 ratio to receive LACTIN-V at 2 X 10<sup>9</sup> cfu/dose or matched placebo. Participants completed a 5-day treatment with topical metronidazole followed by administration of LACTIN-V or placebo once daily for 5 days and once weekly over 2 weeks. Participants returned 10 and 28 days after randomization to determine the colonization efficiency of *L. crispatus* CTV-5 which was defined by a positive culture result for *L. crispatus* CTV-5 at the day 10 and/or day 28 visit. Adverse events (AE) were assessed using the DAIDS Toxicity Table Addendum for Vaginal Microbicide Studies, and acceptability was measured by a self-administered questionnaire.

**RESULTS:** After 4 weeks, colonization efficiency in the LACTIN-V group reached 61% (95% CI: 36-83%) in the intent-to-treat (ITT) cohort and 78% (95% CI: 40-97%) in the according-to-protocol (ATP) cohort. Of 120 total AEs, 108 (90%) and 12 (10%) were mild and moderate in severity, respectively. No grade 3 or 4 AEs occurred. The most common genitourinary AEs included vaginal discharge (46%), abdominal pain (46%) and dysuria (21%). AEs were evenly distributed between the LACTIN-V and placebo groups. Nineteen women (79%) would agree to use the product again.

**CONCLUSIONS:** LACTIN-V appears safe, well tolerated and acceptable to women treated for BV. Colonization results in both the ITT and ATP cohort demonstrate that this product warrants further investigation to determine its effectiveness to prevent recurrent BV.

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**Participant Recruitment and Retention in a Phase II Pharmacokinetics Microbicide Trial in Kampala-Uganda: MTN 001 A Phase II Adherence and Pharmacokinetics Study of Oral and Vaginal Preparations of Tenofovir**

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**BACKGROUND:** Research participant recruitment and retention in clinical trials is widely recognized as the leading bottleneck in the conduct of successful clinical trial. It is often the most labor-intensive and difficult component of clinical trials. Achieving participant recruitment is essential and provides a base for projected participant retention, resulting in evaluative research data. As the design of the clinical trials become more intense, the forces affecting participant enrollment grow more numerous and complex. This paper describes the process, challenges, and strategies utilized during the recruitment and retention of non HIV-infected participants in a microbicide trial at MU-JHU Research Collaboration.

**METHODS:** HIV-negative, sexually active females were screened for the study. Those who met the eligibility criteria were enrolled into the study. Once enrolled, women were expected to visit the clinic at weeks 3, 6, 7, 10, 13, 14, 17, 20 and 21. Each participant is dispensed Tenofovir gel, tablets or both following a cross-over design with close clinical and laboratory monitoring. Pharmacokinetic procedures are performed at weeks, 6, 13, and 20. Participation rates, along with a descriptive summary of retention strategies established prior to and in response to challenges incurred during implementation are presented.

**RESULTS:** The final recruitment rate was 33.5% (230 pre-screened to 77 screened). The enrollment rate was 36.4% (77 screened to 28 enrolled). The overall retention rate is so far 82%. Challenges to retention include a mobile working population, non-disclosure of study participation to partners, and economic constraints. The high participation rates were achievable through various strategies used which include intensive adherence counseling, use of detailed locator information, a participant tracking database, active home visits, provision of high quality medical care, and the formation of close bonds between staff and subjects. All missed clinic visits are followed up promptly

**CONCLUSIONS:** Accrual was met within the protocol specified accrual target of six months. Relatively high enrollment and retention rates have been achieved in this trial through added staff efforts and resources. Community involvement is also crucial to achieve these rates. Recruitment and Retention plans of trial participants should be an on-going process throughout the study and should be modified as needed throughout the study.

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**Acceptability of Optical Coherence Tomography During a Clinical Study**

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**BACKGROUND:** Optical coherence tomography (OCT) has shown promise in animal studies for non-invasive evaluation of microbicide safety. Translating OCT into clinical trials requires participants to tolerate the procedure. Research on colposcopy experiences has focused on the context of diagnostic evaluations. Understanding women's experiences will allow research staff to provide useful information to participants.

**METHODS:** As part of a clinical trial, 30 U.S. women have a pelvic exam and pap smear at a screening visit, and 3 visits with colposcopy and OCT (baseline, 6 hours post-use of 5.5 days of gel use, 1 week later). In qualitative interviews, they were queried about experiences of the gynecologic exam. Participants are reimbursed \$475.

**RESULTS:** Data regarding the experience of OCT and colposcopy have been analyzed for 15 of 26 enrolled women. Data analysis will be completed prior to the meeting. The women are 29 years with 10 being Hispanic, 10 white, 4 African-American and 2 Asian. Women found the process acceptable, were intrigued by having images taken, and appreciated developing better ways to look at the vagina. During the exam, findings verbally reported for documentation had the potential to cause worry since the women heard unfamiliar words (e.g. erythema); one searched the words on the Internet. Some women found that the speculum rotation of the OCT exam caused mild discomfort. The post-product use exam was experienced by some as uncomfortable, believed to be related to irritation from the gel. Others believed the exams got easier as the gynecologist gained experience with their anatomy. With regards to frequency of exams, some believed that the week between exams allowed their vagina to recover and tolerate another exam.

**CONCLUSIONS:** Among women willing to be in a study requiring multiple pelvic exams, colposcopy/OCT were acceptable and intriguing. Women experienced but tolerated mild discomfort from the procedure, and some found the ancillary conversations worrisome. Research staff should provide participants with information about the range of women's experiences, and be sensitive to what they may overhear.

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**Correlates of Missed Visits by Participants Enrolled in an HIV Prevention Clinical Trial in Eldoret Kenya**

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**BACKGROUND:** In clinical trials, retention is critical. In the Partners in Prevention HSV/HIV Transmission Study, Eldoret, Kenya was one of 14 sites. We recruited study participants from a wide multi-ethnic area, to a distance of 150 kilometers. We report on correlates of missed visits in this study.

**METHODS:** The Partners in Prevention HSV/HIV Transmission Study was a phase III, randomized clinical trial to determine if suppression of HSV in the HSV-2/HIV-1 dually-infected members of HIV-1 discordant heterosexual couples could lead to reduction in transmission of HIV to their HIV uninfected partners. The study enrolled 3804 HIV serodiscordant couples where the index participant was also HSV-2 infected from 14 East and southern African sites. The Eldoret site contributed 268 couples. There were 3 polygamous couples hence our data consists of 539 individuals. Missed visit was defined as failure to complete a scheduled visit during the visit window.

**RESULTS:** Of the 539 individuals, 38.4% had at least 1 missed visit. Factors associated with decreased risk of missed visits included age <35 years (OR 0.62; 95% CI 0.43, 0.89); male gender (OR 0.54; 95%CI 0.38, 0.77), having children (OR 0.43; 95% CI 0.23, 0.78). Distance from study clinic <=50kms (OR 2.35; 95% CI 1.54, 3.50) and report of social harm (OR 2.11; 95%CI 1.07, 4.18) were associated with increased risk of missed visits. Distance from clinic <=50kms (OR 3.28; 95%CI 1.86, 5.70) correlated with increased risk of a missed visit among HIV infected partners (N=268). Having children (OR 0.21; 95%CI 0.08, 0.51) and age <=35 years (OR 0.38; 95%CI 0.21, 0.72) were associated with reduced risk of missed visits among HIV uninfected partners (N=271).

**CONCLUSIONS:** Extra retention effort should be directed at HIV infected participants from close to the study clinic. Retention messages should be individualized for each partner in a HIV serodiscordant couple.

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## Managing Expectations of Heterosexual HIV Serodiscordant Couples Referred for Recruitment into an HIV Prevention Clinical Trial in Western Kenya

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**BACKGROUND:** Client expectations and cultural influences are among the factors that contribute to the complexity of the counseling process. This study sought to determine client expectations when they are referred to Partners in Prevention Study Clinic in Eldoret, Kenya.

**METHODS:** A cross-sectional study of healthy heterosexual HIV-1 serodiscordant couples from Western Kenya referred to the Partners in Prevention Study Clinic in Eldoret was conducted between September 2008 and May 2009. The participants were all discordant couples who prescreened and subsequently were screened for eligibility to participate in the Partners PrEP Study, a multicenter study in 9 sites in Kenya and Uganda. At pre-screening, a semi structured questionnaire was used to collect information on client expectations, HIV status, duration since knowing HIV status and residence. Data on age, level of education, income and duration of marriage were collected as part of screening. Descriptive statistics were used to analyze data with couple as the unit of analysis. Associations between the expectations and other variables were explored using the Chi-Square statistic.

**RESULTS:** Data on 355 couples are presented. Additional counseling was the most commonly expressed expectation mentioned by 45.6%. Other expectations included: information on sero-discordancy 6.7%, wanting to participate in research 5.9%, and confirmation of HIV status 5.4%. Couples who had known sero-status for less than 1 year were less likely to expect to participate in research (OR 0.25 95% CI: 0.12–0.53) while they were more likely to expect counseling (OR 1.84 95%CI 1.14–2.95). 32% of couples had more than one expectation.

**CONCLUSIONS:** For recently identified discordant couples emphasis must be on counseling. It is critical to recognize client expectations which shape the counseling content.

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## Testing Partners of HIV Positive Individuals to Identify HIV Serodiscordant Couples for an HIV Prevention Trial: A Novel Recruitment Strategy

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**BACKGROUND:** Epidemiologic studies indicate that the majority of new HIV transmissions in sub-Saharan Africa occur among stable HIV serodiscordant couples. Incidence modeling has found that of new HIV infections in adults (15–49 years) in Uganda in 2008, 43% were among people in discordant monogamous relationships while 46% were among persons reporting multiple partnerships. This highlights a need for new HIV prevention methods for HIV serodiscordant couples thereby making such couples a priority population for clinical trials of new HIV prevention strategies.

**METHODS:** As part of the recruitment strategies for the Partners PrEP Study, a phase III, randomized, placebo-controlled clinical trial of tenofovir and emtricitabine/tenofovir pre-exposure prophylaxis (PrEP) for prevention of HIV acquisition among HIV uninfected partners of serodiscordant couples, the Jinja site (one of nine study sites) initiated efforts of testing partners of known HIV infected individuals in HIV care programs. For HIV infected individuals who consented to approach their partners for HIV testing, community mobilizers (also HIV positive community activists) visited the couples home and prepared them for a day when the study team conducted an outreach for couple HIV counseling and testing.

**RESULTS:** From May to October 2009, 508 couples in which one member was known to be HIV seropositive and the other partner's status was unknown were tested at home; 356 (70%) were HIV serodiscordant. Overall, partner HIV testing accounts for 77% of serodiscordant couples screened, and 58% of those ultimately enrolled at the Jinja site. The screened:enrolled ratio from partner testing at the site is 1:1, compared to 3:1 for other strategies. The cost per couple recruited through partner testing is about \$18 compared to approximately \$92 for VCT referrals and \$505 for mass mobilization.

**CONCLUSIONS:** HIV discordance is high among couples with a known HIV infected partner. Testing partners of known HIV positive individuals under care programs is a high yield strategy and cost-effective way of identifying and recruiting serodiscordant couples for HIV prevention trials. Given their contribution to HIV incidence in sub-Saharan African countries, identifying more discordant couples through partner testing may be an optimal approach to target HIV prevention interventions.

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## Achieving a 99% Retention Rate in a Microbicide Trial at the Isipingo Site in Durban, South Africa

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**BACKGROUND:** The Isipingo site has a history of retaining over 95% of participants in microbicide trials previous to MDP 301. During the implementation of the MDP 301 trial, staff screened 558 women and enrolled 392 within a period of eight months. Recruitment strategies such as one-on-one street intercepts, door to door recruitment drives, and visits to child-grant pay points, clinic waiting areas and bus and taxi ranks were implemented to raise awareness of the trial among community members. The team developed several methods to maximize retention at the close-out visit of the trial, which are described in this abstract.

**METHOD:** The study team developed a hard copy missed-visit log (an e-version was currently in use which was time-consuming to utilize). The log was updated on a daily basis and monitored weekly. Daily meetings with the PI were held to discuss progress in tracking participants who had missed visits, and to ensure focus was maintained on non-compliant participants. A tailored retention plan for each participant was outlined to ensure that women were tracked. Participants who missed scheduled visits were categorized into two groups: those difficult to retain; and those who were easy to track. Resources were allocated based on these categories and a retention team allocated to each group.

**RESULTS:** 115 participants were intensively tracked five months prior to data lock. Missed visits ranged from week 40 to week 52 visits. Home visits were done by study staff during study close-out to encourage participants to attend. A home closeout visit was undertaken approximately 250km away from the site for a participant who had relocated. Close out procedures were performed at their work place for two participants who were not able to take leave. The PI liaised with another MDP site to conduct close out procedures for a participant who had relocated to Johannesburg. 99.2% retention was achieved after exiting 389 of 392 enrolled participants.

**CONCLUSIONS:** The high participant retention rate was the outcome of dedicated staff, improved monitoring of missed visits and innovative methods used to track, and perform closeout visits for, participants.

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**Does Exclusion of Study Volunteers for Renal-, Hepatic-, and Bone-Related Safety Concerns Impact the Representativeness of the Study Population in a Tenofovir trial? Experience of the CAPRISA 004 Trial**

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**BACKGROUND:** Since oral is known to adversely affect renal function and bone mineral density, abnormalities in these laboratory parameters are exclusion criteria in tenofovir studies. Although oral tenofovir is indicated in patients with active Hepatitis B virus infection, hepatic flares could occur when the product is used intermittently or stopped. The safety of extended prophylactic use of 1% Tenofovir gel is a co-primary goal of CAPRISA 004. Hence, the inclusion / exclusion criteria and safety monitoring target renal, hepatic and bone abnormalities. However, the concern is that the enrolled population is substantially different from the general population due to high numbers of exclusions. This study assessed the extent to which women were excluded due to any of these abnormalities.

**METHODS:** Participants for CAPRISA 004 were recruited from an urban and rural setting in KwaZulu-Natal, South Africa. Abnormal renal function was exclusionary. A creatinine clearance of <50 ml/min, estimated using the Cockcroft-Gault method, served as a proxy marker of abnormal renal function. Hepatic and bone function were assessed at enrolment. The prevalence of these abnormalities was assessed and the impact of the exclusions on the representativeness of the enrolled population was assessed.

**RESULTS:** A total of 2160 volunteers were screened between May 2007 to January 2009. None had an abnormal creatinine clearance result; hence, no one was excluded for this reason. In the 898 women assessed at enrolment, Aspartate transaminase (AST) and Alanine transaminase (ALT) levels were within normal limits, except in one woman who had a grade III AST abnormality and one woman had a grade IV ALT abnormality. Phosphate and calcium levels were within normal range in all women. Thirty five women had active hepatitis B virus infection; though this was not an exclusion criterion in CAPRISA 004. Women continued to receive the study product and product hold was instituted when a woman fell pregnant.

**CONCLUSIONS:** Almost all pre-enrolment and enrolment renal, bone and liver function tests in this rural and urban population were within normal ranges. If active hepatitis B virus infection was an exclusion criterion, this may have had an impact of the representativeness of the study population.

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**Structured Tools for Assessing Literacy Levels and Comprehension Assessment in the Informed Consent Process: Experiences from a Microbicide Trial in Rural Kwa-Zulu Natal, South Africa**

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**BACKGROUND:** Facilitating participant understanding of key trial concepts in Microbicide research through the informed consent (IC) process is an imperative pre-study enrollment activity. IC is intended to protect volunteer autonomy and aims to ensure participation is fully informed and voluntary. Establishing participant literacy and comprehension of study information prior to study enrollment are important facets of the IC process. CAPRISA 004, a Phase IIb 1% Tenofovir Gel Trial, utilized an expanded IC process that included a Literacy Assessment (LA) tool for establishing linguistic preferences and literacy levels and a structured comprehension assessment (CA) tool used to assess participant understanding prior to enrollment.

**METHODS:** From May 2007–December 2008 volunteers were screened and enrolled at the rural CAPRISA Vulindlela Research Site. The LA tool was administered to volunteers at screening to assess their ability to read and write in the language of their choice. The outcome guided decision-making on whether to proceed in English or isiZulu and whether an impartial witness is required to observe the IC process. The CA tool was then administered to study volunteers prior to signing the enrollment IC form. The CA is a structured tool used to identify comprehension deficits around study concepts and procedures and stimulate a dialogue between participant and the research team to address identified deficits. The outcome of this assessment guided decision-making on whether the participant should proceed with study enrollment.

**RESULTS:** The LA tool was administered to 1110 volunteers. 32.4% had completed 12 years of schooling. isiZulu was the language of choice for 99% of volunteers. The tool identified 9 illiterate volunteers requiring an impartial witness with 100% correlation between the outcome of the LA tool and ability to proceed with the IC process. Of the 1110 volunteers 6 were unwilling to consent to study participation based on study information received during the IC process. Of the 620 participants eligible for enrollment, 573 demonstrated comprehension of all required points the first time round based on the CA tool and proceeded to enrollment, 45 required reiteration and further discussion of common concepts before enrolling, and 2 were identified with comprehension deficits and required further supportive material to clarify study concepts and were provided with a visual booklet outlining study information to take home and enrollment was deferred.

**CONCLUSIONS:** The LA and CA tools provide a useful objective structured approach to assess linguistic preference, assess literacy and ensure comprehension of study information among potential study participants and have been useful components of enhancing informed and voluntary participation of women in the CAPRISA 004 trial.

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**Improvement of Quality Assurance/Quality Control During Back-to-Back Microbicide Trials in Lusaka, Zambia**

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**BACKGROUND:** Quality assurance/quality control (QA/QC) during clinical trials improves the quality of data generated. In Lusaka, Zambia, we hypothesized that a shift to real-time QA/QC and dedicated QA/QC staff would lower errors in study data.

**METHODS:** Following microbicide preparedness trial HPTN 055 (with 239 local participants from 2004–2005), we implemented a redesigned QA/QC program for microbicides trial HPTN 035 (with 320 local participants from 2006–2008), instituting revised staffing assignments and focused training to provide direct oversight of documentation, including case report forms (CRFs), prior to participants leaving clinic. In 055, QC review was first handled by another study nurse who was also seeing other participants, then by a dedicated QC nurse within 24–72 hours after study visit. In 035, after self-QC by the study nurse, a real-time QC review of time sensitive forms (informed consents, behavioral assessments, and study product supply) was done by a dedicated QC nurse prior to participant leaving clinic. A separate QC nurse reviewed every CRF prior to transmittal of data, generally within 24 hours. Also in 035, retention staff were integrated to oversee generation of locator forms, and data techs were trained to identify errors in CRFs prior to faxing. This real-time QA/QC program was evaluated by monthly and overall database audits by the study data monitoring center.

**RESULTS:** Study roles were redefined for 035, but no additional staff or extra costs were required, compared to 055. QC error rates during 055 were worst at the beginning of study (6–11/100 pages) and improved to 3–4/100 pages by the end of study with a study best of 2/100 pages. For 035, the QC error rate was at its highest during study initiation at 3.5/100 pages. This rate improved to 1.5/100 pages by the end of study, with a study best of 0.5/100 pages. Time to transmit CRFs to the database improved from 3.7 days at the beginning of 035 to 1.1 days at the end, and because of improved data reporting, also resulted in time-savings during post-Datafax editing by our QA/QC department and the data monitoring center.

**CONCLUSIONS:** Data challenges in 055 were overcome during 035 by a fundamental change to real-time QC, redistribution of work to a dedicated QA/QC team, and focused education of study staff in the importance of quality data. The result was a unit capable of delivering data with QC error rates under 1 in 100 pages.

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## Preventing a High Screen-Out Ratio in the FEM-PrEP Trial at the Pretoria Site

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**BACKGROUND:** Participant screen-out is one of the many challenges researchers face in achieving their target sample size in a defined enrollment period. The use of techniques to identify ineligible participants early can help mitigate this problem.

**METHODS:** The Setshaba Research Centre, (Pretoria) initiated recruitment for the FEM-PrEP (randomized placebo-controlled phase III HIV prevention (PrEP) clinical trial) in Aug '09 and has a target sample size of 750 participants. The team anticipated the potential for a high screen: enroll ratio, based on past clinical trial experience, and therefore developed a pre-screening checklist to help identify ineligible participants before the screening informed consent process. Topics included in the checklist are mentioned when recruiting officers speak with potential participants during recruitment in the community as well as during the group trial information session at the clinic. At the end of this session, the checklist is verbally administered with each potential participant. No participant data obtained during the pre-screening phase are recorded. After the IC process, the pre-screening checklist is formally used at two different stages; (1) by the counselor—to confirm eligibility after the participant has signed the IC document and (2) by the investigator—for final confirmation.

**RESULTS:** A total of 285 participants came to the clinic for screening over a period of approximately four months (Aug '09 to Nov '09). Of these 285, 61% (N=174) were pre-screened out based on the use of the pre-screening checklist. Among these, 74% (N=129) were pre-screened out after the trial information session. Primary reasons for participants being identified as ineligible before going through the IC process included: lack of proper ID, not fitting the age criteria (18–35 years) and not being sexually active.

**CONCLUSIONS:** A high proportion—nearly half—of participants who attended the information session was identified as ineligible before the IC process. The use of the pre-screening checklist has thus proven effective in this setting. The pre-screening checklist helps sites in efficient management of staff work load and cost-effective use of limited resources by early identification of ineligible participants.

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## Utilizing Geographic Information Systems (GIS) to Enhance Recruitment for HIV Prevention Clinical Trials

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**BACKGROUND:** The FEM-PrEP clinical trial, which tests the safety and effectiveness of once-daily Truvada to prevent HIV infection in women at higher risk in Sub-Saharan Africa, uses GIS to prioritize areas for recruitment before the trial begins, and to assist in a more efficient recruitment strategy.

**METHODS:** Prior to the clinical trial, 2,011 interviews were conducted with community members using a modified version of the Priorities for Local HIV Control Efforts (PLACE) methodology to identify areas of HIV risk in the community in Bondo, Kenya, and Pretoria, South Africa. The Pretoria interviews with women were collected in 58 bars and bush areas, which were aggregated into areas for the analysis. A formula based on characteristics of bars (distance to clinic and number of patrons) and women's sexual behavior (numbers of total sex partners and new sex partners in the past four months, and condom use) was developed in the GIS to assess spatial trends of higher risk areas in order to prioritize areas for recruitment.

FEM-PrEP also uses the GIS during the trial to strategically monitor recruitment activities in Pretoria and Bondo by producing monthly or quarterly maps of HIV status at screening for areas where women live and areas where women work.

**RESULTS:** GIS identified seven of the 28 areas as areas of higher HIV risk in Pretoria. Areas were then prioritized for recruitment based on the relative degree of risk along with discussions with site staff.

Monitoring recruitment in the priority areas in Bondo via monthly map reports have identified spatial trends in HIV prevalence of women screened, and small anomalous trends regarding where women live or work in relation to where recruitment activities occur (such as women coming from areas where recruitment is not taking place). This monitoring has allowed for recruitment in Bondo to be conducted strategically so as to recruit the target population more efficiently.

**CONCLUSIONS:** The use of a GIS to analyze data on higher risk may enhance the efficiency of recruitment of trial participants. It is a decision-making tool that allows the user to focus recruitment efforts on areas identified to be of higher risk and to monitor whether recruitment continues to be focused in areas of highest risk throughout clinical trial implementation. After trial completion, the use of a GIS will be assessed to fully understand how it might have contributed to successful recruitment.

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## Innovative Strategies in a Phase IIB Microbicide Trial Results in High Retention—Experiences from CAPRISA 004

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**BACKGROUND:** Microbicide trials need to include effective retention strategies to retain study participants in order to ensure scientific integrity and adequate statistical power. Maintaining a retention rate of 90% per annum is especially challenging in rural, poorly-resourced and hard to reach communities. Vulindlela, a rural community in KwaZulu-Natal does not have street maps, street names, house numbers or reliable telephone contact information. The purpose of this study was to assess the implementation of newly developed CAPRISA 004 retention strategies to attain the protocol required retention target of 90% per annum.

**METHODS:** HIV negative sexually active 18–40 year-old women (N=614) were enrolled over 18 months. Follow-up assessments were conducted monthly. The retention strategies were developed through ongoing feedback and dialogue with the resident members, the CAPRISA Community Research Support Group (CRSG) and study participants in Vulindlela. The strategy includes: community mapping with transect walks, a local resource inventory, descriptive maps drawn by participants to illustrate the route from their houses to the CAPRISA rural site; daily information sessions; ongoing information sessions to ensure better understanding of compliance to visit schedule; encouraging male involvement; participant recognition and annual certificate award; communication and relationship skills-building; daily monitoring of retention rates by dedicated retention team with remedial action and flexible clinic hours. Challenges incurred during implementation and regular feedback from participants continually informed the ongoing adjustments to the site retention strategy. Retention rates were calculated by dividing the number attending the clinic by the number expected to attend.

**RESULTS:** Vulindlela is one of two sites in the CAP 004 study. Only the Vulindlela data are reported here. Volunteers were followed for a minimum of 12 months and a maximum of 30 months. With 520 participants in active follow-up, the overall study retention rate was 95.9% at study exit. The average monthly and quarterly retention rate was 90% and 93.8% respectively. Loss to follow-up (n=21), deaths due to natural causes (n=2), inappropriate enrolment (n=1), voluntary withdrawal (n=3) impacted on retention rates in this highly challenging rural environment.

**CONCLUSION:** In this rural community a retention rate of greater than 90% was achieved through involvement of community members, the CAPRISA CRSG, and iterative participant feedback. Retention monitoring is a demanding ongoing process requiring extensive partnership with community members, teamwork from site staff. Adequate resources are critical in achieving high retention rates in rural settings.

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### Urban-Rural Differences in Baseline Characteristics of CAPRISA 004 Trial Participants

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**BACKGROUND:** In Sub-Saharan Africa, young women bear a disproportionate burden of HIV infection and have limited options to reduce their risk of acquiring HIV. Hence there is an urgent need for a microbicide that would enable women to protect themselves from HIV infection. We describe the urban-rural differences in the baseline characteristics of participants in CAPRISA 004, a Phase IIB randomized controlled trial to assess the safety and effectiveness of 1% Tenofovir gel in preventing HIV infection in women in KwaZulu-Natal, South Africa.

**METHODS:** The study was undertaken at CAPRISA's urban (Durban) and rural (Vulindlela) clinical research sites in KwaZulu-Natal between May 2007 and December 2009. Consenting volunteers underwent two screening eligibility assessments prior to enrolment. At the rural site, women were recruited from the adjacent family planning clinic. At the urban site, women were recruited from the adjacent sexually transmitted disease clinic.

**RESULTS:** At baseline, enrolled participants at the rural site (n=614) compared to the urban site (n=278) were younger [mean (sd) 23.3 (4.9) vs 25.0 (5.4) years, p<0.001], reported lower frequency of sex acts in the past 7 days [mean (sd) 1.7 (2.1) vs 2.6 (4.2), p<0.001], reported low male condom use (22.8% vs 42.8%, p<0.001) and were more likely to be living separately from their regular partner (90.4% vs 78.4%, p<0.001). In contrast, women at the urban site reported a higher number of lifetime sex partners [mean (sd) 6.0 (18.4) vs 2.1 (1.2), p<0.001] and having a new partner in the last 30 days (2.5% vs 0.5%, p=0.01).

**CONCLUSIONS:** The urban and rural women enrolled in CAPRISA 004 differ markedly in their sexual behavior and provide suitable diverse groups for the assessment of candidate microbicides. In particular, the high-risk rural women with stable partners and low frequency sex are an appropriate group for establishing the effectiveness of intermittent Tenofovir gel use for prevention of HIV infection.

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### Verification of Reference Ranges for Chemistry in Clinical Trials

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**BACKGROUND:** To assess the safety of daily intake of Truvada® the FEM-PrEP phase III clinical trial determines the AST/ALT, phosphorus and creatinine levels in the study participants at regular intervals. The chemistry analyses are performed by the study site or collaborating laboratory. In most sites, the laboratories do not have the normal ranges for the targeted study population and use the ranges established by the manufacturer, ranges found in the literature or nationally/regionally established ranges. We report on how to verify laboratory reference ranges specific to populations targeted by the FEM-PrEP study.

**METHODS:** The manufacturer's established reference range is used as the reference range until the reference range verification procedure is completed. An initial sampling of 10 representative samples is used to verify the manufacturer's reference range. The samples are representative of the study population and from known clinically healthy participants presenting at screening. The participant mean is compared with the manufacturer's reference range mean. If the % deviation is within the standard established tolerance limits the manufacturer's reference is accepted. If the % deviation is not within the limits, the sampling is continued until a total of 40 samples is analyzed. From the data of the 40 samples the final reference range is calculated. This process is done in each site. We report here on the Bondo, Kenya site.

**RESULTS:** The verification of the reference range for AST/ALT, creatinine, and phosphorus was conducted on serum specimens collected at screening. Participants were considered healthy if not pregnant, not HIV infected, without abnormal laboratory results, and clinically healthy.

Verification of the normal ranges conducted in Bondo, Kenya, using Vitros DT 60 II confirmed the manufacturer's established ranges for ALT (9–52 U/L) and phosphorus (0.81–1.45 mmol/L). New ranges were established for AST (2–27 U/L vs 14–36 U/L manufacturer's range) and creatinine (45–99 vs 62–106 µmol/L manufacturer's range). These new ranges are now used at the site to monitor safety.

**CONCLUSIONS:** Verification and definition of the reference ranges specific to a population are feasible. It is cheaper, faster and more feasible than an a priori establishment of normal ranges. However 2 out of 4 manufacturer's established ranges could not be confirmed, which has an important impact on the re-definition of the toxicity grades and management.

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## The Importance of Normal Ranges in Microbicide Clinical Trials

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**BACKGROUND:** Normal ranges (also known as reference ranges) define the upper and lower bounds of expected results from healthy individuals. They are used in clinical settings to interpret quantitative laboratory results. In clinical trials, they are also crucial for inclusion and exclusion criteria and safety monitoring.

Normal Ranges can vary among different populations because of biological factors or instrumentation. Good Clinical Laboratory Practices recommend that labs locally validate ranges cited in the literature or perform larger studies to calculate them.

**METHODS:** Reference ranges for certain analytes from five US and five African clinical laboratories performing work for the Microbicide Trial Network were compared. No statistical analysis was performed.

### RESULTS:

TABLE 288.1

SITE	PHOSPHATE MG/DL		AST U/L		ALT U/L		CREATININE MG/DL	
	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH
KAMPALA	2.5	5.6	0	32	0	35	0.5	1.2
HARARE	1.9	5.3	10	30	5	44	0.5	1.5
BLANTYRE	2.1	3.8	13	37	8	32	0.6	1.2
DURBAN	1.9	4.3	13	35	7	35	0.6	1.1
LILONGWE	1.9	4.3	0	38	7	35	0.5	1.3
TAMPA	2.7	4.5	5	40	5	40	0.5	1.2
PUERTO RICO	2.7	4.5	0	32	0	33	0.51	0.95
PITT	2.5	4.9	15	37	30	65	0.6	1
UAB	2.7	4.5	15	40	10	44	0.4	1.2
CLEVELAND	2.5	4.5	10	37	7	54	0.6	1.3

TABLE 288.2

SITE	WBC X10 <sup>9</sup> /L		HGB G/DL		NEUTROPHIL X10 <sup>9</sup> /L		PLTX10 <sup>9</sup> /L	
	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH
KAMPALA	2.8	7.7	10.9	16.7	1	5	125	445
HARARE	4	11	11.5	16.5	2	7.5	150	400
BLANTYRE	2.4	7.6	11.4	16.2	NA	NA	117	310
BARC DURBAN	4	12	11.5	16.5	2	7.5	150	450
LILONGWE	2.8	8.4	10.9	17.3	0.8	4.1	130	400
TAMPA	4.6	10	12.2	16.2	2	6.9	142	424
PUERTO RICO	4.8	10.8	12	16	NA	NA	130	400
PITT	3.8	10.6	11.6	14.6	2.24	7.68	156	369
UAB	4	11	11.3	15.2	1.48	8.03	150	400
CLEVELAND	4.4	11.3	12	16	1.2	7.7	150	450

**CONCLUSIONS:** Certain analyte's ranges such as creatinine and WBC were relatively consistent between both sites and continents. Other analytes, including hemoglobin and creatinine, demonstrated differences between continents. Hemoglobin's lower limit of normal was consistently lower in Africa. Phosphate was fairly consistent at the US sites but showed a greater range in Africa and a consistently lower limit of normal.

These results highlight the importance of careful evaluation of normal ranges for specific populations, particularly when changing instrumentation

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### How to Achieve Valid and Reliable Laboratory Data in Multi-Center Clinical Trials?

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**BACKGROUND:** To assess the effectiveness of Cellulose Sulfate for HIV and STI prevention, a phase III clinical trial was conducted in 5 sites: 3 in Africa and 2 in India. The study sites differed in terms of clinical trial experience, infrastructure and capacity in delivering laboratory data meeting the high quality requirements of a clinical trial. To guarantee the reliability and standardization of the laboratory data, a quality assurance (QA) program was designed and implemented at the study sites' laboratories.

**METHODS:** An International Central Reference Laboratory (ICRL) was appointed by the sponsor to design and to manage a QA program. In addition the ICRL provided ongoing advice on the assays to be used, assessed the collaborating laboratories, organized trainings, assisted laboratories in writing and application of standard operating procedures, conducted supervision visits, performed re-tests and manufactured quality control (QC) panels. The laboratories received QC panels to assess their performance in HIV rapid testing and amplification of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG). The ICRL re-tested a specified percentage of the specimens for HIV and CT/NG.

**RESULTS:** Excellent results were obtained for the HIV QC panels, no false results were reported. However, discordant HIV results between the ICRL and study sites were obtained on aliquots re-tested at the ICRL. The discordances were due to clerical and labeling errors, which occurred after testing and reporting. The only study participant who received a wrong result was immediately traced and informed about her correct result. Across the 5 study sites, 168 CT/NG control specimens were distributed in 28 panels. Four and 9 false negative results for CT and NG were reported, respectively. ICRL re-tested 10% of the negative CT and/or NG specimens from the study sites, false negative results were found in 1 site and were less than 1% of the re-tested specimens. The re-testing showed CT and/or NG DNA contamination in all study sites and as part of ongoing corrective action, laboratories were instructed to perform every 2 months an environmental DNA check.

**CONCLUSIONS:** During the course of the study some inaccuracies and problems were detected and promptly solved. Through immediate feedback, guidance, and re-training of the laboratory staff additional inaccuracies were prevented thereby adding to the high quality and reliable laboratory data that were obtained at the end of the study.

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### Strategies for Reducing the Number of Ineligible Women Screened for an HIV Prevention Trial

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**BACKGROUND:** Multiple eligibility criteria must be met for a woman to enroll in FEM-PrEP, a randomized, placebo-controlled PrEP trial. Screening in Bondo, Kenya began in May 2009; the screen failure rate was 65%. In order to reduce the number of women who screened out, new strategies were implemented in August 2009.

**METHODS:** The new strategies introduced included: (1) linking with existing VCT sites, (2) clarifying the high-risk sexual behavior criteria in community meetings and discussions with potential participants, and (3) using a pre-screening eligibility checklist (PSEC) developed by the Pretoria, South Africa site. No information obtained through the checklist was recorded. It includes questions related to eligibility, such as HIV and pregnancy status, pregnancy intentions for the next year, breastfeeding and sexual behavior. The sexual behavior criteria had not been previously discussed in outreach activities due to concerns of stigmatization.

Descriptive statistics were used to evaluate the reasons for screen failures three months before, and three months after, the implementation of the new strategies.

Behavioral site reports provided data on the number of potential participants who pre-screen out using the PSEC, reasons for pre-screening out and number of potential participants who have been referred from VCT clinics.

**RESULTS:** In the three months before the PSEC was implemented, 542 women were screened with 384 (71%) not enrolled. In the subsequent three months, 463 women were screened with 278 (60%) not enrolled. Similar reasons for non-enrollment were identified before and after this new strategies were implemented: HIV-positivity and medical contraindications. However, the number of women screened out because they did not meet the high risk sexual-behavior criteria, dropped substantially, from 12.5% (48/384) before implementation to 0.4% (1/278).

According to the behavioral site reports, the use of the PSEC has pre-screened out 586 women. In addition, 47 potential participants were referred from VCT clinics with 33 (70%) who screened in. The primary reasons for screening out, for those referred from VCT clinics, were medical contraindications and pregnancy.

**CONCLUSIONS:** All three strategies—PSEC, linking with existing VCT clinics, and making the sexual behavior criteria known—have been useful in reducing the number of ineligible women formally screened. These procedures have thereby saved significant participant and staff time and trial resources.

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### Dash Board Meetings as a Strategy for Reaching Recruitment and Enrollment Targets: Lessons from a Microbicide Feasibility Study in KwaZulu-Natal, South Africa

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**Background:** Preparatory epidemiologic studies are necessary prior to the implementation of Phase III Microbicide trials. Recruitment, screening and enrollment of participants for feasibility and cohort studies can be challenging. Communication and team work are critical for successful recruitment of participants to these studies. In this presentation we describe "Dash Board" meetings as a strategy for improving staff morale and resolving recruitment and enrollment challenges in a feasibility study in preparation for a Microbicide trial conducted in Edendale, KwaZulu-Natal, South Africa. This strategy was developed to assist project staff to stay "focused" and "motivated" by setting goals, identifying critical tasks and regularly assessing whether tasks are accomplished.

**Methods:** A cross-sectional and prospective, observational cohort study to estimate HIV incidence in sexually active adult females was conducted from 2008 to date. The target sample was 800 women, in addition, 300 of the screened women who were HIV sero-negative were enrolled into the prospective cohort study for a 12-month follow-up period. All women aged 18 to 35 who were interested were screened for eligibility. The Dash Board strategy was used to communicate the project status to staff on a daily basis. Using the KISS (Keep It Simple) principle, study targets and activities were reviewed; action plans developed and individuals were allocated roles and responsibilities. Daily reviews of the recruitment process were made using an output based system.

**Recruitment Outcomes** Initial recruitment of the targeted sample moved at a slow pace for a variety of reasons including high HIV prevalence. This resulted in 1120 women being screened to enroll 300 HIV sero-negative women into the cohort study. Recruitment and screening challenges were identified and strategies were put in place to overcome these challenges; the Dash Board meeting strategy was one of these.

**Results:** A rise in the recruitment and enrollment numbers was observed at the end of the Dash Board workshop month and in subsequent months until the target was reached in March 2009. A high level of commitment, accountability, motivation and ownership was observed among project staff. The efforts of the study team were acknowledged when the received an award for "outstanding" team work.

**Conclusion:** Dash Board meetings are an effective strategy for improving communication within teams. They encourage open communication, a sense of ownership of the project activities and individual staff responsibility for outputs, in addition to regular assessment by the whole team of whether rates of recruitment and enrollment are on target. This strategy can be easily replicated in similar projects.

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ABSTRACT WITHDRAWN BY AUTHORS

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**External Quality Assurance Program for HIV and BHCG Testing in MDP 301 at the Durban Site**

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**BACKGROUND:** MDP 301 (Microbicide Development Program) was an international, multi-centre, randomized, double-blinded, placebo-controlled trial, to evaluate the efficacy and safety of 0.5% and 2% PRO 2000 gels for the prevention of vaginally acquired HIV infection. The study was conducted at 6 sites within 4 Sub Saharan African. It was imperative that adequate measures were put into place at the site laboratories to ensure accuracy and precision of the HIV and BHCG test results. The Durban site reports on the external quality control programme run for rapid HIV and pregnancy at the 3 MDP 301 Durban on-site laboratories.

**METHODS:** HIV Rapid testing at clinical sites was performed according to site specific operating procedures using FDA approved OraSure OraQuick and WHO approved Abbott Determine rapid HIV assays on plasma. BHCG testing on urine was performed with the Quidel QuickVue pregnancy test. The National Health Laboratory Services (NHLs) HIV EQA program was implemented quarterly and consisted of testing 6 blinded samples on the Rapid HIV tests (n=146). External quality control samples obtained from Royal College of Pathologists of Australasia (RCPA) for rapid BHCG consisted of two samples run every 2 months (n=34). Additionally, an interlaboratory EQA was done: 5% of all urine samples tested for pregnancy (n=1603) and 5% of plasma tested by HIV rapids tests (n=440) were re-tested at the local laboratory (BioAnalytical Research Corporation of South Africa (BARC SA).

Furthermore, 5% of all sero-negatives (n=294) and 10% of screened and excluded sero-positives (n=125) were retested at the Central Laboratory (CLS).

**RESULTS:** NHLs testing and 5 % EQA correlation with BARC SA yielded 100% correlation with the on-site laboratories. 5% interlaboratory BhCG EQA and RCPA yielded 98% and 79 % concordance respectively. Of the 7/34 RCPA samples, there was a discrepancy between RCPA and sites interpretation of equivocal. The 10% screened out participant retest yielded 99% concordance, 1/125 excluded seropositive participants tested HIV negative on CLS retest. Upon re-test, participant tested seropositive, possibly due to sample mix-up at the local lab BARC SA. The remaining panels mentioned above all yielded 100% concordance.

**CONCLUSIONS:** EQA is very important at the clinic level to validate the accuracy and precision of all data generated from sites. The above results are evidence of the outstanding quality produced by MDP 301 Durban sites and measures are in place for continuous improvement as we move forward.

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**An Integrated HIV/AIDS Program/Patient Reporting Solution**

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**BACKGROUND:** Most Health Management Information Systems in HIV/AIDS service settings work in a heterogeneous environment from a database standpoint with mission critical applications for both program and patient management. Their systems run in isolated environments powered by Relational Database Management Systems like EpilInfo, Microsoft Access and Microsoft SQL Server. This poses the following challenges: Data has to be gathered from multiple sources for reporting; Real-time information is hard to get because production databases are heterogeneous and distributed; a lot of time is taken to generate routine lengthy reports and the same reports are generated differently by various personnel based on their individual interpretation. In light of this, a Reporting Solution was developed for TASO to address these challenges.

**METHOD:** Using a free Business Intelligence platform provided by Microsoft SQL Server-SQL Server Integration Services (SSIS) and SQL Server Reporting Services (SSRS), a web based reporting solution was developed. SSIS provided a scalable enterprise data integration platform with high-end Extract Transform and Load; and integration capabilities while SSRS provided a rich enhanced interactive platform for delivering reports, all enabling easy management of data from Microsoft Access, EpilInfo and Microsoft SQL Server. All this data was placed in two databases-one for program reports and the other for patients reports. A tool within Microsoft SQL Server was programmed to schedule periodic synchronization of production and reporting data.

**RESULTS:** Interactive reports can now be generated by end users on demand. Real-time information can be generated for decision making with minimum latency. There is consistency as program performance indicators can now be produced based on a clearly defined and universally understood criterion. Workload statistics can be monitored on a daily basis for planning purposes. Patient management has greatly improved as history can be seen online without paper charts. The system has greatly facilitated continuous quality improvement in service delivery as follow-up can now be prompted by the system in case of patient inactivity.

**CONCLUSIONS:** The system can be implemented in a resource-limited setting as the necessary software is freely available. Data should be entered in time and must be routinely assessed for quality.

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**Participant Verification: A Means to Prevent Co-enrollment in Multiple Clinical Trials in Kwa-Zulu Natal, South Africa**

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**BACKGROUND:** With an estimated HIV prevalence of 28%, Kwa-Zulu Natal faces the brunt of the HIV burden in South Africa. Local, national and international researchers alike have flocked to this province to conduct clinical trials aimed at HIV prevention, treatment and care in an attempt to curb the HIV epidemic.

A vital consequence has been the occurrence of single participants enrolling in multiple trials concurrently. Various reasons for concurrent clinical trial enrollment have been put forth including incentives, altruism and added synergistic benefit against HIV. Consequences of concurrent clinical trial enrolment are many and multifactorial and may have far reaching effects. As such clinical trialists have to maintain and sustain an effective and efficient broad based system to prevent co-enrolment across clinical trials.

**METHODS:** One such offering is the Biometric Verification system from the Medical Research Council, HIV Prevention Research Unit, due for piloting February 2010. This system hosts Sagem software aimed at fingerprinting scanning associated with participant identity. Fingerprinting scanning of two digits per hand will be captured at enrolment onto the system together with participant details consisting of name, surname, South African Identity number and clinical trial details. This system is verified at every subsequent visit.

**CONCLUSIONS:** The key to this is to ensure real time updates to the participant database and to ensure that all research institutes in common Kwa-Zulu Natal districts/regions are collaborating in this regard to prevent any form of co-enrolment institutes and across institutes in Kwa-Zulu Natal.

A feasibility and acceptability survey will be conducted to assess effectiveness and efficiency among users once pilot testing concludes. The Biometric Verification system is a novel approach aimed at preventing co-enrolment in multiple clinical trials in Kwa-Zulu Natal, South Africa.

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**The Use of Quality Control Panels in Clinical Trials**

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**BACKGROUND:** The laboratories collaborating in the FEM-PrEP (Truvada®) phase III clinical trial receive at regular intervals quality control panels for analysis. The aim of the quality control is to assess the performance of the laboratory and to take corrective actions if needed. Quality control panels should cover all analysis as requested by the study protocol and be as much as possible similar to the specimens collected from study participants. Commercially available quality control panels usually do not meet those criteria.

**METHODS:** The following quality control (QC) panels are distributed across the FEM-PrEP collaborating laboratories

ANALYTE	NATURE OF SPECIMEN	STORAGE CONDITION	PREPARED BY
Urine rapid pregnancy test	Urine from pregnant woman	Frozen	Home-made
Urine dipstick	Urine, spiked	Frozen	Home-made
HIV rapid tests	Plasma	Frozen	Home-made
HbsAg rapid test	Plasma	Frozen	Home-made
HbsAb	Plasma	Frozen	Home-made
Syphilis	Serum	Frozen	Home-made
<i>C. trachomatis/</i> <i>N. gonorrhoeae</i> PCR	Diluted PBS, spiked	Frozen	Home-made
Chemistry	Freeze dried	Room temperature	Commercial
Nugent score	Vaginal smear or photograph	Room temperature	Home-made
CD4 count	Fresh blood in stabilization tube	Room temperature	Home-made

The chemistry panels are left-over panels from the Belgian national external quality assessment (EQA) scheme. All study laboratories receive the same panels except for the Nugent score panels.

The freeze-thaw and temperature stability of the panels consisting of frozen specimens was validated. The shipment of the frozen panels to the collaborating laboratories is performed using a dry shipper with temperature registration.

**RESULTS:** Quality control panels are tested by the collaborating study laboratories every 4 months, the HIV rapid testing QC panels are tested every 2 months. Back-up panels are stored at the ITM and tested when discordant results are obtained. The results of the chemistry panel are evaluated by comparing the results with the consensus results obtained by the Belgian laboratories using the same method and analyzer. In the event of a discordant result, the potential reason is investigated in collaboration with the local laboratory, and remediation is sought. The impact on the study results is evaluated.

**CONCLUSIONS:** The inclusion of quality control panels is part of a quality assurance program. It is utmost important that the quality of the control panels itself is guaranteed. Therefore panels should be characterized and validated.

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### Lessons Learned from Building Research Capacity for Phase III HIV Prevention Trials in Beira, Mozambique

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**BACKGROUND:** Successful HIV prevention research requires experienced clinical research sites capable of recruiting and retaining cohorts at higher risk for HIV infection. The Universidade Católica de Moçambique (UCM), Family Health International (FHI), and the Academic Medical Center of the University of Amsterdam (AMC) collaborated to develop a new clinical research site in Beira, Mozambique and to ascertain HIV incidence in women and men at higher risk for HIV in this area. Mozambique has seen practically no HIV prevention research despite high prevalence and suspected high incidence.

**METHODS:** UCM, FHI and AMC established a critical path to build a new clinical research site in Beira, Mozambique, using combined budgets, that included: 1) Obtaining political (national, provincial, municipal and institutional) approvals to develop the research site, and approvals from ethics committees to conduct the study; 2) Consolidating study protocols from sponsor institutions (using cross-sectional and prospective methodologies to determine HIV incidence); 3) Building a new laboratory that meets international quality standards; 4) Creating data management, clinical and financial infrastructure; 5) Recruiting and training local staff; 6) Developing community links for community support, participant recruitment and referrals.

**RESULTS:** It took approximately two years from the time of initial discussions until the first participant was recruited into the incidence studies. During this time, infrastructure was established, cross-sectional and prospective incidence protocols were written, multiple approvals were obtained including from the Ministry of Health and relevant ethics committees, and community outreach was initiated. Study staff were trained in HIV prevention research, good clinical practice, interview techniques, pre- and post-test counseling, clinical and laboratory procedures, study documentation, and standard of care practices.

**CONCLUSIONS:** Development of new HIV prevention research sites is feasible in sub-Saharan Africa, but funders must appreciate the considerable time and effort required. Contingent on the HIV incidence rate, the site is well prepared to conduct Phase III HIV prevention research. Partnership between different funding institutions was vital in providing financial stability and minimizing risk to develop the research site. For sustainability of research capabilities, diversification of research projects and funding will be required.

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### Recruitment of Women by US Sites in MTN 001: Successful Sources

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**BACKGROUND:** To describe the types and relative success of strategies used to recruit women to a phase 2 microbicide trial.

**Methods:** In 2008–2010, four US sites enrolled women into MTN001, an ongoing, prospective, randomized Phase 2 adherence and pharmacokinetics study of oral and vaginal preparations of tenofovir. With 82 of 86 targeted women enrolled to date, frequencies were analyzed from preliminary data on recruitment sources from each of the four US sites (University of Pittsburgh, Case Western Reserve University, University of Alabama, and Bronx-Lebanon Hospital Center). Preliminary recruitment data from women enrolled into the rectal substudy at one site is also included in this analysis.

**RESULTS:** Recruitment data was received on 570 pre-screened, 138 screened and 82 enrolled women. Recruitment sources included prior study registries, newspaper and internet-based advertisements, street outreach, word of mouth and community partnerships. The overall prescreening yield was 14% (82 enrolled/570 prescreened) and internet postings generated 31% of all pre-screenings (176/570). The most successful recruitment sources across all four sites were prior study registries (15 enrolled from 45 prescreened, or 33% yield), word of mouth (10/42, or 24%), and internet postings (20/176, or 11%); these yields varied by site. Enrollment of women in the rectal substudy did not elicit any unusual recruitment challenges.

**CONCLUSIONS:** Internet postings generated many pre-screenings for this study but prior study registries yielded the highest rate of enrollment. Standardized prescreening questions are a valuable way to evaluate site recruitment sources and strategies.

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### Retention of High-Risk Women in a Prospective HIV Prevention Trial

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**BACKGROUND:** Participation and retention of high-risk HIV-uninfected women within heterosexual HIV serodiscordant partnerships is important for HIV prevention trials. We examined socio-demographic correlates of retention of HIV uninfected women within HIV serodiscordant partnerships in a prospective HIV prevention trial.

**METHODS:** Cross-sectional assessment of baseline socio-demographic characteristics of HIV-uninfected women within heterosexual HIV serodiscordant partnerships enrolled in the Partners in Prevention HSV/HIV Transmission Study (a randomized, placebo-controlled trial of acyclovir herpes simplex virus type 2 suppression for HIV prevention) in Kampala, Uganda. The principal site retention strategies included home visit at enrollment, reminder calls, standard medical care, and couples meetings. Correlates of retention were evaluated using logistic regression.

**RESULTS:** Of 450 couples enrolled at the site, 219 (48.9%) were partnerships in which the female partner was HIV uninfected. More than 80% of enrollees were referred to the study site from collaborating VCT centers. Median age for women was 30 years (Interquartile range (IQR); 25, 35), with median duration of partnership of 5.4 years (IQR; 2.8, 11.1). One third of women had an income, with a median monthly amount of US \$30 (IQR; 15, 53). Baseline prevalence of social harm self-defined as a form of verbal, physical or economical abuse from their study partner was 5.9%. Overall, retention rates were 92% and 88% of expected visits at 12 and 24 months of follow-up respectively. The principal motivations for study participation included contributing to research (60%), potential protection from HIV acquisition (30%), and health care benefits (5%). Younger age was significantly associated with lower retention (adjusted odds ratio 0.91, 95% CI 0.85, 0.98). Travel, change of location, and separation from spouse were the principal reasons for missed visits. Notably, duration of partnership, years in school, number of children with partner, income, and baseline social harm were not statistically associated with retention.

**CONCLUSION:** Our data demonstrate the potential for successful retention of high-risk females in HIV prevention trials and are consistent with other studies highlighting challenges in retaining younger participants. These findings suggest that motivation for research participation is dynamic and reinforce that HIV prevention trials must use pragmatic approaches to retention strategies.

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**Computer-Aided Methodology for Assessing Participants' Eligibility for an HIV Prevention Clinical Trial: A Case Study from Mbale, Uganda**

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**BACKGROUND:** Clinical trials have stringent eligibility criteria and research sites encounter challenges in quickly and accurately establishing eligibility of potential trial participants. We developed a computer-aided methodology to assess participant eligibility for an ongoing clinical trial of antiretroviral pre-exposure prophylaxis (PrEP) in Uganda.

**METHODS:** The Partners PrEP Study is a phase III, randomized, placebo-controlled trial of tenofovir and emtricitabine/tenofovir PrEP among HIV uninfected members of HIV sero-discordant couples. The trial is operating at nine sites in Kenya and Uganda. At the Mbale, Uganda site, a database application that accounts for all 21 eligibility parameters including laboratory values, demographic information (age, gender) and other clinical eligibility criteria was developed in Microsoft Access 2003. Prior to enrolling participants in the study, a member of the eligibility committee makes entries into the database for all potentially eligible subjects. For each entry made the computer program alerts for eligibility or ineligibility. A printable report is generated with a bold comment: "Eligible" for eligible and "Ineligible" for ineligible participants. For ineligible participants, the section of the report that has made the participants ineligible is grayed out. This report is further reviewed by at least two members of the eligibility committee who critically verify entries with the source documents.

**RESULTS:** This computer program provides accurate and quick assessment of study eligibility, which increases turnaround and accrual rates of eligible participants. Eligibility of a potential participant can be assessed and determined in less than 1 hour as long all the laboratory results are available; previously, the eligibility committee would wait to convene a weekly meeting for batched review of eligibility.

From the commencement of using this program to assess eligibility, 88.4% (N, 330) of potential participants assessed have been confirmed eligible and enrolled in the study. This computerized eligibility program has overall assessed 71.7% (N, 407) of the participants enrolled at the study site.

**CONCLUSION:** The use of a computer-aided approach has aided eligibility assessment for an HIV prevention trial of pre-exposure prophylaxis. Simple tools can be developed by study sites prior to the inception of clinical trials to assist study staff in eligibility assessment.

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**Improving Participant Retention Interventions During Back-to-Back Microbicide Trials in Lusaka, Zambia**

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**BACKGROUND:** Low retention rates during HIV prevention trials increase expense and introduce bias especially when loss-to-follow-up exceeds the incidence of the primary endpoint. In order to improve retention in Lusaka, Zambia during microbicide trial HPTN 035, as compared to the preceding microbicide preparedness trial HPTN 055, we hypothesized that strategies influencing the participant, her community, and study staff procedures would be required.

**METHODS:** Site-specific methods for participant retention were adapted from interventions utilized during HPTN 055 and retooled for use at the same research unit for HPTN 035. Interventions for participants were: increased education to rebut stigma, rumor, and visit fatigue; improved communication with participants regarding 'planned' absences; and strategies to retain individuals who relocated outside Lusaka by facilitating transport. Efforts to involve community support of retention were: quarterly meetings, ad hoc discussions with neighborhood leaders, and meetings with partners to discuss research, study-specific procedures, and retention. Community sensitization also occurred through drama and posters as well as home visits with clearance from local clinics, trusted peer educators, and police to facilitate community acceptance. Updated staff procedures included: education about the impact of low retention on study outcome; identifying women at risk for study drop-out; regular updating of locator information including detailed community and residence mapping; attainment of three reliable alternative contacts; and participant escorts to residences. Other interventions evaluated were flexible visit scheduling around participant working hours; immediate tracking of missed visits; visible tracking progress tools; and algorithms for improved locating of delinquent participants.

**RESULTS:** Retention during 035 was 96% of 320 participants at 12 and 24 months compared to 93% of 239 participants at 12 months during HPTN 055 by utilizing the retooled retention methods focused on participants, community, and study staff procedures. It required the addition of 18 paid transports, 10 transport reimbursements, 38 after-hours visits, and 52 participant escorts to residences to facilitate participant follow-up.

**CONCLUSIONS:** Excellent retention rates require buy-in by study staff, use of relevant retention tools, and active engagement of participants and community.

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**Implementation of a State-of-the-Art Continuous Temperature Monitoring System in Microbicide Clinical Trials**

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**BACKGROUND:** Optimal temperature control and monitoring is required for the storage of study products and laboratory samples in Microbicide trials. HIV Prevention Research Unit trial sites are located in mostly semi-rural to semi-urban areas that are not easily accessible after working hours. To address this challenge we implemented a continuous temperature monitoring system which can be accessed remotely to assist staff with decisions regarding after hours travel to sites.

**METHODS:** Our system is a live, secure system accessed remotely via the internet. The unit works via a GPRS cell phone network, and uploads data at pre-set intervals onto the web-server. The unit can connect up to 12 different locations within a clinic site which makes the system expandable and cost effective. The system is programmed to provide early warning alerts when it detects changes in factors that could lead to potential protocol deviations. Changes in temperature, humidity, power supply, GPRS signal strength, generator and air conditioner status are detected. Alerts are sent out as cell phone text or email to designated staff until it is acknowledged. "Return to normal" messages are also sent out when the problem has resolved. In the event of an air conditioner failure, the system automatically switches to the stand-by air conditioner.

**RESULTS:** The early warning alerts allow staff the time to troubleshoot problems. The remote access to the system, allows staff to manage problems e.g. generator failure, generator refuel and technical assistance without having to go to site. The detailed information available enables staff to make informed decisions on the urgency of a call out to site. Using the GPRS signal ensures that staff have portable access to the system at any point in time. The return to normal messages prevents call outs due to false alarms.

**CONCLUSIONS:** The ability to access temperature monitoring systems by remote increases the capacity of a site to maintain prescribed storage conditions. Our continuous temperature monitoring system is a valuable tool to prevent protocol storage violations in clinical trials.

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### Overcoming Structural Challenges to Ensure High Retention in Clinical Trials: Experiences Among HIV-Discordant Couples Enrolled in an HIV Prevention Study in Kenya

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**BACKGROUND:** Poor infrastructure in sub-Saharan Africa, the site of a majority of HIV prevention trials, poses challenges to high retention necessary to successfully conduct these investigations. We describe the strategies employed to retain couples in a HIV prevention trial for two years in Kisumu, Kenya.

**METHODS:** 532 HIV-discordant couples were followed up for 24 months at the Kisumu, Kenya site as part of the Partners in Prevention HSV/HIV Transmission Study, a randomized trial of HSV-2 suppression for HIV-1 prevention. HIV-positive participants had monthly visits while HIV-negative partners had quarterly visits to the study clinic. Strategies implemented to retain participants included: obtaining detailed locator information, escorting participants to their homes, providing transportation, phone call reminders and tracing those who missed visits. We established 'express services' for those who were in a hurry. We prepared a list of all participants to identify those who would normally present to the study clinic using 'BEAM' to list participants who came Before their scheduled appointment, and on the Exact day and After the scheduled date including Missed visits. The study organized quarterly 'barazas' or gatherings of enrolled HIV discordant couples to encourage sharing, understanding and adherence with study procedures. We analyzed the correlates of total loss to follow up.

**RESULTS:** Of the 1064 participants, 958 (90%) completed all expected study visits. During follow-up 27 participants died (2.5%) and 79 (7.4%) were deemed lost for the following reasons: participant discontinuation [29 (2.7%)], non-adherence with the protocol [12 (1.1%)], relocation [4 (0.4%)] and not traceable [10 (0.9%)]. The remainder [24 (2.3%)] included failure to obtain consent for extension of the follow up period from 12 to 24 months or closure of the visit window. More urban residents [28 (12.3%)] were lost to follow up than participants from rural areas [51 (6.1%);  $p = 0.03$ ]. No other factors including gender, marital status, years of schooling and number of children was associated with retention.

**CONCLUSIONS:** Study retention was excellent in spite of the challenges. Commitment by the study staff, frequent engagement with couples, and multiple approaches implemented by the site likely contributed to the high retention rate. Future studies should explore the reasons for improved retention among participants from rural regions in comparison to more urban environments.

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### Bacterial Vaginosis (BV) Dynamics Among Women Who Participated in a Four-Arm Microbicide Trial (HPTN 035)

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**BACKGROUND:** Vaginal microbicides can potentially protect against vaginal tract infections. They could also contribute to changes in vaginal flora. These products are used with every sexual act and their impact on the dynamic state of the local vaginal environment is not well established. Continuous monitoring is therefore important. In this observational analysis we describe the patterns of BV (a severe disturbance of vaginal flora) in a large multi-arm microbicide trial.

**METHODS:** HPTN 035 was a Phase II/IIb randomized controlled trial evaluating two candidate active vaginal microbicide gel arms (0.5% PRO 2000 and BufferGel) compared to a placebo gel and no gel (condom-only) arms. Vaginal fluid samples were collected every three months and microscopically examined. BV was defined based on the Nugent score: 7–10, BV positive ("+"); <7, BV negative ("−"). We identified the following changes during the study follow-up period: "+" at any visit during follow-up (Prevalence); "+" to "+" (Persistence); "+" to "−" (Clearance); "−" to "+" (Incidence); "−" to "−" (Maintenance); and "+" to "−" to "+" (Recurrence). We estimated rates of each of these conditions based on the number of events and denominator of woman-years (w-y) of follow-up.

**RESULTS:** Data on 3087 women were analyzed. The rates of BV and other related changes were comparable among the four study arms. Table shows rates (per 100 w-y; 95% CI) for no gel arm, the 3 gel arms combined and overall.

CONDITION	NO GEL ARM	3 GEL ARMS COMBINED	OVERALL
Prevalence (+ at any visit)	160.5 (150.0–170.9)	160.9 (154.6–167.1)	160.8 (155.4–166.1)
Persistence (+ → +)	95.7 (87.2–104.3)	96.3 (91.1–101.5)	96.1 (91.7–100.6)
Clearance (+ → −)	41.1 (37.9–44.3)	41.2 (39.4–43.1)	41.2 (39.6–42.8)
Incidence (− → +)	49.2 (45.5–52.9)	48.8 (46.7–50.8)	48.9 (47.1–50.7)
Maintenance (− → −)	199.2 (188.3–210.1)	193.9 (187.7–200.1)	195.2 (189.8–200.6)
Recurrence (+ → − → +)	11.7 (9.7–13.6)	12.8 (11.6–13.9)	12.5 (11.5–13.5)

**CONCLUSIONS:** Use of vaginal gels (active or placebo) compared to control no-gel use was not associated with changes in vaginal flora

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### A Validation Study of the Gen-Probe® APTIMA Combo2® (AC2) for Detecting *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Rectal Samples.

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**BACKGROUND:** The objective of this study was to validate and compare the use of the Gen-Probe Aptima system and the Becton Dickinson ProbeTec ET System™ for the detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) from rectal swabs. We also evaluated the prevalence of *Trichomonas vaginalis* (TV) and *Mycoplasma genitalium* (MG) in rectal samples using the Aptima system.

**METHODS:** Rectal swabs were collected from 454 people aged 18–64 years, who reported having had at least one episode of anal receptive intercourse. The population was 45% male and 55% female, with 49% Caucasian and 48% black race. A true positive for CT was defined when both NAATs (AC2 and ProbeTec) were positive. A true positive for GC was defined as culture positive or if both NAATs (AC2 and ProbeTec) were positive. Discrepant results between the NAAT tests were repeated using the APTIMA CT or APTIMA GC assay, which target alternate primers, as the confirmatory tests.

**RESULTS:** Of 454 participants, 38 (7.9%) were positive for CT, 15 (3.3%) were positive for GC, 18 (4%) were positive for TV and 34 (7.5%) were positive for MG. Rectal CT and MG were equally common among men and women. GC was twice as common in men compared to women (4.4% vs. 2.4%), while TV was detected exclusively from the rectal swabs from women (7% vs. 0%). The diagnostic sensitivity and specificity of the CT test was 100% and 99.8% for Aptima and 58.3% and 100% for ProbeTec, respectively. The diagnostic sensitivity and specificity for GC was 100% and 100% for Aptima and 73.3% and 100% for ProbeTec, respectively. The diagnostic sensitivity of GC culture was 13.3%. Of the 88 patients who had a positive result for any of the four infectious agents, 14 were positive for 2 different organisms and 1 was positive for 3 of the 4 organisms.

**CONCLUSIONS:** These data suggest that the Aptima system is superior to ProbeTec for the detection of CT and GC from rectal swabs. In addition, this system allows for the detection of TV and MG.

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### Hepatitis B, Hepatitis C, and HIV-1 Coinfection in Two Selected Informal Urban Settlements in Nairobi, Kenya

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**BACKGROUND:** The extent of coinfection with hepatitis and HIV in urban slums in Kenya is unknown, and yet could partly explain the disproportionately high morbidity and mortality associated with HIV infections in these slums

The aim of this study was to determine and describe hepatitis B, hepatitis C and HIV-1 coinfection in Korogocho and Viwandani slums.

**METHODS:** Dried Blood spots and sociodemographic data were collected from 1352 individuals in Korogocho and Viwandani who gave informed consent. Samples were screened for hepatitis B surface antigen (HBsAg), Hepatitis C Ab and HIV Ab.

**RESULTS:** HIV prevalence in these slums is 10.4%. The prevalence of HBsAg and anti-HCV was 13.4% and 0.7% respectively. The rate of HIV/HBV and HIV/HCV coinfection was found to be 4.26% and 0.46% respectively, while 0.3% were co-infected with HBV/HCV. Only two people out of 1352 (0.14%) were co-infected with all the three viruses together. The likelihood of being infected with hepatitis was highest in divorced/separated and in the age bracket of 40-49 years. Prevalence of all viruses was highest in those who don't have any formal education.

**CONCLUSION:** High HBV prevalence was found in this population and it might become a major health problem in future if control measures are not put in place. HCV is not common in this kind of population. Appropriate and compulsory screening of the hepatitis viruses using sensitive methods must be ensured to prevent transmission of hepatitis and HIV.

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ABSTRACT WITHDRAWN BY AUTHORS

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### Modulation of Epithelial Innate Immune Function by *T. vaginalis*

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**BACKGROUND:** Worldwide the extracellular parasitic protozoan *Trichomonas vaginalis* (TV) infects over 180 million people annually and causes trichomoniasis, the most common non-viral sexually transmitted disease. Trichomoniasis has been linked to increased HIV-1 transmission, and other serious public health problems e.g. preterm birth, cervical cancer and bacterial vaginosis. The predominant cell surface glycoconjugate of the parasite, the TV lipophosphoglycan (LPG) plays a critical role in the parasite adhesion and mediates host inflammatory responses to infection. The molecular mechanisms of parasite-host interactions and related risks of viral infection and complications have not been fully understood to date.

**METHODS:** Immortalized and primary cervical and vaginal epithelial cells, TLR- and galectin-deficient cell lines were exposed to multiple strains of *T. vaginalis* or purified *T. vaginalis* LPG and bacterial pathogenic determinants. These exposures were also tested in the presence of physiologic concentrations of steroid hormones. Innate immune responses were measured by multiplex protein, phosphoprotein and mRNA analysis. ANOVA was used to analyse differences between the treatment groups.

**RESULTS:** *T. vaginalis* and LPG induced TLR-4 independent and prostaglandin-enhanced production of proinflammatory mediators e.g. IL-8 and prostaglandin PGE<sub>2</sub> and upregulated the expression of several members of the galectin family of carbohydrate recognizing proteins known to be involved in the HIV pathogenesis. Purified LPG and *T. vaginalis* showed high affinity binding to galectins modulating the vaginal inflammatory responses and HIV replication e.g. gal-1 and gal-3. Galectin-deficient cell lines failed to respond to LPG.

**CONCLUSIONS:** These results suggest pathogenic mechanisms underlying the epidemiologic association between trichomoniasis and HIV infection risk.

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### Recent HIV Seroconverters Are at High Risk for Sexually Transmitted Infections (MTN-015)

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**BACKGROUND:** HIV-STI co-infections are a public health priority because of an increased risk of HIV transmission and the negative impact of STIs in these individuals. We evaluated women enrolled in MTN-015, an observational cohort study of women with HIV-1 seroconversion during microbicide trials, for STI burden and associated risk factors. This knowledge could inform STI prevention strategies in women co-infected with HIV.

**METHODS:** MTN 015 enrolled women who experienced HIV seroconversion during HPTN 035 at 5 African sites. Socio-demographic, behavioral and clinical information was assessed at study entry and clinical testing was performed for STIs including *Neisseria gonorrhoea* (GC), *Chlamydia trachomatis* (CT), and syphilis. Univariate and multivariate logistic regression models were used to assess for characteristics associated with STI risk.

**RESULTS:** 99 HIV-infected women from HPTN 035 were enrolled. At enrollment: median age was 27 years, median time since seroconversion was 18 mo, 49% were married and 99% reported 0 or 1 sexual partner in the prior 3 mo. Median time from the final study visit for HPTN 035 and enrollment was 8 mo. Clinical parameters included median CD4+ T-cell count of 431/mm<sup>3</sup> and a median parity of 2. Prevalence of GC, CT, syphilis or any STI was 8.1%, 10.1%, 1%, and 17.2%, respectively. STI rates varied between the sites with the highest prevalence of any STI at the Zambia and Durban SA sites (26% and 43%, respectively). In univariate analysis, parity of 1 or 0 was associated with increased risk of CT (OR 7.7; 95% CI 1.4, 78.9; p=0.01) or any STI (OR 5.1; 95% CI 1.5, 20.4; p=0.01) compared to women with 2 or more children. Younger age was associated with increased risk of CT (OR 1.4 per year decrease; 95% CI 1.1, 1.8; p=0.01). Marital status, age of primary partner, number of partners, time from HIV seroconversion, and CD4+ T-cell count were not significantly associated with risk of STI. In a multivariate model adjusting for age, site, CD4+ T-cell count and number of partners, parity < 2 increased risk of any STI more than 5-fold (OR 5.3; 95% CI 1.2, 23.9; p=0.03).

**CONCLUSIONS:** Despite prior experience in a longitudinal HIV prevention study, active STI were common among HIV positive women enrolled in MTN 015. With few exceptions, demographic characteristics were not predictive of STI risk. Ongoing surveillance and treatment as well as improved behavioral counseling interventions are needed to modify the risk of STI acquisition.

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### Factors Associated with Sexually Transmitted Infections Among Women Enrolled in a Microbicide Feasibility Study in Northwest Tanzania

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**BACKGROUND:** Well-characterized cohorts of high risk women are needed for future microbicide trials. Since sexually transmitted infections (STIs) are major risk factors for HIV infection, understanding determinants of these infections in such cohorts is important for future microbicide trials.

**METHODS:** Between July 2008 and August 2009, 983 HIV-negative women aged 18–44 working in bars/hotels and other similar facilities in three towns in northwestern Tanzania were enrolled into an observational prospective cohort study. At enrolment, women were interviewed and blood samples were collected for HSV-2 and syphilis serology, while cervical samples were collected for *Chlamydia trachomatis* (CT) and gonorrhea (by PCR). Logistic regression was used to identify independent risk factors for these infections.

**RESULTS:** The majority of enrolled women (60%) were between 18–28 years old. Only 12% have reached secondary education; 49% were widowed, separated, or divorced; and 46% have never used a condom. At baseline, the prevalence of HSV-2 was 74.1%, active syphilis 9.0%, CT 11.4%, and gonorrhoea 12.0%. Risk factors for HSV-2 included: ever drinking alcohol (OR=2.0; 95%CI=1.4–2.8) and being widowed, separated or divorced compared to being married (OR=1.8; 95%CI=1.1; 2.9). Other risk factors for HSV-2 included older age, being less educated, increasing number of lifetime pregnancies, and never using condoms. Active syphilis was significantly associated with young age at first sex (p-value for trend=0.0001) and ever condom use (OR=1.9; 95%CI=1.2–3.3). CT was associated with young age, site of recruitment, and ever receiving money for sex (OR=1.6; 95%CI=1.0–2.5), while gonorrhoea was associated with younger age and ever using a condom (OR=1.9; 95%CI=1.2–2.9).

**CONCLUSIONS:** STI burden at enrolment in this population is high, indicating the need for integrated preventive and treatment services for trial participants. Prevention counseling should emphasize the risk associated with alcohol use and transactional sex, and the importance of consistent and correct condom use. Future trial designs should consider reduction of the STI burden by providing available rapid testing and treatment, adherence counseling and involvement of male partners.

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### Correlation of H<sub>2</sub>O<sub>2</sub> Production by Some Lagos *Lactobacillus* Species with Nugent Score

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**BACKGROUND:** Lactobacilli are the predominant flora in the vagina of women in the reproductive age group. Their presence is believed to inhibit the growth of pathogenic organisms due to ability to produce hydrogen peroxide. In order to assess the vaginal health of women and speculate susceptibility to reproductive infections, this study was done detect hydrogen peroxide production in some *Lactobacillus* species previously isolated, quantify the produced H<sub>2</sub>O<sub>2</sub> and to correlate the amounts produced with the presence or absence of BV

**METHODS:** Hydrogen peroxide detection and quantification was carried out by titration method on 97 isolates of *Lactobacilli* obtained from the Medical Microbiology Department of the College of Medicine, at the Departments of Biochemistry and, Idi-Araba between May and August 2009. Bacterial vaginosis (BV) in the sources was diagnosed by Nugent score. Isolates were subcultured from glycerol broth, re-identified as *Lactobacillus* species if they grew on MRS agar, were gram positive bacilli on Gram stain and catalase negative. Hydrogen peroxide was detected and measured by titration method using dilute sulphuric acid and potassium permanganate.

**RESULTS:** Out of 97 isolates studied (15 from BV and 82 without BV), 76 (78%) were facultative anaerobes, while 21 (22%) were strict anaerobes. The facultative anaerobes were obtained from 11 women with BV and 65 women without BV. Forty nine (50.51%) of the 97 isolates produced H<sub>2</sub>O<sub>2</sub>. Forty four of them from women without BV while five were from women with BV. Majority of the strains obtained from women with BV were non-hydrogen peroxide producing. Rates of H<sub>2</sub>O<sub>2</sub> production by Nugent score were 70%, 43% and 33% in negative, intermediate and BV Nugent scores respectively. There was no significant difference between mean concentration of H<sub>2</sub>O<sub>2</sub> produced by *Lactobacilli* from women with Intermediate Score, BV Score and women with Negative Nugent Score

**CONCLUSIONS:** The overall rate of hydrogen peroxide production was low. While the rates of hydrogen peroxide production correlated with Nugent scores being highest in negative Nugent scores and lowest with BV scores, the concentration of hydrogen peroxide produced had no association with Nugent scores. Nigerian women may have a relatively high rate of susceptibility to vaginal infections.

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### Bacterial Vaginosis as a Risk Factor for Acquiring Sexually Transmitted Diseases

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**BACKGROUND:** Few studies have demonstrated that Bacterial vaginosis (BV) is associated with sexual behavior risk factors similar to those for other sexually transmitted diseases. In the present study, the prevalence of these in a multivariate analysis of data from sexually active women infected with BV and either *Chlamydia trachomatis* (CT), *Treponema pallidum* (syphilis), *Neisseria gonorrhoeae* (NG) or HIV was observed. Non-BV infected women were used as control subjects.

**METHODS:** Data from 788 women screened in the SAVVY HIV gel phase III clinical trial in Accra (West Legon Study Site) from 2004 to 2006 were analyzed. Participants were evaluated for the presence of BV, CT, *Treponema pallidum* (PT), NG, *Trichomonas vaginalis* (TV) and Human Immunodeficiency Virus (HIV), and interviewed in detail with respect to sexual behaviors. Statistical comparisons were made using t-test, chi-squared test (pearson) and logistic regression multivariate analysis.

**RESULTS:** This study has shown a high association between BV and HIV ( $P<0.01$ ) with risk factor (0.4), which does not occurred in the other sexually transmitted diseases like NG, syphilis and Chlamydia with insignificant association ( $P<1$ ) and risk factors (0.6, 0.7, 0.9) respectively. HIV was found to be the most prevalent sexually transmitted disease with 11.2%, Chlamydia 9.2%, TV 2.3%, Syphilis 1.7% and NG the least with 1.5%. Also, BV and candidiasis were found to be the commonest cause of vaginitis in these women. We also observed mixed-infection of the organisms that cause vaginitis in these women.

**CONCLUSIONS:** Bacteria associated with bacterial vaginosis increase female genital-tract infection of HIV but the mechanism by which this happens is not clear. Bacterial vaginosis is not a sexually transmitted disease but predisposes one to HIV infection. It is strongly suggested that all cases of BV both symptomatic and asymptomatic that are presented in the sexual-health clinics should be treated to reduce the risk of PID, preterm delivery, and/or HIV transmission. Also, sexually active and pregnant women should be encouraged to frequently visit sexual-health clinics for BV screening and treatment.

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### Risk Factors Associated with HIV and STIs in Female Prostitutes in Lagos Nigeria

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**BACKGROUND:** Globally, female prostitutes are considered to be at high risk of STIs/HIV infections due to the large number of short-term sexual partners and high-risk behaviours they are involved in like drug and abuse alcohol. This study assessed the other risk factors that influence their acquisition of STIs/HIV infections in Nigeria.

**METHODS:** Four hundred and fifty prostitutes were recruited. Their ages ranged between 15–35 years with a mean age of 23+. A pre- and post-test counseling was conducted after a written informed consent was obtained from them. Participants provided demographic data, information on sexual behaviour, and knowledge of STI/HIV, contraceptives use and douching. Cervical and high vaginal swabs were collected, screened for the presence of sexually transmitted pathogens using standard microbiological methods. The Quick view Chlamydia test kit was used for detection of Chlamydia antigen from the cervical swabs. Their blood samples were screened for HIV antibody using ELISA kits, Western blot tests were used for confirmation, Syphilis antibodies were detected using RPR kits and TPHA test. Prostitutes' positive for any variable received treatment.

**RESULTS:** HIV infections were more prevalent with those that practiced anal (36.0) and oral sex (31.7%) ( $p=0.05$ ). Candidiasis (19.6%) was most prevalent infection in the group that used condom, followed by HIV (16.4%), while Syphilis infection (50%) was most prevalent with IUD users. There was no infection found in two prostitutes who used injectables, spermicides. STI/HIV infection in prostitutes who douched was high (81.5%) and was low (44.7%) in prostitutes who do not douche. HIV infection was significant among the prostitutes that practiced douching ( $2.05 = 0.0002$ ). Candidiasis infection was the most prevalent infection (14.7%) in prostitutes who do not douche.

**CONCLUSIONS:** Anal, oral, douching and improper use of contraceptives have been identified as risk factors to HIV and STIs acquisition in the group of prostitutes studied. Information on the need to access health programmes and appropriate education on proper use of condoms in order to maximize benefits should be made available to them. These will reduce the burden of STI and HIV diseases in the prostitutes. Again, regular surveys on risk factors that lead to acquisition of STIs and HIV in prostitutes will not only enable health authorities to assess the effectiveness of counselling and enlightenment programmes but also help in developing new strategies for control and prevention.

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### Prevalence of Ulcerative Sexually Transmitted Infections (STIs) Among HIV-Positive Women Clinic Attendees in a Nigerian Teaching Hospital

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**BACKGROUND:** Human immunodeficiency Virus (HIV) the most common STI transmitted worldwide. However there are unexplored media for its transmission and these include the presence of STIs especially the ulcer forming STIs e.g., Chancroid, Granuloma Inguinale, etc. There is need for proper exploration of the relationship between sexually transmitted infections and HIV/AIDS. This study sought to determine the association between ulcer-forming locally endemic sexually transmitted infections (STIs), the sexual history of patients and HIV/AIDS infection.

**METHODS:** A seven-year retrospective review of 212 case notes of female patients attending the sexually transmitted diseases clinic was done. Those with ulcer-forming sexually transmitted diseases were then reviewed to determine age at initiation of sex, present number of sexual partners and overall sexual behavioral pattern. The results of laboratory tests taken by the patients were also reviewed. Data was analyzed with the use of the SPSS data editor. Chi square tests were used to determine whether the level of association observed was of statistical significance.

**RESULTS:** Thirty-two patients had been treated for ulcerative STIs (15.1%). The mean age at sexual initiation was 18.7 years. (Standard deviation =1.6, Standard error of the mean =0.375). A total of nineteen cases of those who were confirmed by laboratory tests to have ulcerative STIs were also found to be HIV positive (59.4%). A history of infection with an ulcer-forming STIs had a significant association with HIV infection ( $p=0.01$ ).

**CONCLUSIONS:** Sexually transmitted infections that produce ulcers are associated with HIV infection. A more aggressive management of STIs and more effective contact tracing is needed to reduce the incidence of HIV especially in women.

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### Trends in Human Papillomavirus (HPV) Infection Among HIV-Positive Women before the Highly Active Antiretroviral Therapy (Pre-HAART) and HAART Era in a Nigerian Clinic

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**BACKGROUND:** The prevalence of HIV infection has been on the increase in Nigeria in recent times. HIV-positive patients frequently have anogenital malignancies due to HPV. HAART was introduced in Anti-retroviral (ARV) centers in Nigeria in the year 2002. The aim of the study is to determine trends in incidence of anogenital malignancies among HIV-positive women undergoing treatment in the clinic in the pre-HAART and HAART era.

**METHODS:** retrospective review of 541 case notes of HIV-positive female patients from January 1999 to December 2004 were analyzed by utilizing an on-going observational database at the ARV center. Rate ratios, comparing incidence rates (number of malignancies per 1000 person years) were calculated.

**RESULTS:** Twenty-four (4.43%) of the patients had one form of anogenital manifestation of HPV or the other. The incidence rate for HPV rose from 2.28 in the pre-HAART era to 6.40 in the HAART era (Rate ratio = 3.15; 95% confidence interval (CI) = 1.31–7.44; p = 0.0002).

**CONCLUSIONS:** There has been a significant rise in the incidence of HPV since the introduction of HAART. This may be due to the longer survival of HIV-infected patients, surpassing the latency period for the anogenital malignancies. Care providers should be more vigilant for HIV-associated malignancies as patients live longer in this part of the world.

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### Pap Smears in Clinical Trial Participants: Fears and Challenges Experienced in MDP 301—Vaginal Microbicide Trial

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**BACKGROUND:** Cancer of the cervix is the most common cancer amongst women; however global prevalence has decreased because of early detection by Papanicolaou (Pap) smear. Cervical cancer occurs most commonly between the ages of 30 to 45 years but it can occur as early as 18 years and screening and early detection has been shown to reduce mortality and morbidity. A challenge that exists however is that women are reluctant to have Pap smears done as they have many fears and misconceptions regarding this procedure. In the MDP 301 Trial Pap smears were carried out on all women enrolled into the trial and the experiences of midwives who were responsible for carrying out pap smears on 1000 enrolled women at a Durban site have been collated.

**METHODS:** The observations of 4 midwives with experience of working at a clinical research site were collated, during the face to face interview, pre-discussion prior to pelvic exam, during education and counselling sessions.

**RESULTS:** Although pap smears are readily available at clinics within communities, the majority of the participants who presented to the study clinic were having a Pap smear for the first time. Participants had many misconceptions regarding pap smears and appeared to be uninformed on the importance of having pap smears done. They were also apprehensive of having the procedure and thought that it entailed having some sort of operation. The women were also under the impression that a Pap smear has to be done at every pelvic examination. Their understanding of cancer was poor as many of them believed that cancer was contagious. Additionally, women believed that having a pap smear would negatively impact on their sex life and even went to the extent of attending clinic during menstruation in order to avoid having a Pap smear done at that particular visit.

**CONCLUSIONS:** It has been clearly reflected above that there appears to be major misconceptions regarding pap smears and their role as a screening tool for cervical cancer. It was also noted that participants were uninformed on the importance of having a pap done and were uneducated on the benefits of having this procedure carried out as per national guidelines. These beliefs warrant further research on the current uptake of pap smears in the general population and the development of new tools and strategies to improve understanding and thus the uptake of Pap smears within these communities.

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### Pregnancy Attitudes and Contraceptive Use at Enrolment Among Women Participating in a Microbicide Feasibility Study in Northwest Tanzania

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**BACKGROUND:** Pregnancy is an important exclusion criterion in most HIV prevention trials, including clinical trials of new microbicide products. Information on baseline pregnancy prevalence, attitudes to pregnancy, and factors associated with this condition will inform recruitment strategies for future microbicide trials in high-risk populations.

**METHODS:** Between July 2008 and August 2009, we enrolled into a cohort study 983 women employed in the bars/hotels and other similar facilities in three towns in northwestern Tanzania. At baseline, participants were asked about reproductive health, family planning, and pregnancy attitude. Logistic regression was used to identify independent characteristics associated with positive pregnancy attitude.

**RESULTS:** The median number of lifetime pregnancies was 2 (range=0–12). 31.5% women did not want any additional children, while most women (60.9%) would like 1–2 additional children. Contraceptive use prevalence was 64.8%. The main methods included male condoms (66.7%), Depo-provera (22.1%) and oral contraceptives (14.1%). Positive attitude to pregnancy (i.e. very or somewhat happy if pregnant at the time of baseline interview) was reported by 39.9% women, compared to 51.2% who reported that their partner would have positive attitude. Among those reporting using condoms for contraception, 37.1% reported positive attitude to pregnancy, compared to 51.9% oral pill users and 27.3% Depo-provera users. In multivariate analysis, compared to women aged 18–22, women in older age groups were less likely to be happy if they were found to be pregnant (p-value for trend <0.0001). Those who were using contraception were also less likely to be happy if they were pregnant at the time of interview (OR=0.4; 95%CI=0.3–0.6).

**CONCLUSIONS:** Recruitment of women from this study population to future microbicide trials will require intensive counseling to promote the use of effective contraceptive methods such as injectable and oral contraceptives. Male condoms should be promoted in the context of dual protection. Since there is a discordance of pregnancy attitude between the participants and what was reported for their partners, involving male partners in family planning counseling sessions might be helpful to increase contraceptive use.

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### Unmet Family Planning Needs Among HIV-Positive Participants Screened for FEM-PrEP

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**BACKGROUND:** The public health community has recognized the need for integration of family planning and HIV care services for HIV-infected women as a means of reducing mother-to-child HIV transmission (MTCT). The use of effective contraception by HIV-positive women who do not desire pregnancy is a highly efficacious and cost-effective intervention for HIV prevention, yet an often unmet need.

**METHODS:** A multivariate analysis of FEM-PrEP, a randomized placebo-controlled PrEP trial, baseline data was conducted to examine associations between contraceptive attitudes, use and HIV status at two study sites.

**RESULTS:** The analysis included 1086 women screened for FEM-PrEP who also had complete HIV results at screening, including 936 in Bondo, Kenya and 150 in Pretoria, South Africa. Among those screened, 378 (34.8%) were HIV-positive. The majority of HIV-positive women expressed a desire to avoid pregnancy over the next year: 156 (41.3%) reported that they definitely did not want to get pregnant, 211 (55.8%) preferred not to get pregnant, 9 (2.4%) were unsure if they wanted to get pregnant, 2 (0.5%) reported not minding getting pregnant and none reported hoping to get pregnant. While 97.1% reported a wish to avoid pregnancy in the next year, only 168 (44.4%) reported using *any* method of contraception and only 134 (35.4%) reported using a highly effective contraceptive method (hormonal, IUD or sterilization).

**CONCLUSIONS:** These results reveal a large unmet need for effective contraception among HIV-positive women and an opportunity to prevent MTCT in women with a desire to avoid pregnancy. It may be that a non-negligible number of women identified as HIV-positive at screening were unaware of their status, and thus not currently receiving HIV care. Maximizing the HIV prevention opportunity via contraceptive provision will require integration of family planning and HIV-care services, and also identification of ways to deliver family planning services to the larger community of women desiring contraception, particularly in areas of high HIV incidence.

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### An Analysis of the Relationship Between Non-Menstrual Bleeding and Contraceptive Method for the Durban Centre during the MDP 301 Clinical Trial

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**BACKGROUND:** The MDP 301 clinical trial of the microbicide PRO 2000 was carried out in 4 countries including South Africa; participants were enrolled across 3 clinical trial sites in Durban. Non-menstrual bleeding (NMB) was monitored during the trial, as it can lead to adverse events and possible discontinuation of study product, which in turn carries the risk of effect dilution. The aim of this paper is to identify and quantify factors that affect the distribution of NMB events.

**METHODS:** Contraception type at the time of event in women with NMB and listed clinical reasons for NMB were tabulated. Associations between an NMB event and gel group and age were explored using chi-square tests. Odds ratios for NMB between age groups and contraception mode at enrolment were calculated. Potential confounding between age group and contraception mode at enrolment was examined and a statistical test for interaction was performed.

**RESULTS:** The mean age of the 2391 women enrolled in Durban was 29 (range 18 to 55); women who experienced NMB were younger ( $p < 0.0001$ ). NMB was experienced by 26% of the women enrolled (n=627). Of the women who experienced NMB, 77% were on hormonal contraception at the time of the NMB event. Of these women, 92% were using injectable contraception, and 8% were using oral contraception. The most common reason listed for NMB was "Hormonal" (75%) followed by "Other" (13%), "Not Sure" (11%) and all other reasons <1%.

There was no evidence of difference in number of women who had experienced NMB between gel groups ( $p = 0.301$ ).

The OR for using hormonal contraception over having an NMB event was 1.77 (95% CI 1.46 2.14) Adjusted for age group, the OR became 1.55 (95% CI 1.29 1.89) so there was no evidence of confounding of the effect of hormonal contraception by age. There was also no evidence of effect modification of hormonal contraception by age ( $p = 0.17$ ).

**CONCLUSIONS:** The data suggest that use of hormonal contraception may contribute to the aetiology of NMB in the study population independent of age, although the effect of hormonal contraception may vary with age. The contribution of hormonal contraceptives to NMB merits further evaluation of their use in the context of vaginal microbicide clinical trials.

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### Good Intentions: Risk Factors for Unplanned Pregnancy in the U.S. Cohort of the HPTN 035 Microbicide Trial

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**BACKGROUND:** HIV prevention trial protocols enroll women not planning to conceive, and most require that women who become pregnant discontinue product use. Incident pregnancies can affect trial outcomes, causing increases in interrupted use and decreased treatment effect in intention-to-treat analyses. We hypothesize that within the recruitment pool, there are characteristics that can help separate high from low-risk women with regard to pregnancy risk.

**METHODS:** We designed this mixed-method nested case-control study to uncover risk factors for pregnancy within the U.S. site of HPTN 035. We administered an instrument to assess attitudes/beliefs about fertility control and contraceptive utilization. Cases, women who became pregnant, were matched by time-on-study 1:4 with participant controls. Descriptive statistics compared demographic data and risk factors between the groups. Dichotomous variables were tested with Chi-square or Fisher's exact test; continuous variables with Student's t-test. Conditional logistic regression identified variables independently associated with pregnancy. The qualitative section revealed themes among women who became pregnant.

**RESULTS:** The pregnancy incidence rate was 10.09 per 100 woman-years (95% CI 6.6, 13.6) for all Philadelphia participants. Univariate analyses revealed that contraceptive method change was associated with becoming pregnant (12/26, 46%  $P < 0.01$ ), regardless of whether the adopted method was more or less effective than the original method. Participant/partner desire for future children (OR=4.95) and young age (OR=0.88 annually above age 19) were independently associated with pregnancy. Qualitative data revealed that women take both active and passive approaches to fertility management.

**CONCLUSIONS:** Women who became pregnant in HPTN035 had different attributes than the controls. Desire for a future baby and young age were independently associated with pregnancy. If confirmed in other populations, the information could improve screening and/or help women avoid pregnancy during trials. Future trials could include instruments with more valid assessments of parenthood desires/partner wishes. High scorers, especially among those under 25 years, could prompt referrals for expert contraceptive care.

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### A High Percentage of Unsafe Abortions Among Female Sex Workers in a Microbicide Preparedness Study in Mombasa, Kenya

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**BACKGROUND:** Unsafe abortion remains a major health problem in the developing world, particularly among female sex workers (FSW). In a sub-study of a microbicide preparedness HIV incidence study among 400 female sex workers pregnancy intentions were assessed and consequences of unintended pregnancies were explored.

**METHODS:** Between March and August 2008, qualitative methods were used to assess family planning needs and the consequences of unintended pregnancies. Forty-five of 400 women in the cohort participated in focus group discussions (FGD). FGD participants were grouped by age: 16–19, 20–24, 25–30 and over 30 years. In-depth interviews were conducted with 24 of 109 women who became pregnant within one year of follow up. Data were analyzed using NVivo 7.0.

**RESULTS:** Of the 109 women who became pregnant during the study, 10 intended to get pregnant; 59 did not intend to get pregnant and 40 had unknown intention. There were 56 confirmed pregnancy terminations and 42 confirmed live births. Abortions were induced at home by the pregnant women or were performed in private health clinics and hospitals or by untrained local "experts". The choice of abortion method was mainly determined by cost and the gestational age of the fetus. Products reported to be used to induce home-based abortions included *Aloe vera*, tea leaves, *Omo*® and *Blue*® (washing detergents), sooth, undiluted juice, neem leaves, and overdose of over the counter drugs (aspirin, anti-malaria's, hormonal contraceptives). Makeshift devices such as clothes hangers, crotchets, straws, drip pipes and umbrella/fan spikes were also used. These abortions resulted in psychological trauma, genital infections and broken relationships.

**CONCLUSIONS:** The incidence of unwanted pregnancy and abortion among this cohort of FSW was high despite regular family planning counselling as part of the study and high reported contraceptive use. Health risks associated with unsafe abortion are a major concern in microbicide trials in the developing world and warrant further research.

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### Risk Factors Associated with Prevalent and Incident Pregnancy Among Women at Risk for HIV in Rustenburg, South Africa

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**BACKGROUND:** One challenge microbicide trialists face in testing products in women at high risk of HIV is the corresponding high risk for pregnancy. We evaluated baseline factors associated with pregnancy risk.

**METHODS:** We screened 871 women aged 18–35, where 471 (54.1%) met high-risk inclusion criteria and tested for pregnancy at baseline; of these, 171 participated in a cohort study with up to 6 monthly visits with pregnancy testing. We examined the following baseline factors for associations with pregnancy prevalence and incidence: demographics, partnership characteristics, contraceptive use, fertility intentions, perceived HIV vulnerability and gender role beliefs. Logistic regression models for pregnancy prevalence, and Cox proportional hazard models for incidence, were used. Variables significant at 0.15 were considered in modeling; given the modest sample size, those significant at 0.10 were reported.

**RESULTS:** The cross-sectional sample was 464 (98.5%) Black African, mean age 23.3 years, 261 (59.2%) used condoms and 154 (33.3%) used some form of hormonal contraception. At baseline 42/471 (8.9%) were pregnant and 21.4% reported trying to fall pregnant, but such intentions were not associated with pregnancy prevalence. Prevalent pregnancy was more likely among older women: RR (95%CI) 2.0 (1.0–4.1) (aged 22–35 (23.8 of these 22–24years) vs. 18–21); those with more education 2.1 (0.9–5.0) (diploma vs. <high school); those in shorter relationships 2.5 (1.2–5.5) (<2 years vs. 2–5 years); and less likely among hormonal contraception users 0.1 (0.0–0.5). Median follow-up was 3.6 months; 23 (13.4%) became pregnant: incidence, 43.5/100 woman-years. Use of injectable hormone RR 0.20 (0.1–0.9), but not the pill, was the only factor associated with reduced pregnancy incidence. There was a trend for women with moderately progressive gender role beliefs (compared to those with traditional gender role beliefs) to be at greater risk of pregnancy: 2.3 (0.9–5.9).

**CONCLUSIONS:** Demographics and hormonal contraceptive use are associated with lower pregnancy prevalence, while pregnancy incidence was lower among users of hormonal injections. Women with progressive gender beliefs had double the pregnancy risk, suggesting that beliefs are an important marker for pregnancy risk. Future trials need better screening tools than intention for predicting pregnancy risk. Pregnancy incidence may further be reduced by providing appropriate counseling and offering injectable contraception.

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### Pregnancy Incidence and Outcomes in HPTN 035 in Blantyre, Malawi: A Phase II/IIB Trial of the Vaginal Microbicides PRO 2000 and BufferGel

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**BACKGROUND:** HPTN 035 was a four arm Phase II/IIB randomized controlled trial of two topical microbicides, PRO 2000 and BufferGel, with two control arms, placebo gel and no gel. Women who become pregnant during microbicides studies are taken off study products for fear of potential harm to the fetus. Pregnant women, like all sexually active women in Malawi are at risk of acquiring HIV infection during pregnancy. Some studies show that acquisition of HIV during pregnancy may actually be higher.

**METHODS:** 441 eligible Malawian women who were not planning pregnancies were randomly assigned to one of the 4 study arms (1:1:1:1). At enrollment and at each monthly visit, women had a urine pregnancy test with QuickVue One-Step hCG kit. Pregnant women were excluded from enrolment. Women testing positive for pregnancy had gel use temporarily discontinued. All enrolled women were provided access to contraceptives and counseled on contraceptive and condom use. Self-reported data on gel, condom and contraceptive use were collected at baseline and quarterly visits. At each monthly visit, contraceptive counseling was provided with emphasis on reliable modern contraceptive methods. These methods were available at no cost in the study clinic. All pregnancies were followed for birth outcome. Pregnancy incidence rates were based on number of events (first positive pregnancy test result) and woman-years (WYs) of follow-up.

**RESULTS:** At baseline, 343 (78%) women used highly effective contraception with 279 (63%) women using injectable contraceptives, 34 (8%) birth control pills, 5 (1%) norplant inserts, and 25 (6%) surgical sterilization. 107 (24%) women were ever pregnant during the study. There were 120 pregnancies in 107 women with a pregnancy incidence rate of 17.1 (95% confidence interval:14.0–20.7 ) per 100 WYs. Pregnancy outcomes were full-term live births (61%), premature live births (3%), still births (3%), spontaneous abortion (25%) and therapeutic abortion (8%). No congenital anomalies were identified.

**CONCLUSIONS:** The overall pregnancy rate of 17.1 per 100 WYs, was the highest among the participating study sites in the HPTN 035. Pregnant women during microbicides studies are taken off the study product, leading to loss of important knowledge about the effect of microbicides in preventing HIV infection during pregnancy. Despite intensive counseling and provision of contraception, a considerable number women in Blantyre still became pregnant. It is important therefore that consideration be given to the study of microbicides during pregnancy.

**324****High Uptake of Non-Barrier Contraception by Women Before Randomization into an HIV Prevention Trial in Rural Kenya**

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**BACKGROUND:** Women who become pregnant in biomedical HIV prevention clinical trials are often withdrawn from study products. As a result, time off product is increased and trial statistical power may be compromised. High pregnancy incidence has been a challenge in some trials, especially in regions where contraceptive prevalence is low. The pre-randomization period offers a window of opportunity to provide contraception counseling and initiate contraception prior to study enrollment. We initiated pre-randomization contraceptive counseling and on-site provision of contraception for women screened for an HIV prevention clinical trial of antiretroviral pre-exposure prophylaxis (PrEP).

**METHODS:** The Partners PrEP Study is enrolling HIV-1 serodiscordant couples at 9 sites in East Africa, including a site in Thika, Kenya. We provided contraceptive counseling to all HIV positive and HIV negative women during screening and offered methods to women free-of-charge at the research clinic prior to randomization. Contraceptive prevalence data from the screening and randomization visits were analyzed for 260 HIV discordant couples enrolled between October 2008 and November 2009.

**RESULTS:** Of 260 HIV serodiscordant couples enrolled, 59 (22.7%) were couples in which the HIV negative partner was female. Among 59 HIV negative women enrolled, non-barrier contraceptive use increased from 37.3% [22/59] at screening to 71.2% [42/59] at enrollment [ $P<0.001$ ]. The median time between screening and enrollment was 12 days. The 20 HIV negative women who initiated contraception between screening and randomization, used depot medroxyprogesterone acetate 12 [60%], oral contraceptive pills 3 [15%], implants 3 [15%], and intrauterine devices for 2 [10%]. Among the 201 HIV positive women enrolled, non-barrier contraceptive uptake also increased significantly between screening and enrollment: from 34.3% [69/201] to 61.7% [124/201] ( $p<0.001$ ). The 55 HIV positive women who initiated contraception between screening and randomization, used depot medroxyprogesterone acetate 41 [74.5%], oral contraceptive pills 8 [14.5%], implants 4 [7.3%], and bilateral tubal ligation 2 [3.6%].

**CONCLUSIONS:** Providing contraception counseling and access to contraceptive methods before randomization can lead to a significant increase in contraception uptake by women participating in HIV prevention clinical trials. Early initiation of contraception may reduce pregnancies and time off study product.

**325****Contraception and Family Planning in HIV Prevention Trials: Current Practice and Stakeholder Perspectives**

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**BACKGROUND:** To test the effectiveness of new HIV prevention methods like microbicide, late-stage trials must enroll large numbers of sexually active women. Current safety regulations require women who become pregnant to discontinue use of experimental products. A large percentage of women enrolled in past HIV prevention trials have became pregnant, leading some researchers to suggest that study participants be required to use effective non-barrier contraceptive methods. Others have questioned the morality of mandating contraceptives or restricting reproductive choice.

**METHODS:** To explore this issue further, we used protocol reviews, surveys and key informant interviews to examine: 1) family planning practices in eighteen completed, ongoing or planned phase 2 and 3 clinical HIV prevention trials; and 2) current perceptions of contraceptive services among key stakeholders including international and local researchers, trial sponsors, community advocates, and policymakers.

**RESULTS:** We found that most trials excluded women who indicated that they planned to get pregnant, and that participants who got pregnant were required to stop product use. Many trials also mandated some form of contraceptive use, usually a combined oral or injectable contraceptive. Even among researchers working in the HIV prevention field, however, a majority were opposed to such contraceptive mandates. Policymakers, advocates, and researchers not directly involved in HIV prevention trials were even more uniform in their opposition to mandated use of contraceptives.

**CONCLUSIONS:** Several recent trials have achieved a remarkable reduction in pregnancy rates by providing comprehensive family planning counseling without requiring contraceptive use. Given the ethical questions raised and the attitudes we documented, trial networks and sponsors may want to reconsider the use of contraceptive mandates in favor of enhanced contraceptive provision.

**326****Preference Between Pre-Coital and Continuous Use of Duet® in Zimbabwe**

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**BACKGROUND:** Inadequate adherence to female-initiated methods has undermined the ability of several recent HIV prevention trials to evaluate efficacy. New coitally-independent methods are being developed to simplify use, improve adherence and assessment of their effectiveness. Duet® is a combination microbicide-delivery system and a cervical barrier which can be used continuously or pre-coitally. We explored factors associated with women's preference of one use-regimen versus the other.

**METHODS:** In this cross-over study, 83 women were randomized to 14 days of pre-coital use then 14 days of continuous use of Duet, or the reverse order regimen. Participants were healthy, on hormonal contraceptives, aged 18–40; half (42) had previously used diaphragms and half were diaphragm-naïve (41). Face-to-face interviews were conducted at baseline and study exit. Multivariable logistic regression was used to identify factors associated with use-regimen preference at study exit. Exit focus group discussions (FGD) were conducted with a subset of 41 participants and analyzed thematically.

**RESULTS:** In this sample of married monogamous women, 51% preferred pre-coital use, 39% preferred continuous use and 10% liked both equally. Self-reported product adherence during sex was high (88%) and not associated with regimen preference. Diaphragm experience was the only factor significantly associated with preference for pre-coital use (AOR 2.79, 95% CI 1.01-7.76). In FGD, those who liked pre-coital use said that planning or being prepared for sex with their partner was easy, they preferred using the device only when needed, and/or had hygiene or comfort issues with wearing the device continuously. Those who liked continuous use cited convenience, discreetness, feeling "safe" and being prepared in advance for "sex-on-demand."

**CONCLUSIONS:** Previous experience with using the diaphragm pre-coitally influenced current use-preference for Duet. Different personal and life circumstances may result in varying use regimen preferences. Methods that can accommodate both episodic and daily/continuous use may be advantageous by providing more choice to users. Larger studies in more diverse populations should further explore use modality preferences for this dual purpose method.

# MONDAY POSTERS

## 327

### The Burden of Unwanted Pregnancies in a Female Sex Worker Cohort, Nairobi, Kenya

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**BACKGROUND:** The occurrence of unwanted pregnancies in any clinical trial negates drug reaction and adverse event reporting. Family planning options are widely accessible yet majority of at risk women are not using them. This study was designed to establish barriers to family planning uptake and condom use among female sex workers in a microbicide feasibility study in Kenya.

**METHODS:** In a period of one year, 200 female sex workers were enrolled for study. A standard tool was used to collect data regarding family planning, condom use and sex practices. Thereafter, participants had regular visits for family planning services, diagnosis and treatment of STIs at no cost.

**RESULTS:** Majority 67% were young, aged 18–30 years, with a mean age of 28. Unwanted pregnancies were reported in 12% of the women, fear of partner rejection and infertility were the main reasons for not using family planning method. More than half 53.8% never used condoms with primary partners. HIV prevalence was 11.3%, a figure double that in the general population.

**CONCLUSIONS:** Sex workers in this cohort are at higher risk of unwanted pregnancies and HIV infection which calls for a dual action microbicide preventing pregnancies and STI/HIV/HIV.

## 328

### Contraceptive Use in the MDP 301 HIV Prevention Trial in Rural KwaZulu-Natal, South Africa

H. Ndlovu\*, M. Mzimela, M. Gafos, and the MDP team

Africa Centre for Health and Population Studies, University of KwaZulu-Natal, South Africa

**BACKGROUND:** The Africa Centre for Health and Population Studies was one of six Microbicides Development Programme MDP 301 clinical trial sites. The eligibility criteria included not intending to become pregnant in the following year. All research nurses were specifically trained in family planning and enrolled participants were given regular family planning counselling and provided with free contraceptive services. This analysis evaluates changes in contraceptive use during the 12-month follow-up period.

**METHODS:** Data on contraceptive use were collected monthly at each follow-up visit. Sterilisation, the contraceptive pill and injectable contraceptives were defined as reliable forms of contraception. Contraceptive use at screening and the final 12-month follow-up visit was analysed in STATA 10 to identify changes in contraceptive use. Demographic and socio-economic variables were assessed for association with increased contraceptive use. Univariable and multivariable analysis was conducted; multivariable results are presented here.

**RESULTS:** 1,022 of 1177 women enrolled completed the 12-month follow-up visit and were included in this analysis. At screening 46% of women reported using reliable contraceptives; 29% injectables, 11% sterilised; 6% pill. Controlling for education, employment status, area of residency and socio-economic-status, women over 40 (AOR:0.43 CI:0.31–0.58) were significantly less likely to report contraceptive use. Contraceptive use increased significantly (paired t-test=<0.01) to 64% at the 12-month follow-up visit; 40% injectables, 12% sterilised and 12% pill. At month 12, 4% of women reported stopping using contraceptives and 22% reported starting contraceptive use, mainly injectables. Controlling for the same factors among women not using contraceptives at baseline, women over 40 (compared to 18–24 year-olds: AOR:0.43 CI:0.29–0.64) and urban residents (AOR:0.63 CI:0.41–0.96) were significantly less likely to have started using contraceptives. There were no significant differences on the basis of educational level, employment status, relationship to head of the household or socio-economic-status. The pregnancy incidence rate at the site was 8 per 100 person years.

**CONCLUSIONS:** Contraceptive use increased significantly over the course of the trial, possibly associated with provision of contraceptive services. The increase in contraceptive use assisted in maintaining low pregnancy incidence and could be important in future microbicide trials.

## 329

### Pregnancy Rates in the Durban Site of the MDP 301 Trial: Implications for VOICE and Other PrEP Studies

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Medical Research Council of South Africa, HIV Prevention Research Unit, 123 Jan Hofmeyr Road, Westville, Durban, KwaZulu-Natal, South Africa

**BACKGROUND:** Women recruited into HIV prevention trials are of childbearing age and at high risk for HIV infection. Since the teratogenicity of microbicides and other interventions are unknown, pregnancy is an exclusion criterion in most prevention studies, and researchers make a concerted effort to provide contraception to women participants. We report here pregnancy rates and contraceptive use among women participating at the MDP 301 sites in Durban, and discuss the implications of such observations for the VOICE study.

**METHODS:** The recently completed MDP 301 trial was an international, multi-centre, randomized, double-blind, placebo controlled trial to evaluate the efficacy and safety of 0.5% and 2% PRO2000/5 gels for the prevention of vaginally acquired HIV infection. Women recruited were tested for pregnancy at every visit, and put on study hold if found to be bHCG positive. They were offered condoms, oral and injectable contraceptives; and contraceptive counselling at every visit. Chi-square tests were used to compare the pregnancy rates between different types of contraception use.

**RESULTS:** Of 2391 women who were enrolled at three centres in Durban, 9.5% (n=228) became pregnant during a one year follow-up period. 72% of pregnant women reported using some form of contraceptive at or before the visit at which a positive pregnancy result was determined. The pregnancy rate in those using oral contraception was 17% (59/353) compared to 3% (1182/1216) in those using injectables ( $p<0.001$ ). Of the 34 women who became pregnant and reported injectable use, only three had received the injectable contraception from study staff.

**CONCLUSIONS:** Our results suggest that injectable contraception administered and monitored by study staff may result in greater compliance, and thus avoid time off-product due to pregnancy.

The VOICE study allows oral pill use as a reliable method of contraception. However, given the higher rate of pregnancies among women who use such pills when compared to injectables, intensive counselling and education on compliance will be necessary, or extensive counselling on an alternative form of contraception should be provided.

**330****A Device to Prevent HIV Transmission Through Breast Feeding**

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**BACKGROUND:** The risk of HIV transmission through breastfeeding is difficult to address. The virus is particularly lethal for infants, as roughly 50% of children who acquire it from their mothers die during their first two years of life. Researchers' estimates of babies infected from breast milk range from 50,000 to 150,000. WHO recently recommended the use of anti-retroviral drugs (ARVs) to prevent transmission by breast milk, but the use of ARVs entails various issues.

**METHODS:** We developed this device concept and the first prototype at a 2008 MIT workshop, the International Development Design Summit. Since then we have made improved prototypes for further testing.

**RESULTS:** We have developed a novel, low-cost modified Nipple Shield device to be used by HIV-positive mothers to prevent MTCT of HIV through breastfeeding. The device is based on combining the following: (1) an approved medical device, a nipple shield, (2) a virucidal agent on FDA's "Generally Recognized As Safe" food additive list which is in toothpaste, sodium dodecyl sulfate (SDS), and (3) a small disk of non-woven felt-like textile material to hold the SDS in the tip of the Nipple Shield.

To prevent HIV transmission through breast milk, the tip of our novel Nipple Shield holds a disk made of a non-woven material impregnated with SDS that can kill HIV. The unique element of this device is the non-woven disk. To use the device, the impregnated textile material would be cut into appropriate size disks and put in blister packs. A mother would open a blister pack and insert a disk into the tip of a modified Nipple Shield. The mother would place the device over her breast and feed her baby. As breast milk passes through the Nipple Shield and disk, SDS is released from the disk and kills HIV in breast milk without disrupting breastfeeding. Previous research has shown that low concentrations of SDS will inactivate HIV. We are also considering other virucidal agents.

To date, we have developed prototypes of our device and have demonstrated the ease of fluid flow through an SDS-impregnated disk. We are currently (1) optimizing an SDS sustained release formulation that will release an adequate concentration of SDS into breast milk during one or more feeding sessions, and (2) analyzing data from an acceptability study in Kenya.

**CONCLUSIONS:** A modified nipple shield that could deliver a microbicides might provide an alternative to ARVs for the prevention of HIV transmission through breast milk.

**331****Knowledge Attitudes and Practices Towards Prevention of Mother-to-Child Transmission of HIV Among Antenatal Care Mothers in Gedio, South Ethiopia**

D. Gebreegziabher

**BACKGROUND:** HIV/AIDS is currently a major public health problem in Ethiopia and mother-to-child transmission (MTCT) is by far the largest source of HIV infection in children below the age of 15 year. For women to take advantage of measures to reduce transmission, they need to know their HIV status. The objective of the study was to assess knowledge, attitude and practice of the PMTCT of HIV among antenatal care mothers.

**METHODS:** A health institution based cross-sectional study was conducted in gedio zone from a June to September 2008. A total of 461 pregnant mother were interviewed from three health centers and one hospital. Proportional distribution of samples was carried out to attain the required sample size. data were entered and processed into the computer using EPI info version 6 and SPSS version 10 statistical packages.

**RESULTS:** Almost all the ,457(99.1%) respondents had heard about HIV/AIDS of which,419(92.7%) mentioned the major routes of transmission and 437 (94.8%) knew that HIV could transmitted from an infected mother to her baby. Most of the respondents 433 (93.9%) knew that MTCT of HIV is preventable. Four hundred fifty seven (99.1%) of the pregnant mothers have positive attitudes towards VCT 323(84.6%) of the mothers were tested for their current pregnancy and among 301 (78.8%), reason for testing was to protect "MY" child from HIV. Pregnant women with two to three and more than three visits were less likely accepting PMTCT as compared to only first visit [OR=0.10, 95% CI 0.03,0.36],[OR= 0.12,95% CI 0.04,0.38] respectively.

**CONCLUSIONS:** Most mothers knew that HIV could be transmitted from mother to her fetus and its preventive methods. Health education targeted on male partners, and community at large on PMTCT and VCT would have paramount importance using different sources.



The background of the image is a soft-focus, abstract representation of organic forms in a light green or lime-yellow hue. It features various rounded, bulbous, and elongated shapes that suggest cells, leaves, or perhaps microscopic organisms, creating a sense of depth and biological complexity.

Abstracts

Tuesday  
May 25, 2010

# ORAL SESSIONS

## SESSION 35

### Plenary

**Moderators:** Gita Ramjee, Renee Ridzon

Tuesday, May 25, 8:00 am–9:00 am

Ballroom

## 332

### Putting Vaccines Back into the Prevention Puzzle

Susan Buchbinder

AIDS Office, San Francisco Dept. of Health, USA

The HIV vaccine field has evolved considerably since the news in September 2007 that the MRK Ad5 gag/pol/nef trivalent HIV vaccine was not effective in preventing HIV infections nor in reducing early plasma viral load. Most recently, promising data has been published from the RV144 trial of a canarypox vector HIV vaccine with a gp120 boost; a 31% reduction in HIV acquisition was seen in vaccinees compared with placebo recipients. A number of lessons have been learned from both trials. Animal models alone could not have predicted the potential importance of pre-existing immunity to HIV vaccine vectors, nor the importance of understanding the mucosal immune response. Detailed laboratory studies are identifying early positive effects of the MRKAd5 trivalent vaccine; detailed studies of potential immune correlates of protection in the RV144 study are underway. Many lessons from microbicide trials have been applied to understanding HIV vaccine trial results, and results of HIV vaccine trials may, in turn, inform microbicide studies. The HIV prevention landscape may become increasingly complex as partially effective strategies are found, but this complexity should be embraced.

## 333

### From Research to Reality: Implementation of Biomedical Prevention

James McIntyre, BMChB, FRCOG

Perinatal HIV Research Unit, Univ. of the Witwatersrand, South Africa

Biomedical HIV prevention research over the past decade has faced a series of disappointingly negative or “flat” trial results. Recent promising vaccine trial results and a renewed focus on the use of antiretroviral agents for prevention suggest that effective biomedical prevention interventions may now be closer. The prevention of mother-to-child transmission of HIV (PMTCT) provides some lessons on the challenges faced in moving successful prevention research strategies into practice. More than decade after the first successful trials of antiretroviral PMTCT interventions, less than half of HIV-positive pregnant women globally have access to appropriate prophylaxis. A number of factors have hindered wider implementation, including cost, the need for HIV testing, health system infrastructure, health provider attitudes and community stigmatisation. Many similar factors will impact on the use of any newly proven biomedical prevention strategies, although the worldwide progress towards universal access of HIV treatment is likely to add to an enabling environment for new interventions. Consideration of implementation challenges and planning for the rapid introduction of effective biomedical interventions must proceed in parallel with research efforts.

## SESSION 36

## Oral Abstracts (OA18): STIs, Bacterial Vaginosis, and Lactobacilli

**Moderators:** Lorna Rabe, Carolyn Deal

Tuesday, May 25, 9:30 am–11:00 am

Ballroom

**334**

**Can We Quantitatively Follow up Lactobacillae Species with Real Time PCR Methods for the Safety Evaluation of Microbicides and Mucosal Vaccines?**

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Institute of Tropical Medicine, Antwerp, Belgium

**BACKGROUND:** The gold standard to assess the vaginal flora in microbicide vaginal safety trials consists of culture methods and Nugent scoring (Gram stain). Nowadays we have real time PCR methods to quantify Lactobacillae species including fastidious species. This study evaluated the added value of quantitative real time PCR methods to Nugent scoring in women recovering from bacterial vaginosis (BV).

**METHODS:** Twelve women with BV (Nugent score 7–10) and twenty nine women free of BV were examined twice. Women with BV received oral metronidazole at the first visit. Real time PCR for *L. crispatus*, *L. iners*, *L. jensenii*, *L. gasseri* and total *Lactobacillus* sp. were performed on vaginal specimens at both visits.

**RESULTS:** *L. crispatus* was significantly more present (Table 334.1) in women free of BV compared to women with BV at the first visit and similarly, *L. crispatus* and overall *Lactobacillus* logs were significantly higher. The 10 women that recovered from BV after treatment had, despite a normal Nugent score, still significantly lower *L. crispatus* logs than the women free of BV at both visits.

TABLE 334.1 Number (%) of Women with Specific *Lactobacillus* Species

	N (%)	<i>L. crispatus</i>	<i>L. iners</i>	<i>L. jensenii</i>	<i>L. jensenii</i>
<b>VISIT 1</b>					
<b>BV Present</b>	12	5 (42%)	10 (83%)	3 (25%)	1 (8%)
<b>BV Free</b>	29	23 (79%)	25 (86%)	15 (52%)	7 (24%)
<b>P-value (Fishers Exact test)</b>		0.029	NS	NS	NS
<b>VISIT 2 No BV</b>					
<b>Recovered from BV</b>	10	3 (30%)	9 (90%)	3 (30%)	3 (30%)
<b>BV Free at Study Start</b>	18	16 (89%)	16 (89%)	8 (44%)	8 (44%)
<b>P-value (Fishers Exact test)</b>		0.003	NS	NS	NS

**CONCLUSIONS:** This study shows that the quantification up to the species level of Lactobacillae is feasible. It can pick up subtle changes and provides additional information to the Nugent score even in small sample sizes. Real time PCR is therefore a valuable tool for the evaluation of the safety and impact of microbicides and mucosal vaccines on the vaginal flora.

# ORAL SESSIONS

## 335

### Aptamers that Neutralize Herpes Simplex Virus Type-2 as Potential Components of a Multi-Virus Microbicide

M. Moore<sup>1</sup>, D. Bunka<sup>2</sup>, P. Spear<sup>3</sup>, I. McGowan<sup>4</sup>, P. Stockley<sup>2</sup>, William James<sup>1</sup>

<sup>1</sup>Sir William Dunn School of Pathology, University of Oxford, Oxford, UK; <sup>2</sup>Astbury Centre for Structural Molecular Biology, University of Leeds, UK;

<sup>3</sup>Department of Microbiology-Immunology, Northwestern University, Chicago, USA; <sup>4</sup>Magee-Womens Research Institute, Pittsburgh, PA, USA

**BACKGROUND:** HSV-2 is a sexually transmitted infection affecting about 1 in 4 people in the USA. It infects epithelial cells of the GU tract and associated neurons following binding to the cellular receptors, Nectin-1 and HVEM through its envelope glycoprotein, gD. Infection can be prevented *in vitro* using antibodies to gD. In order to develop a candidate anti-HSV-2 agent for use in a prospective multivalent microbicide, we have isolated 2'-F-pyrimidine-substituted RNA aptamers that bind to the gD envelope glycoprotein.

**METHODS:** A starting RNA aptamer library of over  $10^{14}$  different sequences was created by T7 transcription using 2'-OH purines and 2'-F pyrimidines. Recombinant, Fc-tagged gD glycoprotein was used to partition binding from non-binding nucleic acids in a high-throughput SELEX procedure. Immobilized control Fc protein fragments were used in negative selection cycles. Samples of the polyclonal nucleic acid libraries were tested at each gD-binding enrichment cycle for their ability to reduce infectivity of HSV-2 in a standard *in vitro* plaque assay. Individual clones isolated from neutralizing pools were assayed for their potency against HSV-2. CHO cell lines engineered to express only one HSV2 receptor, either Nectin-1 or HVEM, were used to identify which receptor binding site on gD the aptamers affected. The secondary structures of all neutralizing aptamers were modeled, and conserved motifs identified.

**RESULTS:** We have screened numerous monoclonal aptamers for ability to neutralize HSV-2 infectivity of Vero cells. Multiple aptamers proved capable of reducing infectivity of HSV-2 by a factor of 5-fold or more at 100 nM, and IC50s were determined to be typically in the order of 30–50 nM. Preliminary data also suggests that we have isolated aptamers able to discriminate between the two receptor binding sites of gD.

**CONCLUSIONS:** 2'-F RNA-based aptamers, selected for binding to HSV-2 gD protein, can neutralize HSV-2 and prevent it from initiating infection.

## 336

### Genital Secretions from HIV Infected Women Exhibit Decreased Activity against HSV-2

R.P. Madan, N.M. Torres, A. Huber, A.C. Boyd, E.E. Bruce, D. Futterman, S. Coupey, M. Kim, M. Keller, B.C. Herold  
Albert Einstein College of Medicine

**BACKGROUND:** Genital tract mucosal immunity may be modified in the setting of HIV and could impact risk for HIV shedding, acquisition of STI, and response to microbicides. We conducted a pilot study to quantify endogenous antimicrobial activity and immune mediators in cervicovaginal lavage specimens (CVL) collected from sexually active, HIV infected and uninfected women ages 15–24.

**METHODS:** Anti-HSV-2 activity in CVL was quantified by plaque reduction assay, antimicrobial proteins were quantified by ELISA, and cytokines by Bioluminex. Anti-HIV and anti-E. coli activity will also be measured. Categorical variables for HIV+ and uninfected women were compared by Fisher exact test. Continuous variables were compared by unpaired t test or nonparametric equivalent, with Spearman's correlation coefficient to assess for possible associations.

**RESULTS:** HIV+ subjects (n=10) were older than uninfected subjects (n=8) (21 vs. 17.5 years, p=0.02) but were similar for race, hormonal contraception and tobacco use, HSV-1,2 serostatus, history of abnormal Pap smear in the past 6 months, and vaginal pH ( $p>0.05$ ). All HIV+ subjects were Category A (asymptomatic), 2 subjects were infected perinatally, 2 subjects received HAART, median years from HIV diagnosis was 3.5, and median CD4+ T cell count was 548 cells/ml. 8 subjects had detectable viral loads (median 2169 copies/ml). CVL from HIV uninfected women had significantly more activity against HSV-2 than CVL from HIV+ women (70.33% inhibition vs. 40.1, p=0.046). CVL from HIV+ women had significantly lower concentrations of IL-6 ( $p=0.03$ ) and a trend towards lower levels of HNP1-3 ( $p=0.18$ ), lactoferrin ( $p=0.13$ ), lysozyme ( $p=0.07$ ), IgA ( $p=0.2$ ), IL-8 ( $p=0.18$ ), and IFNy ( $p=0.08$ ). For all subjects, anti- HSV-2 activity in CVL correlated with HNP 1-3 ( $r=0.89$ ,  $p=0.001$ ), lysozyme ( $r=0.82$ ,  $p<0.001$ ), and IL-8 ( $r=0.54$ ,  $p=0.03$ ). Mean plasma concentrations of HNP 1-3 were similar between HIV+ and uninfected subjects ( $p=0.1$ ) and were lower than CVL concentrations ( $p<0.001$ ). Plasma and CVL concentrations did not correlate with one another, suggesting that genital tract HNP1-3 is produced locally.

**CONCLUSIONS:** Young women with HIV infection, even in the early, asymptomatic stages of disease, may have local immune dysregulation in the genital tract resulting in diminished antimicrobial activity, lower levels of key immune mediators, and possibly increased risk of STI acquisition and differential response to vaginal microbicides.

## 337

### Establishing and Sustaining a Healthy Vaginal Environment: Results from a Randomized Trial of Oral Periodic Presumptive Treatment for Vaginal Infections

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**BACKGROUND:** Vaginal infections including bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and *T. vaginalis* have been associated with increased risk of HIV-1 acquisition. Data from a randomized, double-blinded placebo controlled trial of directly observed oral periodic presumptive treatment (PPT) to reduce vaginal infections among Kenyan women was analyzed to assess the effectiveness of the intervention in establishing a healthy vaginal environment.

**METHODS:** Eligible participants were randomized to receive 2g metronidazole plus 150mg fluconazole or matching placebo administered monthly for 12 months. An intent-to-treat analysis using Andersen-Gill proportional hazards models was conducted to estimate the effect of the intervention versus placebo on the incidence of a healthy vaginal environment (Nugent score 0–3 with no yeast or *T. vaginalis* on wet mount). The effect of the intervention on sustained vaginal health, defined as the presence of a healthy vaginal environment for ≥ 3 consecutive visits, was assessed using Kaplan-Meier survival analysis and Cox proportional hazards models in the subgroup of women who attended 3 or more follow-up visits.

**RESULTS:** The trial enrolled 310 participants (155 in each arm). This analysis included follow-up to 15 months from enrollment. Incidence of a healthy vaginal environment was 608/100 person-years in the intervention arm versus 454/100 person-years in the placebo arm (HR = 1.36; 95% CI: 1.17–1.58). Among 287 women who attended 3 or more follow-up visits, the incidence of sustained vaginal health was 65/100 person-years in the intervention arm versus 47/100 person-years in the placebo arm (HR = 1.62; 95% CI: 1.17–2.22). Median time to first episode of sustained vaginal health was significantly shorter in the intervention arm compared to the placebo arm (21.4 weeks versus >60 weeks, respectively ( $p=0.003$ )).

**Conclusions:** Compared to placebo, PPT increased both the overall frequency of having a healthy vaginal environment and the incidence of sustained vaginal health. PPT could be used to promote vaginal health and potentially reduce susceptibility to HIV-1 and sexually transmitted infections. Interventions to preserve or promote normal vaginal flora could also be used to augment the effect of microbicides directed at specific pathogens, including HIV.

**338****Validity of Clinical Diagnosis of Bacterial Vaginosis by Human Immunodeficiency Virus Infection Status**

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**BACKGROUND:** The control of bacterial vaginosis (BV) could hold an important role in limiting the transmission of HIV. BV typically is diagnosed for patient care on the basis of its clinical manifestations (Amsel criteria). In contrast, for research purposes, BV often is diagnosed with a semi-quantitative method of scoring bacterial morphotypes seen on Gram-stained vaginal smears (Nugent scoring). We assessed the validity of clinical diagnosis of bacterial vaginosis, overall and by HIV infection status.

**METHODS:** Women with HIV, or at risk for HIV, participated in the HIV Epidemiology Research Study (HERS), a prospective study conducted in four US sites. At enrollment and follow-up visits, scheduled at six-month intervals for  $\leq 5$  years, participants received gynecologic examinations, had specimens collected, and underwent standardized interviews. We used McNemar's test statistic to evaluate agreement between Amsel criteria and Nugent scoring. Using Nugent scoring as the reference standard, we calculated sensitivity and specificity for Amsel criteria and for three other classifications of clinical BV.

**RESULTS:** Results are based on data collected from 9,140 study visits by 862 HIV-infected women and 421 HIV-uninfected women. Amsel criteria and Nugent scoring did not agree in the classification of BV cases ( $P$ -value  $< 0.01$ ). Amsel criteria had poor sensitivity (60%; 95% confidence interval [CI], 58%–61%) and specificity (90%; 95% CI, 89%–91%). Alternative classifications of clinical BV suggest that in the typical patient care setting, which usually requires vaginal discharge to prompt testing for BV, the sensitivity of Amsel criteria would be even lower, without any gains in specificity. Also, relaxing the clinical diagnosing of BV to require a minimum of two (instead of three) of the Amsel criteria, did improve sensitivity but with an accompanying decline in specificity. We found no differences in diagnosing BV by HIV infection status.

**CONCLUSION:** The under- and over-diagnosing of BV clinically suggests that the accuracy of Amsel criteria for routine screening of asymptomatic women might be lower than previous estimates; that clinicians need more rigorous training to apply subjective Amsel criteria accurately; or that wide heterogeneity in cases might prevent agreement between clinical and laboratory diagnoses, with future research needed to better understand the criteria or morphotypes associated with specific adverse outcomes.

# ORAL SESSIONS

## SESSION 37

### Mini-Symposia (MS7): What Comes After Tenofovir?

**Moderators:** Ward Cates, Helen Rees

Tuesday, May 25, 9:30 am–11:00 am

Rooms 301–304

# 339

### Designing Studies Without a Placebo

Doug Taylor

*Family Health International, USA*

Recent trials of tenofovir gel and other oral tenofovir formulations offer the hope that a new and proven effective HIV prevention technology may be available in the near future. When one of these formulations becomes established as a standard of care, however, subsequent placebo controlled trials of HIV prevention methods (e.g. vaccines, vaginal rings, and other oral, topical, and injectable microbicides) may become ethically impossible in many populations. We consider some study designs that do not include a placebo, including superiority and non-inferiority trials using tenofovir as a standard of care, as an active control agent, or in a combination product. Also considered are uncontrolled (i.e. single arm) trials analogous to those used to assess new contraceptive methods. The various study designs are judged both in terms of feasibility (size and cost) and challenges to valid interpretation of results. The interplay of product efficacy, adherence, and HIV exposure status are found to be key determinants in the likely success of such future studies.

# 340

### Alternative Antiretroviral Drugs for HIV Prevention

Daria Hazuda

*MERCK Research Laboratories, USA*

The focus on nucleosides-based antiretrovirals for prevention is a bias largely founded in historical origins. With the expanding antiretroviral armamentarium and an increasing understanding of the biology of transmission, multiple alternatives which have an equal if not a greater theoretical basis for consideration should be also be explored. The biology of the newest agents, integrase inhibitors and CCR5 antagonists, makes them attractive candidates for prevention trials; in addition, the licensure of potent second generation PIs and NNRTIs suggests earlier first generation drugs in particular those which are no longer in use clinically could be also be exploited as topical preventions. The pros and cons of each of these approaches and early data from monkey studies on some of these agents will be reviewed.

# 341

### Combination Antiretroviral Microbicide Products

Joe Romano

*International Partnership for Microbicides, USA*

The availability of anti-retroviral agents (ARVs) with distinct mechanisms of action allows for their combined development as microbicides. Combining ARVs has proven an effective strategy in treatment of HIV infection, as well as in the prevention of mother-to-child transmission. Various formulation technologies are available which allow for drug combinations to be developed as both single use and sustained release dosage forms. Advantages of combination microbicide development include potential increased potency, and a broadening of the scope of effectiveness, particularly against the transmission of virus with some level of resistance against one of the mechanisms of action in the combination formulation. With greater availability of ARV based treatment in areas of the world likely to use microbicides as a prevention strategy, expanded protection against transmission of resistant virus could become more important. There are also meaningful challenges associated with combination microbicide product development, including formulation development, cost, and regulatory approval. In this presentation, strategies and risks for combination microbicide product development will be discussed. Specific efforts to develop combinations of reverse transcriptase/entry inhibitors will be described for both single-use and vaginal ring dosage forms. Combination of ARVs with contraception and other anti-infectives will also be discussed.

# 342

### Combining HIV Prevention and Contraceptive Technologies

Gustavo Doncel

*CONRAD, USA*

Millions of women, especially in developing countries, need protection against sexually transmitted diseases, in particular HIV/AIDS, and family planning methods to prevent unwanted pregnancies. In many of these countries, the AIDS pandemic is intricately intertwined with overpopulation, poverty, malnutrition, and gender inequality. Therefore, there is an urgent need to develop multi-purpose prevention technologies. The main strategies pursued for the development of microbicide contraceptive combinations include two drugs with different targets and mechanisms of action (MOAs), one drug with contraceptive and microbicidal properties, and drug-device combinations with two or more MOAs. Dual-protection delivery systems currently in development by CONRAD and others include sustained-release intravaginal rings (IVRs), barrier devices and gels delivering anti-HIV and contraceptive drugs. Development of these dual-protection technologies requires a comprehensive preclinical/early clinical testing algorithm providing data on safety, efficacy and pharmacokinetics/pharmacodynamics for both MOAs (i.e., microbicide and contraceptive), and a regulatory strategy that encompasses both applications. Among other technologies, an IVR delivering a progestin/reverse transcriptase inhibitor combination is currently undergoing preclinical assessment.

## SESSION 38

### Oral Abstracts (OA19): Rectal Sex: Frequency and Impact

**Moderators:** Jim Pickett, Kevin Fenton

Tuesday, May 25, 9:30am–11:00am

Rooms 319–321

## 343

### Anal Intercourse Among Female Sex Workers Participating in Microbicides Preparedness Studies in Mombasa, Kenya and Kigali, Rwanda

N. Veldhuijzen<sup>\*1</sup>, S. Luchters<sup>2,3</sup>, C. Ingabire<sup>4</sup>, W. Bosire<sup>2</sup>, S. Braunstein<sup>5</sup>, M. Chersich<sup>3,6</sup>, J. van de Wijgert<sup>1</sup>

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<sup>2</sup>International Centre for Reproductive Health (ICRH), Mombasa, Kenya; <sup>3</sup>International Centre for Reproductive Health (ICRH), Department of Uro-Gynaecology, Ghent University, Ghent, Belgium;

<sup>4</sup>Projet Ubuzima, Kigali, Rwanda; <sup>5</sup>Columbia University, New York, NY, USA; <sup>6</sup>Reproductive Health and HIV Research Unit, University of Witwatersrand, South Africa

**BACKGROUND:** Microbicide effectiveness studies are conducted in areas and among populations with high rates of heterosexual HIV transmission. In many of these settings, the prevalence of heterosexual anal intercourse (AI), its correlates, and contribution to the spread of HIV is not well established. AI may impact vaginal microbicide effectiveness. Even though it has been calculated that the effectiveness of a vaginal microbicide with 80% efficacy in the context of exclusive vaginal intercourse (VI) may be reduced by 25% after one year if 5% of intercourse is AI, trials rarely take AI into account. We performed a secondary analysis on the incidence and prevalence of, and factors associated with, AI reporting by female sex workers (FSW) participating in microbicide preparedness studies conducted in Mombasa, Kenya and Kigali, Rwanda.

**METHODS:** Sexual and other risk behaviors, including frequency of AI, were assessed in two cross-sectional surveys of FSW in Mombasa, Kenya (n=820) and Kigali, Rwanda (n=800). At both sites, HIV-negative, non-pregnant FSW (n=400 in Mombasa, n=397 in Kigali) were followed for one year. AI prevalence and cumulative incidence rates were calculated and univariate analyses on survey characteristics were performed using STATA 9.2 (StatCorp, TX, USA).

**RESULTS:** AI was reported by 4.3% and 5.5% of FSW participating in the cross-sectional surveys, and cumulative incidence was 4.5% and 2.2% after 12 months of follow-up, in Mombasa and Kigali respectively. FSW reporting AI had multiple other risk factors for HIV transmission including inconsistent condom use, higher number of sexual partners and alcohol use before sex compared to FSW not reporting AI. AI reporting was not associated with HIV prevalence or incidence.

**CONCLUSIONS:** The reporting of AI among FSWs underscores the need to better understand the prevalence and correlates of AI in the context of microbicide trials and for eventual product introduction. This is further accentuated by the association we found between AI reporting and other high-risk behaviors. Underestimating AI in the study population might decrease the power of microbicide trials to demonstrate a protective effect and may lead to overestimation of the potential public health impact of the product. Finally, risk reduction counseling in microbicide trials, as well as in HIV prevention programs, should include AI practices.

## 344

### Interest of At Risk Boston Men Who Have Sex With Men in Oral and Topical Antiretroviral Chemoprophylaxis

K.H. Mayer<sup>\*1,2</sup>, M.J. Mimiaga<sup>1,3</sup>, W. O'Brian<sup>1</sup>, C. Dolezal<sup>4</sup>, A. Carballo-Diequez<sup>4</sup>

<sup>1</sup>The Fenway Institute, Boston, MA, USA; <sup>2</sup>Brown University/Miriam Hospital, Providence, RI, USA;

<sup>3</sup>Harvard Medical School/Massachusetts General Hospital, Boston, MA, USA; <sup>4</sup>New York State Psychiatric Institute/Columbia University, New York, NY, USA

**BACKGROUND:** Studies are underway to determine whether orally or topically delivered antiretrovirals can decrease HIV incidence. Understanding prior knowledge, attitudes, experience and interest in these approaches may facilitate their uptake and effectiveness.

**METHODS:** HIV-uninfected MSM who reported unprotected receptive anal intercourse with at least one HIV status unknown or infected partner in the prior year were enrolled at a Boston community health center in a study to determine the acceptability of rectally administered placebo gels and suppositories. Participants filled out a demographic and behavioral questionnaire at enrollment. Logistic regression procedures were used to examine demographic and behavioral factors associated with an increased likelihood to use different forms of antiviral chemoprophylaxis.

**RESULTS:** The 95 MSM participants' mean age was 39.1 years; 70.5% had more than a high school education; 24.0% were African-American and 8.6% Latino. During the prior 2 months the mean number of sex partners was 4.3. While 44.8% had heard of PEP, only 20% had heard of PrEP. Once informed about oral (PEP, PrEP) and rectal microbicides, 59.9% indicated that they would be likely to use PEP, 39.0% likely to use PrEP, and 50.0% likely to use a rectal gel for prevention. There were no demographic differences between the men who were most likely to use any one of the different chemoprophylactic approaches and those who did not indicate a high likelihood of use. MSM who were most likely to use rectal microbicides were less likely to use cocaine (AOR=0.05; 95% C.I. 0.01, 0.63), but more likely to use erectile enhancement drugs (AOR=4.90; 95% C.I. 1.13,21.34) and were more likely to use PrEP (AOR=6.64; 95% C.I.=2.04, 21.66). In a final multivariable logistic regression model adjusting for participant's age, race, and educational attainment, significant predictors of likelihood of PrEP use were increased likelihood to use PEP in the future (AOR=12.37; 95%CI = 3.27, 46.85) and likelihood of topical microbicide use(AOR=3.86; 95%CI = 1.28, 11.62).

**CONCLUSIONS:** Very few at-risk HIV-uninfected Boston MSM had prior experience with antiviral chemoprophylaxis, but a substantial subgroup, which did not have distinctive demographic characteristics, indicated interest in both oral and rectal approaches once educated, suggesting potential for wide use.

# ORAL SESSIONS

**345**

## What Impact Could We Expect at a Community Level from an Effective Rectal Microbicide Used by MSM in Peru?

H. Prudden<sup>\*1</sup>, H.C. Johnson<sup>1</sup>, A.M. Foss<sup>1</sup>, J. Peinado<sup>3</sup>, J. Sanchez<sup>3</sup>, J.R. Williams<sup>2</sup>, C. Celum<sup>4</sup>, P.T. Vickerman<sup>1</sup>, C.H. Watts<sup>1</sup>

<sup>1</sup>London School of Hygiene & Tropical Medicine, UK; <sup>2</sup>Imperial College London, UK; <sup>3</sup>INMENSA and IMPACTA, Lima, Peru; <sup>4</sup>University of Washington, USA

**BACKGROUND:** Past research into the potential public health impact of microbicides has mostly focused on vaginal microbicides with a few additional analyses of rectal microbicides used by men who have sex with men (MSM) in the USA. In Peru, the HIV epidemic remains concentrated in high-risk groups including MSM. This project aims to use mathematical modelling to estimate the potential impact of a rectal microbicide on the HIV epidemic among MSM in Lima, Peru.

**METHODS:** A deterministic compartmental model was developed, parameterised and fitted to detailed epidemiological and behavioural data for MSM in Lima. The MSM were categorised into three subgroups, based on sexual identities and risk behaviours, and the model was used to simulate the joint transmission dynamics of HIV, herpes and syphilis. The potential future HIV epidemic was explored, taking into account current levels of reported condom use, with and without a 5-year microbicide intervention. The microbicide was assumed to reduce the probability of HIV transmission to the receptive partner per anal sex act by 60%, as an illustrative case example. Scenarios of either 30% or 80% of the total MSM population in Lima being reached by the intervention were considered. It was assumed that these MSM would use the microbicide between half and all of the time that a condom is not used during anal sex while assuming that 20% fewer sex acts are protected by a condom amongst those men that are using microbicides.

**RESULTS:** Assuming a microbicide with an efficacy of 60%, preliminary model findings suggest that 2–5% of HIV infections may be averted over a 5-year period when 30% of the MSM population are reached and they use the microbicide in 50–100% of acts. In contrast, if the intervention reaches 80% of MSM, the impact could rise to 6–12% of infections averted, assuming other factors remain the same. These low and high coverage scenarios equate respectively to approximately 240 to 1200 potential infections among MSM in Lima being averted over a 5-year period with a 60% effective rectal microbicide.

**CONCLUSIONS:** These preliminary findings highlight the potential public health benefit of an effective rectal microbicide that is widely distributed among the MSM community in Lima. These are the first estimates of the potential impact of a rectal microbicide in a Latin American setting. This study emphasises the importance of pursuing further research and development into this new technology.

**346**

## Potential Impact on HIV Transmission of a Rectal Microbicide Used by Men Who Have Sex With Men in Southern India

A.M. Foss<sup>\*1</sup>, H.C. Johnson<sup>1</sup>, H. Prudden<sup>1</sup>, J.R. Williams<sup>2</sup>, A.E. Phillips<sup>2</sup>, B.M. Ramesh<sup>3,4</sup>, Reynold Washington<sup>3,4,5</sup>, M.C. Boily<sup>2</sup>, P.T. Vickerman<sup>1</sup>, C.H. Watts<sup>1</sup>

<sup>1</sup>London School of Hygiene & Tropical Medicine, UK; <sup>2</sup>Imperial College London, UK; <sup>3</sup>Karnataka Health Promotion Trust, Bangalore, India; <sup>4</sup>University of Manitoba, Winnipeg, Canada; <sup>5</sup>St John's Research Institute, Bangalore, India

**BACKGROUND:** The HIV epidemic in India remains concentrated in high-risk groups including men who have sex with men (MSM). Given the challenges of condom use, interest has been growing in the potential role of a rectal microbicide for MSM. Prior to this study, there were no estimates of the likely public health impact of a rectal microbicide in any low- or middle-income country. This research project aimed to model the potential impact of a rectal microbicide on the HIV epidemic among MSM in Bangalore, India.

**METHODS:** A deterministic compartmental model was developed, parameterised and fitted to detailed epidemiological and behavioural data from MSM in Bangalore. The model was used to simulate the joint transmission dynamics of HIV, syphilis and herpes between three subgroups of MSM, based on sexual identities/risk behaviour. Potential evolution of the HIV epidemic was investigated in the absence and presence of a 5-year microbicide intervention, with moderate underlying levels of condom use. It was assumed that 30% of the total MSM population of Bangalore Urban District would be reached by the intervention and use the microbicide between half and all the time when a condom is not used during anal sex (allowing 20% fewer sex acts to be covered by a condom following access to microbicide). Microbicide efficacy scenarios of a 35%, 60% or 85% reduction in the probability of HIV transmission to the receptive partner per anal sex act were considered.

**RESULTS:** For a microbicide with 35% HIV-efficacy per sex act, preliminary model findings suggest that approximately 4–8% of HIV infections among MSM in Bangalore could be averted within a 5-year period, given the assumptions stated above. Preliminary results imply that this impact may increase to averting about 10–20% of HIV infections if the microbicide's per sex act HIV-efficacy is 85%. In all these scenarios described, approximately 800–4,000 infections among MSM in Bangalore could be averted over 5 years through the use of an effective rectal microbicide.

**CONCLUSIONS:** This study highlights the importance of pursuing further research and investment for developing rectal microbicides. The public health benefit from an effective rectal microbicide could be considerable. These preliminary findings are in some sense conservative since they do not include the onward infections averted among the female partners of MSM.

**347****Safety and Anti-HIV Activity of Over-the-Counter Lubricant Gels**

J. Russo<sup>1</sup>, L.C. Rohan<sup>1,2</sup>, B. Moncla<sup>1,2</sup>, R.P. Na Ayudhya<sup>1</sup>, L. Wang<sup>1</sup>, M. Cost<sup>1</sup>, K. Pryke<sup>1</sup>, M.A. LeBlanc<sup>3</sup>, J. Pickett<sup>1</sup>, C.S. Dezzuti<sup>\*1,2</sup>

<sup>1</sup>Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, PA, USA; <sup>2</sup>University of Pittsburgh, Pittsburgh, PA, USA; <sup>3</sup>International Rectal Microbicide Advocates, Chicago, IL, USA

**BACKGROUND:** Because lubricants may decrease trauma during coitus, it is hypothesized that they could aid in the prevention of HIV acquisition. However, the safety and anti-HIV activity is currently unknown for over-the-counter (OTC) lubricant gels.

**METHODS:** Based on an International Rectal Microbicide Advocates survey, 6 OTC lubricant gels were tested: 5 aqueous-based (Astroglide, Elbow Grease, ID Glide, KY Jelly, and PRÉ) and 1 condom compatible silicone-based (Wet Platinum). Formulation characteristics (pH, osmolarity, and viscosity) were determined. Viability of *Lactobacillus* species and cells [Caco-2 (colorectal), HEC-1-A (uterine), and TZM-bl (cervical) epithelial cell lines and peripheral blood mononuclear cells (PBMCs)] exposed to the lubricants was evaluated. For cells, dilutions of each aqueous-based lubricant were made; Wet Platinum was used undilute. Transepithelial resistance of Caco-2 and HEC-1-A cell lines was measured to determine the impact of lubricants on epithelial cell monolayers. The anti-HIV activity was tested with the TZM-bl cell line. Colorectal and ectocervical safety was evaluated by the MTT assay and histology after apical application to polarized explant cultures.

**RESULTS:** PRÉ was pH 7, isosmolar, with moderate viscosity. Elbow Grease, ID Glide, and KY Jelly were pH 4 to 5, 9 to 13-fold above isosmolar, with varying degrees of viscosity. Astroglide was pH 4, 21-fold above isosmolar, with low viscosity. KY Jelly which contains chlorhexidine had a complete loss of *Lactobacillus* viability, but the other lubricants had < log<sub>10</sub> loss of bacteria and were considered safe. PRÉ was not toxic up to 1:10 dilution for the PBMCs and cell lines. Elbow Grease, ID Glide, and KY Jelly were not toxic up to 1:100 to 1:200 dilutions. Astroglide was not toxic up to 1:1500 dilution. Wet Platinum had no toxicity. PRÉ had no impact on the epithelial cell monolayers whereas the other aqueous-based lubricants disrupted the epithelial cell monolayers. All lubricants retained colorectal and ectocervical explant viability by MTT assay. Histology showed intact epithelium for PRÉ and Wet Platinum, while epithelial striping was observed for Astroglide, Elbow Grease, ID Glide, and KY Jelly. Lubricants had no measurable anti-HIV activity.

**CONCLUSIONS:** Our data suggests that PRÉ and Wet Platinum were safest. The hyperosmolar nature of the other lubricant gels was associated with cellular toxicity and may lead to increased risk of HIV infection.

**348****Rectal Lubricant Use and Risk for Rectal STI**

P.M. Gorbach<sup>\*1,2</sup>, R.E. Weiss<sup>1</sup>, R. Jeffries<sup>1</sup>, E. Fuchs<sup>3</sup>, M. Hezera<sup>4</sup>, S. Brown<sup>4</sup>, A. Voskanian<sup>2</sup>, E. Robbie<sup>1</sup>, P. Anton<sup>2</sup>, R.D. Cranston<sup>5</sup>

<sup>1</sup>School of Public Health, University of California, Los Angeles, CA, USA; <sup>2</sup>David Geffen School of Medicine, University of California, Los Angeles, CA, USA; <sup>3</sup>Johns Hopkins University School of Medicine, Baltimore, MD, USA; <sup>4</sup>AIDS Research Alliance, Los Angeles, CA, USA; <sup>5</sup>University of Pittsburgh, PA, USA

**BACKGROUND:** Use of sexual lubricant products (lube) before and during receptive anal intercourse (RAI) is common among men and women. Lubes may increase vulnerability to rectal sexually transmitted infections (STIs) possibly via mucosal irritation. This association was examined in a study of rectal health and behaviors of 896 men and women in Los Angeles and Baltimore.

**METHODS:** From October 2006–December 2008, men and women from the UCLA IPCP U19 0606414 in Los Angeles and Baltimore completed computer-administered self interviews about sexual and hygiene behavior, were tested for rectal STIs (Gonorrhea and Chlamydia) and 302 of the 896 reported practice of RAI in the past month (men) or in the past year (women). Frequencies for lube use before last RAI and associations with demographics, HIV status, and other behaviors are presented. Associations of lube use with rectal STI were tested using univariate and multivariate regression.

**RESULTS:** Overall 76% reported lube use before last RAI and 8.3% tested positive for a rectal STI (5.6% of women and 10.2% of men). 11.7% of lube users were positive for rectal STI vs 4.5% who did not use lube ( $p<.05$ ). Lube users reported on all types of lubes used, many reported using > 1 type. Most reported water-based lube use (76%) but silicon, oil-based, and numbing lubes were reported by 28%, 17%, and 6% respectively. Fewer African Americans and Hispanic/Latinos than Whites reported lube use (38.5%, 58%, and 72%, respectively  $p<.0001$ ). More HIV positives reported lube use than HIV negatives (57% vs. 40%,  $p=.006$ ). There was no difference by age, numbers of sex partners in the past month, and partner type in lube use before RAI. In multivariate logistic regression analysis lube use was associated with rectal STI (AOR 3.15, 95% CI 1.23, 8.04) after controlling for gender, HIV status, city, condom use, and number of sex partners in the past month.

**CONCLUSIONS:** Findings suggest use of some rectal lubricant products may increase vulnerability to rectal STIs, highlighting a need for more research on types of rectal lubricant products, their use during RAI, and potential mechanisms for how rectal lube use may facilitate transmission of rectal STIs and HIV.

# ORAL SESSIONS

## SESSION 39

### Oral Abstracts (OA20): Consent, Ethics and Community Participation

**Moderators:** Morenike Ukpong, Liza Dawson

Tuesday, May 25, 9:30 am–11:00 am

Rooms 403–405

## 349

### Ongoing Informed Consent Comprehension Assessment: Results of a Pilot Conducted in HPTN 035

A. Coletti<sup>1\*</sup>, C. Kelly<sup>2</sup>, N. Coumi<sup>3</sup>, S. Hurst<sup>4</sup>, F. Martinson<sup>5</sup>, M. Mlingo<sup>6</sup>, J. Prince<sup>7</sup>, C. Reid<sup>8</sup>, and C. Woodsong<sup>9</sup> for the HPTN 035 Protocol Team

<sup>1</sup>Family Health International, Research Triangle Park, NC, USA; <sup>2</sup>Fred Hutchinson Cancer Research Center, Seattle, WA, USA; <sup>3</sup>Medical Research Council, Durban, South Africa; <sup>4</sup>College of Medicine-Johns Hopkins University Research Project, Blantyre, Malawi; <sup>5</sup>UNC Project, Lilongwe, Malawi; <sup>6</sup>UZ-UCSF Research Programme, Harare, Zimbabwe; <sup>7</sup>Virtua Health Systems, Marlton, NJ, USA; <sup>8</sup>University of Alabama at Birmingham, Birmingham, AL, USA; <sup>9</sup>University of Zambia and Centre for Infectious Disease Research in Zambia, Zambia; <sup>9</sup>International Partnership for Microbicides, Silver Spring, MD, USA

**BACKGROUND:** The challenges of obtaining informed consent for HIV prevention trials are well-recognized. Although considerable attention has been paid to participant comprehension of informed consent topics at the time of enrollment, limited data are available on comprehension over time. In trials with extended follow-up, comprehension could decline as a result of misunderstandings at enrollment, poor recall, and/or rumors or misinformation that may arise during the course of a trial. An assessment of ongoing comprehension was piloted in HPTN 035, a Phase II/IIb safety and effectiveness trial of BufferGel and PRO 2000 Gel.

**METHODS:** HPTN 035 was conducted among 3099 women from 6 sites in sub-Saharan Africa and 1 site in the US. All women took part in an enrollment informed consent process that included administration of a standardized informed consent comprehension (ICC) checklist. Comprehension of all checklist items was required prior to enrollment. In February 2007, a modified 12-item version of the enrollment ICC checklist was administered to a random sample of 140 women (20 per site). The ongoing ICC checklist assessed participant comprehension of the study objectives, randomization/blinding, risks, benefits, unknown effectiveness of the study gels, and withdrawal from the study.

**RESULTS:** Ongoing ICC assessments were administered 13–715 days after enrollment (median=206). At least 75% of women demonstrated comprehension of 9 of the 12 checklist items. The 3 items that were less well understood related to the safety objective of the study and the associated potential risks of study participation. For most items, comprehension was not associated with time since enrollment; however, some items showed a modest downward trend and comprehension of the unknown effectiveness of the study gels improved over time. In response to observed findings, a targeted information and counseling message was developed and administered to all participants; site-specific action plans also were carried out in response to site-specific trends.

**CONCLUSIONS:** Ongoing ICC assessments can be incorporated into large-scale HIV prevention trials and can provide important quality assurance information to guide action in support of ongoing comprehension. Inclusion of such assessments should be considered for future trials. Comprehension of safety objectives may reflect therapeutic misconceptions; participant education for effectiveness trials may require added emphasis on safety objectives.

## 350

### Using Informed Consent Quizzes to Determine Enrollment Eligibility and Level of Trial Understanding for HIV-negative Women in the FEM-PrEP Clinical Trial

K. McKenna<sup>1\*</sup>, W. Rountree<sup>1</sup>, A. Cornelius<sup>1</sup>, V. Mtimkulu<sup>2</sup>, K. Ahmed<sup>2</sup>, K. Agot<sup>3</sup>, J. Odhiambo<sup>3</sup>, C. Wong<sup>1</sup>, M. Lanhman<sup>1</sup>, L. Van Damme<sup>1</sup>

<sup>1</sup>Family Health International, Research Triangle Park, NC, USA; <sup>2</sup>Setshaba Research Centre, Soshanguve, South Africa; <sup>3</sup>Impact Research & Development Organization, Bondo, Kenya

**BACKGROUND:** In order to be admitted to FEM-PrEP, a phase 3, multi-center, double-blind, randomized, placebo-controlled trial of Truvada PrEP for HIV-negative women, all potential trial participants (PTPs) go through informed consent (IC) at the screening and enrollment visits. Despite an in-depth IC process, PTPs may misunderstand the purpose of research and concepts such as randomization and the use of placebo.

**METHODS:** As part of the IC process, PTPs are administered quizzes at screening and enrollment to assess their understanding of research and the trial requirements. The quizzes follow a true/false format. To be eligible to participate, PTPs are given three attempts to answer at least 6 (75%) of 8 questions correctly from the screening IC quiz (SICQ), and three attempts to answer all 10 (100%) questions correctly from the enrollment IC quiz (EICQ).

**RESULTS:** Quiz results were examined using descriptive statistics from SAS. Of the 1,164 women who took the SICQ, 536 (46%) answered all questions correctly and 1,081 (93%) answered at least 75% of the questions correctly on the first attempt. At the second and third attempts, 73 (88%) of 83 and 7 (70%) of 10 answered 75% of questions correctly, respectively. Three women were unable to pass the quiz. The two questions most often answered incorrectly were: 1) If you are found eligible to take part in the clinical trial, you can come back to the study clinic to enroll at any time, and 2) All people who take part in the screening process can enroll in the clinical trial.

Of the 412 women who took the EICQ, 252 (61%) answered all questions correctly on the first attempt. At the second and third attempts, 129 (81%) of 160 and 23 (74%) of 31 answered all questions correctly, respectively. Eight women were unable to pass the quiz. The two questions most often answered incorrectly were: 1) There are drugs adults can take to prevent HIV infection before they are exposed to the virus, and 2) Being in this clinical trial will definitely prevent you from getting HIV.

**CONCLUSIONS:** Preliminary data suggest that nearly all women who went through the IC process were able to understand specific concepts of the FEM-PrEP trial. However, there are limitations to the true/false quiz format when assessing the understanding of PTPs. Data on concepts that PTPs have difficulty understanding are used to improve the explanation of concepts to new PTPs during the IC process and to improve the translation of quiz questions.

## 351

### "No Man Can Serve Two Masters:" The Intersection of Recruitment and Education by Community Educators

N. Barnabas<sup>\*1</sup>, G. Wolnitzek<sup>2</sup>, S. Cooper<sup>3</sup>, M. Wigley<sup>3</sup>

<sup>1</sup>Global Campaign for Microbicides, PATH, Johannesburg, South Africa; <sup>2</sup>Global Campaign for Microbicides, Washington, DC, USA; <sup>3</sup>Family Health International, Durham, NC, USA

**BACKGROUND:** Attention to the importance of community preparedness and education at HIV prevention research sites has escalated in recent years, particularly following public controversies that resulted in the 2004–05 discontinuation of tenofovir PrEP trials in Cambodia and Cameroon. The benefit of engaging and educating trial communities as outlined in UNAIDS GPP, DAIDS guidelines is now generally accepted; and the need to meet study recruitment and retention targets is well established. However how to implement effective community education and engagement and whether community educators and outreach workers should be actively involved in recruitment/retention activities is still under debate.

**METHODS:** Using survey methods and in-person discussions, we gathered a range of perspectives on this from: 1) investigators who are responsible for the successful meeting of recruitment/retention targets and completion of the study; 2) community program managers who struggle to find program resources to support both community education, recruitment/retention; and 3) community educators and study recruiters who are tasked with the business of education and recruitment.

**RESULTS:** In the absence of any concrete guidance on the issue and in the face of resource limitations, the line between general community education and targeted study recruitment are often blurred. Strong opinions exist about the utility of distinct roles in this area. Some of those surveyed believe that community education and recruitment activities should be separated to avoid the conflict of interest (real or perceived) that community educators may experience if they are also required to meet recruitment targets. Others argue that such a separation is neither possible nor desirable. Some advocate for the complete outsourcing of community education from the research entity to an independent organization.

**CONCLUSIONS:** As we head into the next generation of microbicide clinical studies and contemplate the best use of resources for the HIV prevention field in general, it is vital that we: 1) acknowledge and address the potential challenges introduced by the desire to conduct effective, credible community education and the need to meet study recruitment/retention targets; and 2) learn from experiences of past studies as we turn toward a new chapter of HIV prevention research.

## 352

### UNAIDS Good Participatory Practice Guidelines and Principles Implemented in the HPTN 035 Trial

N.S. Morar<sup>\*1</sup>, R. White<sup>2</sup>, M. Ukpong<sup>3</sup>, L. Seyama<sup>4</sup>, E. Chihota<sup>5</sup>, J. Prince<sup>6</sup>, M.Z. Chileshe<sup>7</sup>, P. Kumwenda<sup>8</sup>, on behalf of HPTN 035 Community Educators and MTN Community Working Group

<sup>1</sup>HPRU, Medical Research Council, South Africa; <sup>2</sup>Family Health International, Research Triangle Park, North Carolina USA; <sup>3</sup>Obafemi Awolowo University, Osun State, Nigeria; <sup>4</sup>Malawi College of Medicine-Johns Hopkins University, Blantyre, Malawi; <sup>5</sup>University of Zimbabwe-University of California at San Francisco Collaborative Research Programme, Harare, Zimbabwe; <sup>6</sup>University of Pennsylvania Health System, HIV Prevention Research Division, Philadelphia, PA USA; <sup>7</sup>Centre for Infectious Disease Research in Zambia, Lusaka, Zambia; <sup>8</sup>University of North Carolina Lilongwe Clinical Research Site, Lilongwe, Malawi

**BACKGROUND:** Good Participatory Practice (GPP) guidelines note that effective community engagement during and beyond the life-cycle of biomedical HIV prevention trials enhances the quality and outcome of research. HPTN 035 community engagement activities commenced in 2004, three years prior to the GPP guideline being published. This analysis presents the experiences of HPTN 035 in community engagement in relation to the GPP guidelines. Further, changes that were implemented at various sites to ensure culturally sensitive and effective community engagement programmes are described.

**METHODS:** Prospective quarterly site community reports were reviewed and, at the close of the study, community educators at each site consulted with research staff to complete a questionnaire on community challenges and efforts related to participant accrual, retention and visit adherence. An evaluation of the reports to assess level of community involvement with the HPTN 035 research lifecycle using the part III of the GPP was conducted using a grading scale of 1–5 on the following elements: site development, study initiation, study conduct, study closure, data analysis, validation, dissemination, site maintenance and plans to ensure future access to products.

**RESULTS:** HPTN 035 scored high (scores 4 and 5) on study initiation, study conduct, study closure, data analysis, validation, dissemination and plans and efforts made with MDP 301 to ensure future access to products. It obtained an average score (3) on site development, and site maintenance between trials

**CONCLUSIONS:** HPTN 035 demonstrates the possibility of successful community engagement in relation to the GPP benchmark for trials by ensuring time sensitive responses to community concerns and interest. The average score on site development and site maintenance between trials may be due to the site community staff having to transition to new studies and focus less on the HPTN 035. Active community engagement throughout the research lifecycle in a culturally sensitive way can ensure scientific and ethical integrity of trials is maintained even in the absence of guidelines.

## 353

### Empowering the Communities through the Training of Laypersons on Ethics Board

M. Ukpong, O. Akanni, O. Ezechi, K. Oyedele, A. Amumuziam, C. Amechi  
New HIV Vaccine and Microbicide Advocacy Society

**BACKGROUND:** IRBs are recognised for their critical role in making decisions about the design and implementation of study protocols. These researches are equally expected to be socio-culturally relevant and so need to be reviewed with the perspective of making them so. In view of these, IRBs are expected to have layperson(s)—persons with expertise in terms of understanding the culture and norms of the immediate community and who can bring this expertise to bear on the design and implementation of research—as members. In Nigeria, the National ethics board would not register an IRB that does not have a layperson on its membership composition. How does this translate to community inputs in research protocol review? NHVMAS shares on its experience with the training of laypersons on IRBs in Nigeria.

**METHODS:** NHVMAS organized a 5-day training on how to review a protocol and provide constructive feedback for 20 laypersons engaged on IRBs. A pre- and post-test questionnaire was administered prior to and after the training respectively. Feedback were also received 3 months and 12 months post training on their experience in the field.

**RESULTS:** Prior to the training, none of these laypersons serving on the IRBs had had basic training on research ethics. Only 10% of the trainees had ever had to handle protocols—most are just invited to IRB meetings. The 2 (10%) who had reviewed protocols in the past felt they could have done much better based on the new skills and knowledge they had acquired. Prior to the training, they really could not identify their critical role on the IRB and so stayed on as members out of obligation. The training did impact significantly on their skills and knowledge base on the post training assessments. 16 (80%) of the 20 trained laypersons were now actively reviewing protocols and providing feedback on the protocols 12 months after the training.

**CONCLUSIONS:** Laypersons are important on IRBs. However, there is the need to empower this critical group of stakeholders to facilitate their effective engagement with protocol review and providing constructive feedback. This is one way of further facilitating community engagement in research.

# ORAL SESSIONS

## SESSION 40

### Oral Abstracts (OA21): Novel Approaches

**Moderators:** John Mellors, Fulvia Veronese

Tuesday, May 25, 11:30 am–1:00 pm

Ballroom

# 354

### Lactic Acid is a Natural Microbicide with HIV Virucidal Activity

C. Conza<sup>1,2</sup>, T. Zakir<sup>1</sup>, A. Johnson<sup>1</sup>, S. Tan<sup>1,2</sup>, D. O'Hanlon<sup>3</sup>, R. Cone<sup>3</sup>, T. Moenck<sup>4</sup>, G. Tachedjian<sup>\*1,2</sup>

<sup>1</sup>Burnet Institute, Melbourne, Victoria, Australia;

<sup>2</sup>Monash University, Melbourne, Victoria, Australia;

<sup>3</sup>Johns Hopkins University, Baltimore, MD, USA;

<sup>4</sup>ReProtect Inc, Baltimore, MD, USA

**BACKGROUND:** Lactic acid (LA) present in the vagina of healthy women is produced by Lactobacilli and acidifies the vagina to pH~3.8 by maintaining a ~1.0% racemic mixture of D and L isomers of LA. LA has a pKa of 3.8, and at pH 3.8 comprises uncharged LA and lactate anion. We have discovered that LA has potent and broad-spectrum microbicidal activities including inhibiting bacteria associated with bacterial vaginosis (BV) and inactivating HSV, with L-LA more potent than D-LA and acid alone. L-LA is also less toxic than D-LA in a mouse susceptibility model. Since LA inactivates HSV we hypothesized that LA also inactivates HIV.

**METHODS:** HIV<sub>Ba-L</sub> was treated with L-LA adjusted to pH4.5, compared to pH4.5 alone (HCl-adjusted) and incubated with continuous monitoring and adjusting of pH and temperature. HIV<sub>Ba-L</sub> was treated with DL-LA, D-LA and L-LA without pH adjustment at 37°C for 30 min and L-LA was further tested against several HIV isolates and in the presence of 75% human seminal plasma (SP) to mimic a 1:4 dilution of LA during coitus. Sodium lactate was also evaluated for virucidal activity. Following incubation, buffered medium was added to dilute out the effects of LA and infectious virus titre determined in TZM-bl cells.

**RESULTS:** 1.0% L-LA at pH4.5 reduced HIV<sub>Ba-L</sub> (CCR5 strain) infectivity by 35-fold compared to pH4.5 (HCl-adjusted) alone at 37°C for 30 min. Inactivation of HIV<sub>Ba-L</sub> (10<sup>5</sup>-fold) by 1.0% L-LA (pH ~3.8) was observed within 5 min at 37°C. HIV<sub>Ba-L</sub> treated with 1.0% L-LA followed by immediate neutralisation resulted in a similar 10<sup>5</sup>-fold reduction in titre compared with 1.0% L-LA not adjusted to pH7.0. 0.3% L-LA was ~10<sup>2</sup>-fold more potent than D-LA in inactivating HIV<sub>Ba-L</sub>. Similar results were observed with a clade A CCR5 strain. 0.4% L-LA inactivated (>10<sup>3</sup>-fold) HIV-1 clades A, EA, C and three clade B patient isolates. Importantly, 1.0% L-LA retained maximum HIV virucidal activity even in the presence of 75%SP (final pH 5.0). Sodium lactate (1%) was not virucidal.

**CONCLUSIONS:** LA inactivation of HIV, as for HSV, demonstrates chiral dependence, suggesting a viral protein target, and is rapid, irreversible and more potent than acid alone. LA and not the lactate anion is virucidal. LA's HIV virucidal activity in the presence of SP and its ability to inactivate a broad-spectrum of HIV strains (CCR5, CXCR4 and dual-tropic) is significant for its future development as a microbicide that directly blocks male to female transmission of HIV.

# 355

### Functional Characteristics of MucoCept *Lactobacillus* Strains upon their Adherence to Human Cervical and Vaginal Epithelial Cells

R. Fichorova<sup>1</sup>, H. Yamamoto<sup>\*1</sup>, V. Tang<sup>1</sup>, H. Yuan<sup>1</sup>, Y. Liu<sup>2</sup>, L. Jia<sup>2</sup>, X. Liu<sup>2</sup>, Q. Xu<sup>2</sup>

<sup>1</sup>Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; <sup>2</sup>Osel, Inc, Santa Clara, CA, USA

**BACKGROUND:** The development of an HIV vaccine has proven to be a struggle due to the high mutation rate of the virus and difficulties in inducing protective mucosal responses. The development of a topical microbicide is investigated as an alternative method in prevention of HIV infection. A promising candidate is MucoCept: a *Lactobacillus jensenii* bacterium naturally occurring in the human vaginal microflora, genetically engineered to express an anti-HIV protein cyanovirin-N (CV-N). We applied a *L. jensenii*-cervicovaginal adherence model to define functional characteristics with relevance to microbicide safety and effectiveness.

**METHODS:** *Lactobacillus* strains engineered to produce CV-N, β-glucuronidase, or green fluorescent protein with confirmed purity were tested in parallel with wild type *L. jensenii*. In three independent experiments, multiple batches of all strains were adhered to immortalized and primary cervical and vaginal epithelial cells for 24 hrs. Adherence efficiency was measured by colony-forming units of lactobacilli attached to epithelial cells. Inflammatory, immunoregulatory and apoptotic markers of cervical and vaginal epithelial cells were measured by multiplex protein and mRNA technologies. NF-κB and AP-1 activation were assessed by a Luciferase reporter assay. CN-V produced in co-culture supernatants was measured by Western blot and its anti-HIV activity was confirmed by a gp120 binding assay.

**RESULTS:** The adherence of *Lactobacillus* to human cervical and vaginal epithelial cells was comparable between wild type and bioengineered strains, and between bacterial batches. TLR-mediated response of cytokines, chemokines and inflammatory mediators induced by *L. jensenii* was weak and not significant, with only moderate difference between strains. Only NF-κB showed activation by *L. jensenii* with modest difference between strains. No significant increase in apoptotic markers was observed in epithelial cells adhered with *L. jensenii*, compared to no bacteria (negative control). Similar levels of full length CV-N expression were observed among the CV-N expressing strains. In addition, the CV-N expressed in the context of epithelial adherence retained anti-HIV properties.

**CONCLUSIONS:** These results suggest the MucoCept strains reproducibly adhere to cervical and vaginal epithelial cells without induction of immunotoxicity or significant upregulation of proinflammatory responses. These findings will be further tested in women.

**356****Microbicide Testing for Effects on *Lactobacillus* Species: Appropriate Methodology**B.J. Moncla<sup>\*1,2</sup>, K. Pryke<sup>2</sup>, L.C., Rohan<sup>1,2</sup><sup>1</sup>University of Pittsburgh, Pittsburgh, PA, USA;<sup>2</sup>Magee-Womens Research Institute, Pittsburgh, PA, USA

**BACKGROUND:** Microbicides should prevent infection by HIV without disturbing the protective normal vaginal microflora. Prior to animal and human clinical trials microbicides must be proven safe for the vaginal microbiota. In the past, microbicides were evaluated using the Minimum Cidal Concentration (MCC) assay that detects decreases in microbial viability  $\geq 10^4$  colony forming units/ml (CFUs). Using the MCC assay on gel and film based microbicides is problematic since product viscosity requires considerable dilution before testing; resulting in concentrations far below that expected during use. Thus, MCCs underestimate deleterious effects, undermine safety testing against *Lactobacillus* and may misrepresent the efficacy of the drug. We developed a new product and excipient testing method for use with *Lactobacillus* species.

**METHODS:** Octylglycerol (OG), a well characterized microbicide was used in Polyvinyl alcohol based films to develop a new method. Placebo films and methylcellulose based gels were also used. *Lactobacillus* species were suspended in to a density of 1–2 McFarland units in saline and CFUs determined. The “*Lactobacillus* safety test (LST)” was performed as follows: Test materials (0.5 gm) in a 50 ml conical tube was dissolved in 0.5 ml of PBS, 1.0 ml of bacterial suspensions added and mixed under various conditions. After 30 min at 37°C the CFUs were enumerated by dilution and plate count. The different parameters involved in the LST were evaluated. *Bacillus atrophaeus* spore suspensions were also used.

**RESULTS:** The LST gave an MCC of 0.1% for OG, the reported value, but gave precise values for the changing of CFUs over a 5-log range. *L. jensenii* strains were more sensitive to killing by vortex mixing than others, killing of all species was time dependant. Killing was observed in conical but not round bottom tubes; mixing times and protective agents were important parameters of the assay. Killing of test bacterial less than 1 log<sub>10</sub> can usually be considered background.

**CONCLUSIONS:** The *Lactobacillus* safety test gave results equivalent to the MCC test but was not confounded by viscosity and it was more sensitive. Certain species were more sensitive to killing to vortex mixing than others. Mixing times, protective agents and tube shape were important parameters for viability. Killing of *Lactobacillus* and *B. atrophaeus* spores was time dependant.

**357****Toward an RNAi-based Microbicide: CD4 Aptamer-siRNA Chimeras Inhibit HIV-1 Infection in Primary Cells and in Polarized Human Cervical Explants**

Lee Wheeler\*

Harvard Medical School

**BACKGROUND:** The therapeutic use of small interfering RNAs (siRNA) to prevent or treat HIV infection requires an effective means for *in vivo* delivery into susceptible target cells. Aptamers, small structured nucleic acid sequences that bind with high specificity to individual proteins, provide an attractive approach for cell-specific targeting.

**METHODS:** We designed a chimeric RNA, which was transcribed *in vitro* and complexed with the complementary 21-nucleotide active siRNA strand. We hypothesized that the partially-duplexed RNA would be selectively internalized into CD4+ cells, and subsequently processed into an active siRNA capable of silencing target genes.

**RESULTS:** Internalization of Cy3-labeled aptamer-siRNA chimeras was first demonstrated by fluorescence microscopy and flow cytometry. Delivery was restricted to those cells expressing CD4. Functional silencing was verified by showing robust knockdown of host genes (e.g. CCR5, CD45 & lamin), at both the protein and mRNA level. To investigate whether this system could be used to suppress HIV infection, CD4-chimeras were designed to encode siRNAs targeting the viral genes gag, vif and the HIV co-receptor, CCR5. These inhibited HIV infection in both macrophages and CD4+ T-cells by 60-90%. Efficient inhibition of HIV replication was also demonstrated in polarized human cervical explants. Importantly, chimera treatment was non-cytotoxic to tissues and did not induce an interferon-mediated inflammatory response.

**CONCLUSIONS:** This data suggests that aptamer-siRNA chimeras could be an effective, cell-type specific therapeutic gene silencing approach to topically prevent sexual transmission of HIV, providing the framework for developing an HIV microbicide using RNA-interference that is economical, easily produced, and amenable to use in resource-poor settings.

## ORAL SESSIONS

**358**
**5'-O-Fatty Acyl Ester Derivatives of 3'-Fluoro-2',3'-dideoxythymidine (FLT) as Microbicidal Agents**
K. Parang<sup>\*1</sup>, H. Agarwal<sup>1</sup>, G. Doncel<sup>2</sup><sup>1</sup>*University of Rhode Island, Kingston, RI, USA;*  
<sup>2</sup>*CONRAD, Eastern Virginia Medical School, Norfolk, VA, USA*

**BACKGROUND:** Alovudine (3'-fluoro-2',3'-dideoxythymidine, FLT) is a thymidine nucleoside analog and a potent human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) inhibitor. HIV-1 replication can also be inhibited by heteroatom-containing analogs of myristic acid, targeting myristylation of several proteins in HIV life cycle (e.g., capsid protein p17, Pr160<sup>gag-pol</sup>, Pr55<sup>gag</sup>, p27<sup>nef</sup>). We synthesized and tested dual-substrate FLT-myristic acid analogs with enhanced cellular uptake and anti-HIV potency.

**METHODS:** A number of bifunctional 5'-O-fatty acyl derivatives of FLT were synthesized. The compounds were evaluated for anti-HIV activity against cell-free and cell-associated and multidrug resistant virus, and vaginal cell cytotoxicity. Selected FLT derivatives were further studied to determine their cellular uptake and physicochemical properties (e.g., solubility, Log P, pKa).

**RESULTS:** Among these compounds, the 5'-O-(12-thioethyldodecanoyl) derivative of FLT (KP-17) was found to be one of the most potent and least cytotoxic analogs, displaying EC<sub>50</sub> = 0.1–1.1 ng/mL against X4 and R5 cell-free and cell-associated HIV in a single-round infection assay. KP-17 was significantly more active than FLT and the physical mixture of FLT and the fatty acid against cell-associated virus. FLT derivatives showed no signs of cytotoxicity against HeLa cells, peripheral blood mononuclear cells and human vaginal cells. KP-17 was further evaluated against clinical isolates, displaying similar high potency against wild-type and multidrug-resistant (MDR) isolates. KP-17 showed activity against B and C clades with an IC<sub>50</sub> around 0.5 ng/mL. Long-chain FLT analogs showed >12 times higher cellular uptake profile than the same analog with short chain. The pKa of the 12-azidododecanoyl derivative of FLT was 9.67 ± 0.02 and its aqueous solubility, 510 nM.

**CONCLUSIONS:** Several FLT ester conjugates were found to have better anti-HIV activity profile than FLT and other RT inhibitors such as tenofovir and emtricitabine. The higher activity of these compounds is possibly due to their increased uptake and intracellular hydrolysis yielding two antiviral agents, FLT and fatty acid analog, which inhibit different HIV targets.

**359**
**Intravaginal Gene Silencing Using Biodegradable Polymer Nanoparticles Densely Loaded with Small-Interfering RNA**
K.A. Woodrow<sup>\*1</sup>, Y. Cu<sup>2</sup>, C.J. Booth<sup>3</sup>, J. Saucier-Sawyer<sup>2</sup>, M.J. Wood<sup>2</sup>, W.M. Saltzman<sup>2</sup><sup>1</sup>*Department of Bioengineering, University of Washington, Seattle, WA, USA;*  
<sup>2</sup>*Department of Biomedical Engineering, Yale University, New Haven, CT, USA;*  
<sup>3</sup>*Section of Comparative Medicine, Yale University, New Haven, CT, USA*

**BACKGROUND:** Delivery of small-interfering RNA (siRNA) targeting specific viral and bacterial pathogens offer new opportunities for microbicide development. Liposomal delivery of siRNA has been shown to suppress HIV-1 infection and protect against HSV-2 challenge in murine models. However, concerns about the safety and stability of siRNA lipoplexes may preclude their use as microbicides. We describe an approach to formulate and deliver siRNA nanoparticles to the vaginal mucosa *in vivo* using FDA-approved materials. We hypothesized that polymeric nanoparticles, applied topically in a single dose to the vaginal mucosa, would be retained and penetrate deep within the tissue thereby providing sustained-release of siRNA and reduced gene expression.

**METHODS:** SiRNA precomplexed with polyamines were entrapped into poly (lactide-co-glycolide) (PLGA) nanoparticles using a double-emulsion solvent evaporation technique. Dynamic light scattering (DLS) and electron microscopy were used to characterize the particles size, morphology, and surface charge. The physicochemical properties of the encapsulated siRNA were evaluated by quantifying with Quant-iT™ PicoGreen and gel electrophoresis. Gene silencing was evaluated *in vitro* using cell culture and *in vivo* using transgenic GFP mice. Gene expression was measured by qRT-PCR, quantitative immunoblots, and image analysis of tissue sections. Inflammation and pathology were evaluated from haematoxylin-eosin and anti-CD45 stained sections of reproductive tracts. The distribution and retention of nanoparticles in the vaginal tissue were analyzed using an IVIS 200® imaging system and multiphoton microscopy.

**RESULTS:** Over 1,000 molecules of siRNA were entrapped per polymer nanoparticles of <200 nm diameter. Nanoparticles were observed to penetrate deep into the epithelial tissue (75–120 nm) and be retained within the tissue (7 d) after only a single topical application to the vaginal mucosa of transgenic GFP mice. The siRNA nanoparticles produced significant levels of gene knockdown (>50%) and were less irritating and inflammatory compared to delivery of siRNA lipoplexes. The siRNA nanoparticles reduced GFP expression proximally (in the vaginal lumen) and distally (in the uterine horns) to the site of delivery, and gene silencing was sustained for at least 14 d.

**CONCLUSIONS:** This is the first report demonstrating that polymeric nanoparticles provide a safe and effective method to deliver siRNA to the vaginal mucosa and leads to efficient and sustained gene silencing.

## SESSION 41

### Mini-Symposia (MS8): Mice, Monkeys, and Humans

**Moderators:** Ron Veazey, Natalia Teleshova

Tuesday, May 25, 11:30am–1:00pm

Rooms 301–304

## 360

### Comparing Mice and Monkeys

Paul Denton

*Center for Infectious Diseases, University of North Carolina School of Medicine, USA*

Transitioning candidate microbicides from concept to the clinic requires animal studies. Ideally, once potential topical inhibitors are identified and shown effective *in vitro*, safety and efficacy studies in animals then funnel only the most promising candidates towards human clinical trials. The ability to screen microbicide candidates *in vivo* for their efficacy to prevent HIV transmission has the potential to guide these efforts. Unfortunately, to date no animal model has been shown to be predictive of the human condition. Non-human primates have been extensively used for the *in vivo* testing of microbicides. More recently, humanized mice have been shown to have several desirable qualities that indicate their potential as a yet another system for the *in vivo* evaluation of microbicides capable of preventing HIV transmission. Evaluation of six different candidate microbicides in humanized BLT mice indicate that this model can effectively stratify them between highly efficient to completely ineffective to prevent vaginal HIV-1 transmission. Three of the six inhibitors utilized have also been tested in non-human primates (TC247, C52L, and FTC/TDF). This represents the first opportunity to compare results from primate studies to those from the BLT model. The fact that comparable results were obtained with both systems is highly encouraging regarding their potential efficacy in humans. In addition, these results serve to corroborate that efficacy results obtained with SIV in macaques might be representative of what could be expected with HIV-1 infecting human cells as modeled in BLT mice.

## 361

### Using Human Explants Models to Predict Safety and Efficacy

Charlene Dezzutti

*Magee-Womens Research Institute, University of Pittsburgh, USA*

Microbicides are at the fore-front of the HIV prevention efforts. The recent experience with several trials where potential toxicity and/or lack of efficacy was identified in the late phase of clinical development show the importance of extensive pre-clinical evaluation for product safety and efficacy. *Ex vivo* organotypic cultures such as ectocervical and colorectal explant models offer an essential bridge between *in vitro* culture systems and clinical studies by providing a controlled environment where microbicides can be comparatively evaluated for 1) toxicity against the mucosal epithelium and 2) anti-HIV-1 activity in target cells present within the submucosa of human tissue. We will discuss how explant models are used and their limitations. The use of the *ex vivo* organotypic models along with other sensitive *in vitro* measurements can help provide a comprehensive evaluation of new microbicides and formulations that may be predictive of clinical success.

## 362

### Bridging Preclinical Observations to Phase 1 Clinical Trials

Peter Anton

*David Geffen School of Medicine at UCLA, USA*

Expediting microboidal drug development is essential. As Phase II/III trials are expensive in terms of human, financial and physical resources, increased attention is focusing on extracting as much information as possible from pre-clinical and Phase 1 trials. While safety is the keystone of all human trials, especially early/Phase 1 types, intensification of these early trials with detailed secondary and/or ancillary endpoints can provide a wealth of data from a variety of research angles (acceptability, pharmacokinetics and pharmacodynamics, mucosal immunoreactivity, *ex vivo* infectivity, etc) to guide later trials. Inherent in most Phase 1 trials is the safety evaluation of a new drug, often in a new formulation, requiring an FDA-approved IND. While the “pipeline” metaphor is useful, the working model is more akin to multiple, parallel highways merging nearly at the same time into a large toll road. The success in these endeavors rests as much on the excellence of each highway’s record as it does on the oversight committee coordinating that merging, in a timely manner.

In practical terms, these efforts require alignment of research laboratory expertise (assays/questionnaires), the formulation and drug development (study product), the scientific rationale and development of the concept/protocol (trial design) with close, flexible, seasoned regulatory expertise (for IND, IRB etc). The latter is likely the most critical factor in moving forward smoothly and quickly. Certain requirements are immutable and must be anticipated, practically, and intercalated into the timeline. Examples of bridging efforts toward two Phase 1 microbicide trials will be presented.

# ORAL SESSIONS

## **SESSION 42**

**Session Cancelled**

## SESSION 43

### Oral Abstracts (OA22): HIV Seroincidence—Finding the Communities at Risk

**Moderators:** Connie Celum, Lut van Damme

Tuesday, May 25, 11:30am–1:00pm

Rooms 403–405

## 364

### HIV Seroconversion Patterns in MDP301

U. Jentsch<sup>1</sup>, P. Lunga<sup>2</sup>, C. Lacey<sup>3</sup>, J. Weber<sup>4</sup>, J. Cairns<sup>4</sup>, G. Pinheiro<sup>1</sup>, S. Joseph<sup>2</sup>, W. Stevens<sup>1</sup>, S. McCormack<sup>2</sup>

<sup>1</sup>School of Pathology University of the Witwatersrand, Johannesburg, South Africa; <sup>2</sup>MRC Clinical Trials Unit, London, UK; <sup>3</sup>University of York, York, UK; <sup>4</sup>Imperial College School of Medicine, London, UK

**BACKGROUND:** We describe HIV seroconversion during MDP301: a clinical trial carried out in 9385 women at 6 centres in 4 Sub-Saharan African countries between October 2005 and August 2009. MDP 301 was a phase III, multi-centre, randomized, double-blind, placebo-controlled trial evaluating the safety and efficacy of 0.5% and 2% PRO 2000/5 microbicides.

**METHODS:** HIV seroconversion was identified at the clinics on the basis of a double negative HIV Rapid test result and at least one positive Rapid test after enrolment. Such a result triggered the shipment to and testing of archived sets of serum and Buffy Coat samples according to the "MDP HIV Testing Algorithm" at a central facility in South Africa. The algorithm utilized the Abbott AxSYM HIV Antigen-Antibody (Ag-Ab) and BioRad Genetic Systems HIV-1 ELISAs in a parallel testing strategy. Additionally, Western Blot, P24, HIV DNA and RNA PCR testing was done.

**RESULTS:** 543 of 9385 (6%) gave a positive HIV Rapid test. 419 (77%) of these were confirmed to be HIV seroconversions using the algorithm. 63 (12%) were identified to be positive at screening/enrolment and 61(11%) were confirmed HIV negative. Using the archived samples, the algorithm detected 60 (14%) seroconversions at an earlier visit, compared to the first positive HIV Rapid result. In 30 cases this was because of its ability to identify acute infections. When including the 63 participants that were positive at or before enrolment, 54 had no detectable HIV antibodies (BioRad ELISA negative). However, 44 of these had a positive Ag-Ab ELISA and a confirmatory positive p24 or PCR test, indicating acute infection.

In 4 cases, there was serological evidence of seroconversion with a negative HIV PCR.

**CONCLUSIONS:** Our results demonstrate the HIV testing algorithm to be user-friendly, accurate and able to detect acute HIV. In clinical trials conducted in Africa, such as MDP301, where the primary endpoint is HIV infection, these properties are critical to exclude HIV infection before or at enrolment and to detect true HIV seroconverters in the follow-up period.

## 365

### Cross-Sectional Estimation of HIV Incidence by BED-CEIA and Avidity Index Assays in Vaginal Microbicide Preparedness Studies in Rwanda and South Africa

J. van de Wijgert<sup>1,2</sup>, S. Braunstein<sup>3</sup>, J. Vyankandondera<sup>2,4</sup>, C. Louv<sup>5</sup>, E. Hellstrom<sup>6</sup>, J. Hugo<sup>5</sup>, M. Kamupira<sup>7</sup>, A. Nel<sup>7</sup>

<sup>1</sup>Academic Medical Center, Center for Poverty-related Communicable Diseases, Amsterdam, The Netherlands; <sup>2</sup>Projet Ubuzima, Kigali, Rwanda; <sup>3</sup>Columbia University, New York, USA; <sup>4</sup>Belgian Technical Cooperation, Kigali, Rwanda; <sup>5</sup>Madibeng Centre for Research, Brits, South Africa; <sup>6</sup>Be Part, Mbekweni, Paarl, South Africa; <sup>7</sup>International Partnership for Microbicides, Paarl, South Africa

**BACKGROUND:** Estimation of HIV incidence rate (IR) in cross-sectional surveys via a serological testing algorithm for recent HIV seroconversion (STARHS) may be beneficial for microbicide research. Two serological tests used for this purpose, BED-CEIA (BED) and Avidity Index (AI), are not well described in sub-Saharan Africa.

**METHODS:** Female sex workers in Kigali, Rwanda, and sexually active women at two research centers in South Africa (Madibeng and Mbekweni), were HIV tested in 3 cross-sectional surveys with 800 women each. HIV-positive women were tested by BED and AI. In Kigali, CD4 counts and assay false-recent rates (FRR) were also determined. Subsets of HIV-negative women (400 in Rwanda and 300 at each center in South Africa) were enrolled in prospective HIV seroconversion studies. BED and AI-based IR were estimated using published formulas with adjustments for FRR (all sites) and CD4 count (<500 cells/ $\mu$ L excluded, Kigali only). Cross-sectional IR were compared with longitudinal seroconversion rates. BED and AI sensitivity and specificity were calculated using serial specimens from seroconverters in Kigali.

**RESULTS:** In Kigali, Madibeng, and Mbekweni, baseline HIV prevalences were 24%, 24% and 22%, and seroconversion-based IR (95% CI) were 3.5 (1.6, 5.4), 6.0 (3.0, 9.0), and 4.5 (1.8, 7.1) per 100 person-years, respectively. Adjusted BED-based IR (95%) were 7.2 (4.0, 10.3), 7.4 (4.7, 10.1), 6.1 (3.7, 8.5); AI-based IR (95% CI) were 8.2 (5.1, 11.4), 9.1 (6.1, 12.9), 4.1 (1.9, 6.3); and combined BED/AI IR (95% CI) were 5.1 (2.8, 7.5), 6.0 (3.1, 8.9), 2.2 (0.4, 4.0) for the 3 centers, respectively. BED and AI-based IR were generally higher than prospective IR, but all 95% CI overlapped, and rates were similar in the first 6 months of cohort follow-up, when HIV incidence was highest. Sensitivity and specificity based on serial specimens from 19 seroconverters were 100% (88–100%) and 43% (27–61%) for BED; 92% (74–98%) and 43% (27–61%) for AI; and 91% (72–97%) and 55% (33–67%) for BED/AI combined. Assay specificity improved markedly with time from seroconversion. Assay FRR, representing longer-term specificity, were 6.4% for BED, 16.3% for AI, and 2.8% for BED/AI combined in Kigali.

**CONCLUSIONS:** Current BED and AI assays cannot replace longitudinal studies when precise HIV IR are required, but could be used to identify risk groups and monitor trends over time. Work is ongoing to improve assays and algorithms for assessing recent HIV infections cross-sectionally.

# ORAL SESSIONS

## 366

### Time to Think Locally: Investigating the "Hot Spots" for HIV Infection in Communities of Durban, South Africa Which Were Targeted for Microbicide Studies

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**BACKGROUND:** Currently, the province of KwaZulu-Natal is at the most advanced stage of the HIV/AIDS epidemic. Identifying what causes HIV to explode in this region is likely to be complicated. However, it is now known that the severity of the epidemic varies by geographic location in this region. Therefore, it is important to determine the "hot" spots in this region.

**METHODS:** The aim of this study was to characterize the geographical variation of HIV infection in 158 sub-censuses in Durban, South Africa. Geographical data from a cohort of women who were recruited for various microbicide site preparation and feasibility studies were used to determine the areas with high HIV prevalence and incidence by using geostatistical methodologies.

**RESULTS:** This study identified three significant clusters (one primary and two secondary) of high HIV prevalence in small rural communities of Durban. Among women who tested HIV positive at screening, 458 were geographically clustered. Of these 146 (32%) were determined to be centered within a 4.5 km radius and exclusively from west of Durban. This study also identified areas of high HIV incidence which were broadly consistent with high prevalence areas.

**CONCLUSIONS:** Geographic excesses of HIV infections were detected in rural communities of Durban where the women were exclusively targeted for microbicide studies. Results reinforce the inference that risks for HIV infection are associated with definable geographical areas. The regions identified are critical in controlling the HIV epidemic and may provide important direction for future interventions.

## 367

### Comparison of Partner Testing with Home-based HIV Counseling and Testing (HBHCT) for Recruitment of Young Sexually Active HIV Sero-discordant Couples into an HIV Prevention Trial: A Case Study from Mbale, Uganda

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**BACKGROUND:** Recruitment of HIV sero-discordant couples into HIV prevention clinical trials is challenging, as only 10–30% of persons in Africa who are tested for HIV do so as a couple. A high priority for HIV prevention strategies, including antiretroviral pre-exposure prophylaxis (PrEP) which is being tested for efficacy, is targeting young HIV serodiscordant couples. We compared the age of the HIV-negative partner among serodiscordant couples identified through testing of partners of known HIV positive persons ('partner testing') to home-based HIV counseling and testing (HBHCT) in north eastern Uganda.

**METHODS:** The Partners PrEP Study is a phase III randomized trial of PrEP for HIV prevention in HIV discordant couples. HBHCT was carried out in Kumi and Bukedea districts adjacent to Mbale between January 2006 and September 2008 where over 120,000 individuals were tested and over 600 couples were found to be serodiscordant; these serodiscordant couples were later referred to Mbale for trial participation. The second highest yield recruitment strategy was partner testing, primarily by testing partners of unknown serostatus whose sexual partners were TASO HIV positive clients. Age of the HIV-negative partner among the 336 couples identified by partner testing and HBHCT strategies by recruitment strategy was compared.

**RESULTS:** 111 couples were identified by partner testing and 225 couples by HBHCT in Mbale; the mean age of the HIV-negative partners was 36 years (s.d. 7) and 37 years (s.d. 8) for partner testing and HBHCT respectively as recruitment strategies. Other strategies that contributed a smaller number of participants included referrals from the TASO HIV care center (N=36, Mean age =39, s.d=8) and from collaborating VCT centres (N=30, Mean age=36, s.d=7).

**CONCLUSIONS:** The study showed that testing partners of HIV-infected persons (partner testing) recruited a significant number of new HIV serodiscordant couples in Mbale, Uganda into the Partners PrEP study, but they were of almost similar age to participants recruited by other strategies. Multiple, innovative strategies are needed to recruit HIV serodiscordant couples, particularly for recruitment of younger couples.

## 368

### Who Screens for HIV Prevention Research and How Do They Compare with a Representative Community Sample? Implications for Microbicide Trials in South Africa

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of Psychology, University of KwaZulu Natal, South  
Africa

**BACKGROUND:** Selection into clinical trials raises issues of generalizability to the larger population, which is especially an issue in microbicide trials as they target women at high risk of HIV, who must avoid pregnancy and incorporate a new method into their sex life while participating. We compared HIV risk profiles and method use between a representative community sample of Rustenburg, and persons from the same South African community recruited to our research centre to screen for a cohort study of HIV incidence.

**METHODS:** Trained interviewers administered two cross-sectional surveys using the same questions to a representative community sample (n=351), and clinic attendees (n=1815). For the representative survey we used census data to determine the sampling frame. We performed random sampling of neighbourhood, stand, and within a household, person. The clinic survey was administered to all recruited to our research centre whom we expected were at high risk for HIV. To adjust for skewed racial and age distributions in the two samples, analysis was limited to 18–35 year old Black participants. Logistic regression models, adjusting for age, gender, education and employment, compared the clinic and community samples on characteristics in the 3 months prior to interview.

**RESULTS:** Compared with community, clinic attendees were more likely to: have multiple sex partners AOR (95% CI) 1.70(1.10–2.63); have new sex partners 1.97(1.30–2.99) and; be using coitus interruptus 3.97(2.11–7.50), but were no more likely to have STI symptoms 1.41(0.89–2.24). There was no difference in condom use 0.81(0.58–1.12), but clinic attendees felt less able to convince their partners to use them 0.42(0.31–0.59) and were more fatalistic about HIV 2.24(1.23–4.08). Clinic attendees were less likely to be on injectable contraception 0.64(0.46–0.90).

**CONCLUSIONS:** This is the only study of which we are aware, to examine generalizability of a research sample, an issue the prevention field will face if an effective microbicide is found. Clinic recruits had a riskier behavior profile yet were no more likely to be using condoms, and exhibited little evidence that they could initiate their use, which bodes poorly for microbicide uptake. Further, clinic recruits were at greater pregnancy risk underscoring the challenge microbicide trialists face in testing products in high risk populations. However, if efficacy can be shown in such samples, community-wide uptake of microbicides may be wider spread.

# POSTER SESSIONS

SESSION 44  
Tuesday, May 25, 2010

## SESSION 44

### Poster Discussion (PD9): Standards of Care in Prevention Trials

**Moderators:** Mitzy Gafos, Jeff Spieler

Tuesday, May 25, 3:00 pm–4:00 pm

Rooms 317–318

**370**

#### How Much Is Enough?: Standards of Prevention in HIV Prevention Trials

S. Philpott<sup>1,5</sup>, E. McGrory<sup>\*2</sup>, C. Hankins<sup>3</sup>, L. Paxton<sup>4</sup>, L. Heise<sup>1,6</sup>, the Participants in the 2009 GCM/CDC/UNAIDS Consultation on Standards of Prevention in HIV Prevention Trials

<sup>1</sup>Global Campaign for Microbicides (GCM), PATH, Washington, DC, USA; <sup>2</sup>Consultant to GCM, Nyack, NY, USA; <sup>3</sup>Joint United Nations Programme on HIV/AIDS (UNAIDS), Geneva, Switzerland; <sup>4</sup>United States Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA; <sup>5</sup>Union Graduate College-Mt. Sinai School of Medicine Bioethics Program, Schenectady, NY; <sup>6</sup>Current address: Gender Violence & Health Centre, London School of Hygiene and Tropical Medicine, London, UK

**BACKGROUND:** As effective new HIV prevention tools are developed, researchers face ethical and logistical questions about how and when to include them in the standard prevention package of ongoing and future HIV prevention trials. Current UNAIDS/WHO guidance recommends that decisions about adding new tools to the prevention package be made in consultation with “all relevant stakeholders” but leaves open questions of process and implementation. In March 2009, GCM, UNAIDS, and CDC convened an international consultation in Uganda to develop practical approaches to these questions.

**METHODS:** Through presentations, participatory exercises and debate, 59 diverse participants (including researchers, ethicists, advocates and policymakers) worked to reach agreement on key criteria to guide decision-making on whether and when to include new HIV prevention tools in future trials, and whether ongoing trials should be stopped or modified when another trial shows a new method to be effective.

**RESULTS:** Participants developed a set of specific criteria to inform decision-making, including:

- whether the method has been recommended by international bodies or adopted at a national level
- size of the effect and the weight of evidence
- relevance to the trial population
- whether the tool has been approved or introduced in the trial country
- feasibility
- whether adding the tool to a current trial would lead to futility of trial continuation
- the extent to which adding it might compromise ability to evaluate future methods
- outstanding safety issues
- status of the trial

Researchers and sponsors must clearly justify and communicate to all stakeholders any decision not to include a new method in a prevention package. While no consensus emerged regarding how researchers should balance their opportunity and responsibility to provide state of the art prevention and care and concerns about providing a method that cannot be sustained in that setting after the trial, the consultation made significant progress in developing practical criteria to help researchers, sponsors and other stakeholders address some critical trial issues facing the HIV prevention field.

**CONCLUSIONS:** It will be important to widen the discussion and continue to adapt the criteria and points of agreement with different stakeholders and as new data emerge. Additional work is also needed to develop, implement, and evaluate approaches to ensuring stakeholder participation in decision making.

**386**

#### Common Decimals on Community Perspectives on the Ethical Conduct of Clinical Trials: A Review of the Oral Tenovofir Controversy

M. Ukpong, K. Peterson, O. Akanni  
New HIV Vaccine and Microbicide Advocacy Society

**BACKGROUND:** There has been lots of effort to promote community engagement in biomedical HIV research over the last decade. As such, there is an increasing recognition of the benefits of engaging the communities as research evolve far beyond the sole purpose of preventing trial disruption. Despite this growing interest and support for communities to engage in biomedical HIV research, the pace has remained slow. This paper shall review the events that resulted in community agitations and controversies around some oral tenofovir pre-prophylaxis studies in the past. It will share insights into the multiple community interest and concerns about the trial across several countries.

**METHODS:** A review of anecdotal reports of key players involved with raising concerns around the planned oral tenofovir trials in Cameroon, Cambodia, Thailand and Nigeria. A review of the literature on the subject matter. Concerns that were common to all the countries are highlighted.

**RESULTS:** Communities were primarily interested in reviewing the trial oral tenofovir research protocols in order to ensure that trial participants' health related issues were addressed. Health related concerns include issues of standard of care and the standard of prevention. In addition, despite the fact that the research team conducted community consultations in the form of FGDs in these countries, these did not translate to community consultation from the perspectives of advocates. Advocates will rather have a consultative process that focused on facilitating community inputs into trial design (their main desire) rather than consultations which primarily aimed at achieving support for trial implementation. The conduct of a phase II trial with no Phase I data was also a source of concern. The use of the word ‘unethical’ did take on a different meaning for the community; different from the working definition by the research team.

**CONCLUSIONS:** It appears that health concerns are of paramount interest to communities when it comes to trial design and implementation. The communities also have mechanisms for consultation on issues which facilitates self education. When the rule and norm of science needs to change, extensive community discussions and consultations may help prior to its utilization in research design as communities equally keep themselves updated on these issues.

# POSTER SESSIONS

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### Addressing Coercion in the Conduct of Clinical Trials on Microbicides

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**BACKGROUND:** There has been a lot of ethical debate around the concept of undue incentives and coercion in the field of microbicide research. Many consider the probability of compensation acting as undue inducement for enrolment in such clinical studies while a few differ on the subject matter. However, there have been few discussions on other possible forms of subtle coercion in the field of clinical trial practice in Africa. This abstract reviews some of these forms of ethically acceptable subtle coercions and argues for the consideration of compensation of research participants' time and effort expended in microbicide research as not being considered as undue inducement.

**METHODS:** A review of the literature in the field on the subject matter. Reflection of events on microbicide clinical trials on the field in Africa

**RESULTS:** A few articles in the literature notes that when there is emphasis on ensuring risk minimization in trials, compensation should no longer be perceived as an undue inducement for risk taking. In the conduct of microbicide clinical trials in Africa, forms of subtle coercion do exist in the field. This include the provision of high standards of health care within trials that can otherwise not be obtained outside the trial setting; provision of gifts and services as a form of compensation for retention in trials. Also, the pressure for meeting recruitment and retention targets on time also makes the use of some subtle coercion to ensure that trial participants continue to stay on in the trial.

**CONCLUSIONS:** 'Ethically acceptable' subtle coercion goes on in the conduct of microbicide clinical trials in Africa. There is however the need to address disparity in access to health services as a form of coercion in microbicide trials. There is also the need to re-evaluate the current ethical definition of compensation of trial participants' time and effort invested in the research process as undue incentive as such compensation are economic empowerment tools rather than undue inducement in trials where risk is considerably minimized. Finally, it would be advocated that compensation should be not be viewed as undue inducements as IRBs are saddled with the responsibility of minimizing risks in such trials more so that true voluntarism is the guiding philosophy behind trial compensation in clinical research.

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### Standards-of-Care in Microbicide Trials: Do Research Participants have Special Entitlements?

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**BACKGROUND:** Whether or not researchers are obligated to provide participants in HIV prevention trials with antiretrovirals (ARV) has been the subject of intense international debate for more than a decade. The current consensus of the universal treatment access movement is that, where possible, participants in efficacy trials should be provided with ARV, but the ethical basis of this obligation is contested. It is not clear, for example, whether participants have a special entitlement to receive scarce goods due to their status as research participants or as part of a community-wide benefit predicated on a universal right to health. This study maps trends in the provision of ARV in HIV prevention studies and examines the moral justification for current practice.

**METHODS:**

- Systematic review of standard-of-care and ancillary care provisions in microbicide trials from 2000 – 2009, through analysis of trial protocols, research publications and related 'grey' literature describing the benefits of trial participation to participants.
- Qualitative analysis of statements made by researchers about their decisions regarding provision of ARV to trial participants

**RESULTS:** Eight microbicide studies (phases IIb and III) were included in the review. Access to ancillary care such as antiretrovirals for participants who seroconvert on microbicide trials has changed markedly since 2000, with a shift from no provision of ARV to full access (through linkages; not necessarily provided by sponsor). Reported provision of free condoms and contraception varied between trials. There is conflicting evidence as to how and why researchers recognise specific obligations towards research participants, with variation occurring between and even within trials.

**CONCLUSIONS:** Provision of ARV to trial participants is now the norm, but the cost is not born solely by research sponsors, and may be more a function of improvements in the care of people with HIV than any shift in research practice. Research participants do however have exclusive access to some ancillary care in some circumstances. This suggests that conflicts between the putative universal right of all people to life-saving treatment, the particular obligations of researchers to participants and the doctor-patient dynamic force researchers and research communities into high-stakes moral negotiation regarding benefits.

## 380

### Community Monitoring of the Rollout of Male Circumcision for HIV Prevention and its Implications for Women

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<sup>5</sup>Namibia Women's Health Network; <sup>6</sup>Swaziland for Positive Living; <sup>7</sup>Mama's Club; <sup>8</sup>ATHENA Network

**BACKGROUND:** Pending the rollout of male circumcision for HIV prevention, women in affected communities have identified the potential for increased rates of infection from newly circumcised partners who do not abstain prior to wound healing as well as from a possible increase in men's risk behaviors. Women have also expressed concern that spending on women-focused prevention might decrease and that greater stigma and blame could be directed at HIV-positive women. In recognizing the need to monitor male circumcision for HIV prevention and to amplify women's concerns and inform its scale up to ensure that the intervention is beneficial to men and women, AVAC and ATHENA launched the Women's HIV Prevention Tracking Project (WHiPT) in 2009.

**METHODS:** To inform policies and programs related to male circumcision, WHiPT country teams were formed in Kenya, Namibia, South Africa, Swaziland and Uganda out of networks of women living with HIV who work predominantly at the community level. Each team developed a work-plan tailored to their context and trained women in qualitative data collection to capture local women's impressions of male circumcision. WHiPT teams developed a standard interview questionnaire and focus-group template to be adapted to local contexts. Teams met to evaluate data across countries for common and context-specific themes. An advocacy agenda was shaped according to the data and implemented at a global, country and local level.

**RESULTS:** The central outcome of WHiPT is to build the capacity of women stakeholders to understand and engage with HIV prevention research. WHiPT members gained skills in research literacy with a focus on male circumcision, data collection and analysis, grant writing, report writing and advocacy. The women have built linkages between themselves and researchers/policymakers; strengthened their HIV prevention research literacy; and learned how research translates into policies and programs.

**CONCLUSIONS:** The WHiPT project is a thriving mechanism by which community-based women, who often stand outside of research and policy circles, are able to engage. Further, the project is a vehicle by which a particular cohort of HIV affected communities—HIV-positive women and their networks—participate in the AIDS response, specifically in HIV prevention research, implementation and advocacy.

## SESSION 45

### Poster Discussion (PD10): Sexual Behavior and HIV Prevention

**Moderators:** Pamina Gorbach, Patrick Ndase

Tuesday, May 25, 3:00 pm–4:00 pm

Rooms 301–304

**389**

#### A Comparison of Peer-mediated HIV Prevention Interventions Under Different Environments for Female Sex Workers in Kenya

P. Mwangi

*Bar Hostess Empowerment and Support Programme*

**BACKGROUND:** Many salonists (hair dressers and beauticians) double up as part time sex workers in Kenya. Peer-mediated HIV prevention interventions among female sex workers (FSW) have been the cornerstone of most sex worker organizing programs in Kenya. A comparison of different the role of the environment under which the interventions are carried out was evaluated

**METHODS:** A pre-intervention survey in Nov.2007, recruited 342 FSW using snowball sampling. 16 peer educators were trained (8 full time sex workers, 8 salonists/part time sex workers). Thereafter, the peer educators provided STI/HIV education, condoms, and facilitated HIV testing, treatment and care services. In Nov. 2009, data were collected using identical survey methods, allowing comparison with historical controls, and between FSW who had received peer education from salonists, sex workers or had not received peer interventions

**RESULTS:** Over the two years consistent condom use with clients increased from 52.0% (178/342) to 82.4% (285/346) as well as the likelihood of refusing clients who were unwilling to use condoms. In Nov.2009, FSW who received peer interventions from salonists (35.1%, 120/342), had more consistent condom use with clients compared with FSW who received peer education from other sex workers and those unexposed FSW (90.1% versus 87.5%; 58.4%). HIV prevalence was 10.6% (8/85) in FSW attending salon peer education sessions compared with 10.9% (11/101) in those attending sex worker (streets/ bars) peer education, and 18.3% (13/71) for FSW not attending peer education sessions. FSW who attended salon sessions indicated they spent more time with their peer educators compared with FSW who were attended to by other sex workers in the streets/ bars on average (35 versus 20 minutes). They were more comfortable to read IEC materials and collect condoms from the salons than from other sex workers on the streets

**CONCLUSIONS:** Peer-mediated interventions were associated with an increase in protected sex. Though peer-mediated interventions remain important, sex workers feel more comfortable receiving information/ prevention materials in a free and secure environment. Drop in centres in selected salons are an effective intervention in HIV prevention for sex workers

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#### Predictors of Delayed Sexual Debut Among Youth in the Nyanza Province, Kenya

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<sup>1</sup>Center for Microbiology Research, Kenya Medical Research Institute; <sup>2</sup>Population Services International

**BACKGROUND:** Describe the distribution of age at sexual debut by gender and identify predictors of delayed sexual debut (after 16 years of age) among 15- to 24-year-old youth in the general population within Kisumu, Kenya.

**METHODS:** We conducted a cross-sectional study in which every fourth household from 40 clusters throughout Kisumu District was systematically sampled. Everyone aged 15–49 who slept in the house the night before the interview was eligible to participate. Informed Consent was obtained and/or assent and then collected data on socio-economic and demographic attributes, and behavioral and cultural factors. Cox proportional hazards model was used to analyze data.

**RESULTS:** Of the 1655 participants in the study, 910 (55%) were youth aged 15–24 years. Of these youth, 55% were female and 53% had at least a primary level education. The average age of sexual debut was 16 years. The median abstinence time before first sex was the same (18 years) for both males and females. However, after age 18 the proportion of females abstaining from first sex was consistently higher than for males. None of the variables considered were independently associated with delayed sexual debut.

**CONCLUSIONS:** In this population with high risk sexual behavior and STI prevalence, none of the socio-economic and demographic factors considered could independently predict delayed sexual debut. This could be an important finding for programs aimed at delaying sexual debut to focus on all the youth regardless of their socio-economic and demographic attributes.

# POSTER SESSIONS

## 392

### **Sexual Networking in a Nigerian University: Implication for HIV Transmission**

B. Olaiya, O. Olibamoyo  
Obafemi Awolowo University

**BACKGROUND:** Tertiary institutions world over provide a platform for young people to get educated, enhance their personalities and build skills that enable success in their chosen career paths. However, these institutions have also been identified as "hotspots" for the transmission of sexually transmitted infections including HIV/AIDS. This study aims to identify the social and environmental factors involved in sexual networking with a view to informing evidence-based policies and programs targeted at HIV prevention amongst youth.

**METHODS:** The study population employed a two-stage sampling method aimed at selecting eligible people.

Study was cross sectional in design, and involved 480 randomly selected unmarried undergraduates. Data was collected using self-administered questionnaire. Data was analyzed using bivariate and multivariate analysis.

**RESULTS:** Males constituted 51.5%, and 64% of respondents were between 18–24 years. A higher proportion of males (69.6%) were sexually experienced compared to females (57.2%) ( $p=0.006$ ). While the majority of sexually experienced people did not have first sexual experience till late adolescence, 21.8% of males and 8.8% of females had their first sexual experience before the age of 15. The first sexual partner was older than the respondent in 36.1% of sexually experienced males and 94.3% of females. 8.6% of male and 9.4% of female respondents indulged in homosexuality. 60.1% of males and 51.4% of females used condom consistently in the six month prior to the survey. Significantly higher proportion of males were engaged in sexual networking (70.6%) compared to females (47.1%). Binary logistic regression showed only sex as a significant factor for multiple sexual engagement ( $OR=2.98$ ; 95% C.I. = 1.49–5.95). Factors significantly associated with sexual networking included sex ( $p=<0.001$ ) age of first sexual exposure ( $p=<0.001$ ), level of income ( $p=0.03$ ) and off-campus residence ( $p=0.017$ ). Knowledge of HIV/AIDS was not significantly associated with networking.

**CONCLUSIONS:** There is high level of sexual networking among undergraduates in tertiary institutions, and implies that campus environment could be high-risk locations for HIV transmission. Thus, there is an urgent need to intensify HIV behavior change communication programs in campuses.

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### **Unmet Need for Safe Sex Practice and Opportunities for Vaginal Microbicides among HIV-Uninfected Female Sex Workers (FSW) in India**

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National AIDS Research Institute, Pune, India

**BACKGROUND:** Prevention of HIV transmission in core groups such as Female Sex Workers (FSW) is likely to reduce further transmission to bridge populations and the general population. Possible HIV prevention options among FSW include vaginal microbicides. Objective of this paper is to explore the vulnerability and safe sex practices among HIV uninfected FSWs in India.

**METHODS:** Data are drawn from the first round of Integrated Biological and Behavioural Assessment (IBBA) study that aimed at evaluating "Avahan, the India AIDS initiative," a large HIV prevention programme among high risk populations. District-wide probability sampling methods such as conventional cluster sampling or time-location sampling were used to recruit FSWs from 29 of 83 Avahan districts in 4 states of India in 2007. The paper reports on data from over 8000 HIV uninfected FSWs among those surveyed.

**RESULTS:** 60% of the FSWs were illiterate and they were practicing commercial sex work for over 6 years with at least 2 different types of partners. Types of sexual partners included occasional clients, regular paying clients and regular non-paying clients. Consistent condom usage with regular paying and regular non paying partners was 66% and 14% respectively. Anal sex was also reported by 13% and 69% had used condom at the last anal sex. Nearly 20–25% FSWs reported condom breakage or inability to convince clients to use condoms in the previous month. Of the one in three FSWs who had awareness and risk perception about HIV transmission; half had their HIV test done. Symptoms of Sexually Transmitted Infections (STI) were reported by 49% in the last one year and currently 29% were symptomatic. Symptoms of Sexually Transmitted Infections (STI) were reported by 49% in the last one year and 29% were symptomatic at the time of the survey. Of these HIV negative FSWs, 2300 were examined for four STI pathogens. 66% tested positive for at least one; 9% tested positive for syphilis by RPR, 2.7% for Gonococci by GC NAT, 5.1% for Chlamydia trachomatis by CT NAT and 63% for HSV2.

**CONCLUSIONS:** Data clearly highlight huge gaps in safe-sex practices among FSWs in India, predisposing them to HIV and STI. This unmet need for protection can be fulfilled by vaginal microbicides.

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### **Exploring Adolescent Girls' Protection Strategies against HIV in Lusaka, Zambia**

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<sup>1</sup>Population Council, New York, New York;  
<sup>2</sup>Institute of Social and Economic Research, University of Zambia

**BACKGROUND:** For microbicides to deliver on their promise of preventing a significant number of HIV infections, they need to be adopted by young women, among whom incidence rates are higher than men. Yet what do we know about how this target group perceives risk and strategies for protecting themselves? Both before and within marriage, young women are disproportionately infected and affected by HIV/AIDS in Zambia. Prevalence is 5.7% among 15- to 19-year-old females (vs. 3.6% for males), and 11.8 % among 20- to 24-year-old females (vs. 5% for males). Like the adult female population, young female youth are twice as likely to be infected as male youth.

**METHODS:** A cross-sectional survey of 821 young women ages 15–24 was conducted in four urban slums of Lusaka, Zambia to explore their notions of risk and safety, perceptions of HIV risk in particular. The survey sample was randomly selected from four compounds using WHO cluster survey methodology. The survey was supplemented by qualitative data from focus groups and in-depth interviews with a subset of girls in two of the study communities.

**RESULTS:** Overall knowledge levels about HIV/AIDS was high; 86% of respondents correctly knew measures to reduce risk of HIV, 73% knew where to go for HIV testing. Risk perception of HIV/AIDS was high; 38% believed they were at risk, and 33% had been tested at some point. 66% had heard of the female condom, however only 2% had ever used them. Lack of safety in the home, school, and community arose as a significant hazard for girls in this study. 37% reported being threatened by physical violence, 21% reported having been forced, pressured, coerced or tricked into having sex. The vast majority (79%) reported doing something to protect themselves. Key "protection strategies" included: not walking alone, avoiding certain places, having only one partner (24%), abstinence (67%), and condom use (17%).

**CONCLUSIONS:** Sexual violence and coercion, lack of physical safety, and economic dependence on men leave many young women vulnerable to unsafe sex, and given the high HIV prevalence, to HIV. Delivering effective HIV prevention programs and products (i.e. microbicides) to this target group requires attention to these contextual variables and broader structural interventions.

## SESSION 46

### Poster Discussion (PD11): Advocating for HIV Prevention

**Moderators:** Lori Heise, Samukeliso Dube

Tuesday, May 25, 3:00 pm–4:00 pm

Rooms 319–321

**441**

#### Tapping into the Strength of Faith-Based Organizations: An Effective Strategy in Promoting Microbicides

S. Babtunde

*Church of Nigeria (Anglican Communion) Diocese of Lagos West*

**BACKGROUND:** In some resource-poor countries information about new HIV prevention technologies is still not as wide spread as it should be, religious leaders can support enlightened attitudes, opinions, policies and laws, redirect charitable resources for spiritual and social care and raise new funds for HIV prevention, promote action from the grass roots up to the national level, use their pulpits to spread messages about new HIV prevention technologies as well as help disseminate accurate information and influence opinion about microbicide development and research

**METHODS:** A quasi-experimental study was carried out at a yearly retreat for members of clergy in a faith-based organization; people in attendance include those in training seminaries and those who are already working in various parishes. A structured self-administered questionnaire was used to collect information on day 1 of the retreat after which a 30 minute lecture was given on microbicides, on the 5th day of the retreat during the closing ceremony the questionnaire was again readministered.

**RESULTS:** A total of 267 people attended the retreat; all were men, computer literate and had post secondary education. Pre intervention analysis of data revealed that only 5 (2%) had heard of microbicides in the past therefore they could not answer any of the other questions. Post intervention data revealed that 91% could explain what microbicides were, 89% were willing to talk about microbicides when talking about ABC prevention strategies, ways of disseminating information to their congregation suggested include incorporating microbicide information into church HIV/AIDS IEC materials, updating the theological HIV/AIDS curriculum to include lectures on microbicides, talking about microbicides during pulpit sermon on HIV/AIDS. The participants were of the opinion that if they have adequate information about microbicides they will be in a good position to serve as advocates.

**CONCLUSIONS:** Faith-based organizations have a broad reach through numerous channels for social mobilization coupled with creativity in delivering messages, leadership and influence, affiliations with large numbers of people. This opportunity can be tapped into and used to effectively mobilize communities for microbicide research, development and acceptance.

**444**

#### Identifying Priorities, Gaps and Opportunities in Prevention Research Through Community Advocacy: How the AVAC-GCM HIV Prevention Research Advocates Fellowship Program Is Nurturing Emerging Leaders

M. Chatani<sup>1</sup>, E. Bassi<sup>1</sup>, A. Kaggwa<sup>1</sup>, B. Patel<sup>2</sup>, N. Barnabas<sup>2</sup>, D. Baron<sup>2</sup>

<sup>1</sup>AVAC: Global Advocacy for HIV Prevention;  
<sup>2</sup>The Global Campaign for Microbicides

**BACKGROUND:** Global advocacy around HIV prevention research is most meaningful and sustainable when rooted in local priorities. Yet there are few resources to support local community advocates and networks to be effective at national and global levels. This identified need guided the development of the “AVAC-GCM HIV Prevention Research Advocacy Fellowship” in 2009. The program goal is to expand the capacity of civil society advocates and organizations, especially in the Global South, to monitor, inform and advocate around HIV prevention research. It also aims to increase the cadre of skilled advocates who can contribute to setting the prevention research advocacy agenda.

**METHODS:** The project was designed to attract individuals interested in HIV prevention research advocacy and/or the rollout of HIV prevention options. Applicants identified a priority area and local host organization, and developed a project that would fill a critical gap. A broad call for applications was launched in May 2009. Applications were reviewed by an independent committee of various stakeholders.

**RESULTS:** Of the 112 applications from Africa, Asia and South America, nine Fellows were selected. Inaugural Fellows are from: Kenya (1), Malawi (1), Rwanda (1), South Africa (2), Uganda (2), and Zimbabwe (2).

The Fellows were selected based on stringent criteria and on projects that explore innovative approaches to address gaps in prevention research advocacy. Project foci include: monitoring broad community engagement during the trial life-cycle, developing media training across technologies, facilitating dialogue with diverse communities in rollout plans, engaging national stakeholders around trials and bolstering the capacity of HIV advocacy campaigns to integrate research literacy.

About half of the applications were not considered as they focused on trial-specific activities such as improving recruitment, increasing adherence and developing advisory boards.

**CONCLUSIONS:** The Fellowship Program has identified emerging leadership in prevention research advocacy, diverse activities that community players consider important, as well as significant gaps in trial-specific activities. Priority should be put on identifying additional resources to support advocacy activities and tools to measure the ongoing impact of them on the nature and quality of research conduct. It is also vital that trial sponsors and advocates seek new mechanisms to address gaps identified in trial-specific activities.

# POSTER SESSIONS

**445**

## New Prevention Technologies and Sex Workers: Protection for Whom and at What Price?

A. Forbes, MSS, M. Ditmore, PhD,  
Network of Sex Work Projects

**BACKGROUND:** As a group highly vulnerable to HIV infection, sex workers are frequently cited as potential targets for future microbicide and/or PrEP introduction. Social science research among sex workers on microbicide acceptability has produced important data but does not address how access to new prevention HIV tools may interact with the social, economic and physical parameters of sex work. The Asia Pacific Network of Sex Workers (APNSW) 2009 report entitled, "Sex work and the new era of HIV prevention and care," raises fundamental questions on this issue for consideration by social scientists and access planners as we expand the universe of HIV prevention technology.

**METHODS:** A 2006 cross-training held by APNSW, the Network of Sex Work Projects and the Global Campaign for Microbicides led to creation of a joint advocacy agenda of issues requiring additional investigation and attention. GCM is using this agenda to raise awareness of these issues among sex worker advocacy groups and other NGOs. Since sex worker organizations are profoundly stigmatized and have almost no funding with which to mobilize, their issues rarely get attention on the HIV/AIDS research agenda. To date, sex worker marginalization has exceeded even that experienced by MSM, injection drug user communities and other high risk groups. One illustration of this is that US-imposed regulations policies such as "abstinence-only-until-marriage" sex education, "no promo homo" and the syringe exchange funding ban are dropping away because HIV/AIDS and SRHR advocates have shown their counterproductive effects on HIV prevention. While contested, however, the PEPFAR-mandated "prostitution pledge," remains in force.

**RESULTS:** This presentation will outline the results of this mobilization effort; the challenges confronted, and successful and unsuccessful aspects of our strategies.

**CONCLUSIONS:** Sex workers and their advocates are the experts on the real consequences that the introduction of microbicides and PrEP are likely to have on their working lives. The HIV prevention research field must attend to these issues before product introduction to assure mitigation of foreseeable and potentially harmful effects wherever possible. Generating advocacy for this demand is complicated by the profound stigma against sex work but can be achieved. Once recognized as both a human rights issue and a pragmatic necessity, the specific HIV prevention needs of sex workers can be addressed just as the unique risks and needs of MSM, injection drug users and other high risk populations have been.

**449**

## Leveraging Web-Based Technology to Build the Capacity and Knowledge of Advocates

G. Wolnitzek<sup>\*1</sup>, R. Webb<sup>1</sup>

<sup>1</sup>Global Campaign for Microbicides, PATH, Washington, DC, USA; <sup>2</sup>Global Campaign for Microbicides, London, UK

**BACKGROUND:** The Global Campaign for Microbicides (GCM) launched a virtual classroom in June 2009. By September 2009, fourteen European and African students successfully completed the course with demonstrable proficiency. The virtual classroom drew content from the *Microbicides Essentials* on-line course, a self-instructional tool created by GCM.

**METHODS:** Over the course of four months, students from as far apart as Zambia, Denmark, Rwanda and Portugal came together to participate in seven cyber-space study sessions. Equipped with headsets, computers, and phones, students engaged with expert guest speakers and GCM staff, viewed scientific animations and participated in interactive activities. Topics examined in depth included female condoms, vaginal and rectal microbicides and pre-exposure prophylaxis (PrEP).

**RESULTS:** By blending the self-instructional aspects of the *Microbicides Essentials* course with group sessions via a virtual classroom, students were able to gain greater understanding of the material, enabled them to apply their new-found knowledge with confidence in their advocacy role.

**CONCLUSIONS:** The use of asynchronous and synchronous distance learning activities provides an opportunity for advocates to strengthen their capacity and expand opportunities to engage with research and clinical trials productively. Web-based training tools can provide organizations with a cost-effective way to provide interactive training and connect participants from different time-zones and countries.

This presentation will provide an overview of the content, the technology utilized, as well as the successes and the challenges of training advocates on microbicides research literacy using asynchronous and synchronous distance learning.

## SESSION 47

### Poster Discussion (PD12): Partners and Prevention

**Moderators:** Clemensia Nakabiito, Sharon Riddler

Tuesday, May 25, 3:00 pm–4:00 pm

Rooms 403–405

## 408

### Behavioral Recall Accuracy in a Microbicide Trial: Effects of Psychological Stress, Serostatus and Timing of Interview

K.M. MacQueen<sup>\*1</sup>, F. van Logerenberg<sup>2</sup>, M. Weaver<sup>1</sup>, S. Succop<sup>1</sup>, N. Majola<sup>2</sup>, O. Abdool Karim<sup>2</sup>, S.A. Karim<sup>2</sup>

<sup>1</sup>Family Health International, Durham, NC, USA;

<sup>2</sup>CAPRISA, University of KwaZulu-Natal, Durban, South Africa

**BACKGROUND:** Nested case control studies comparing seroconverters and nonseroconverters may provide an efficient way to investigate relationships between adherence, exposure, and product effectiveness in microbicide trials. However delays in timing of interviews and differential stress may lead to biased recall if participants are interviewed after receiving positive or negative HIV test results. We assessed recall accuracy as part of a case control study nested with the CAPRISA 004 microbicide trial evaluating safety and effectiveness of tenofovir gel.

**METHODS:** Trial participants with indeterminate or positive rapid HIV test results at any study visit (n=80) and a randomly selected comparison group with negative rapid results (n=216) were recruited to participate in an in-depth behavioral assessment. 120 participants were recruited prior to post-test counseling (107 negative controls, 13 indeterminate/positive cases) and 176 after counseling (109 controls, 67 cases). All participants completed a Perceived Stress Scale (PSS), an Impact of Event Stress Scale (IES), and questions assessing accuracy of recall of procedures conducted at the most recent previous study visit (RPS). Responses were evaluated using two-sample T-tests.

**RESULTS:** No significantly different results were observed in total PSS, total IES or total RPS scores for cases and controls interviewed prior to counseling. For those interviewed after counseling all scores differed significantly by serostatus (total PSS p=0.031, total IES p=0.007, total RPS p<0.001); there was also a greater delay between testing and timing of the interview for cases (mean = 17 days) compared to controls (mean = 3 days).

**CONCLUSIONS:** Knowledge of non-negative HIV test results is likely to lead to delays in the timing of interviews and to induce psychological stress; both may impact recall accuracy for self-report data. The resulting potential for bias needs to be addressed in the design and implementation of nested case control studies assessing relationships between adherence, exposure, and product effectiveness in microbicide trials.

**BACKGROUND:** Studies conducted in Buenos Aires, Argentina, repeatedly show high HIV incidence per 100 person years among MSM. Rates were 6.7 in the Vignoles et al. (2006) cross-sectional study that used STARHS, and 3.9 in the Segura et al. (2007) study of a prospective cohort followed up for 12 months (retention: 97.2% at 6 months and 91.5% at 12 months). With prevention efforts limited to condom promotion and no locally developed, proven effective, behavioral interventions, new strategies are urgently needed. We assessed HIV prevalence and incidence and studied acceptability of microbicides among MSM in Buenos Aires.

**METHODS:** 500 MSM were recruited through Respondent Driven Sampling. They provided blood samples and underwent CASI interviewing on microbicide acceptability. HIV-positive plasma samples were tested using a detuned version of an HIV-1 enzyme immunoassay (Vironostika HIV-1 Microelisa System; bioMerieux Inc, North Carolina, USA) to sort out potential recent infections (less than 6 months) from chronic infections using the STARHS strategy. Microbicide acceptability was measured with questions on willingness to use a gel microbicide for anal sex measured on 10-point Likert scales ranging from 1= completely unwilling to 10 = completely willing.

**RESULTS:** Sample HIV prevalence was 15.7 % (CI: 11.8-20.2), being higher among gay identified men (30.6 %) than non-gay identified MSM (12.9%,  $\chi^2$  p <.001). When the 85 HIV-positive plasma samples were tested using a detuned version of the HIV-1 enzyme immunoassay, 23 cases were identified as possible recent infections, this yielding an HIV incidence of 11.4 per 100 persons/year. Incidence was significantly higher among gay men (22.6) than non-gay identified MSM (8.3). Concerning willingness to use a gel microbicide during anal sex, although the mean score was 6.0 (neither willing nor unwilling), gay identified men scored significantly higher (7.1, willing range) than non-gay identified men (5.6).

**CONCLUSIONS:** Gay identified MSM in Buenos Aires have high HIV prevalence and incidence and are willing to use gel rectal microbicides. Furthermore, the research infrastructure (i.e., laboratory facilities, demonstrated participant recruitment and retention success, record of scholarly activity of the University of Buenos Aires/Nexo Asociación Civil, and effective collaboration with international partners) suggests great potential for successful collaborations in Phase 2 and 3 micro trials.

## LB6

### High HIV Incidence and Willingness To Use Rectal Microbicides Among Argentine MSM: Potential for Rectal Microbicide Studies

A. Carballo-Díéguez<sup>\*1</sup>, I. Balan<sup>1</sup>, R. Marone<sup>2</sup>, M. Pando<sup>3</sup>, C. Dolezal<sup>1</sup>, C.S. Leu<sup>1</sup>, V. Barreda<sup>2</sup>, M. Avila<sup>3</sup>

<sup>1</sup>HIV Center for Clinical and Behavioral Studies at New York State Psychiatric Institute and Columbia University, New York, NY, USA; <sup>2</sup>Nexo Asociación Civil, Buenos Aires, Argentina; <sup>3</sup>Universidad de Buenos Aires, Centro Nacional de Referencia para el SIDA, Facultad de Medicina, Buenos Aires, Argentina

# POSTER SESSIONS

## 411

### I Am Forever Scared: The Sexual and Reproductive Health Needs and Rights of Women Newly Diagnosed with HIV

S. Abbott<sup>\*1</sup>, D. Cooper<sup>2</sup>, J. Harries<sup>3</sup>, S. Cooper<sup>4</sup>, L. Katzen<sup>1</sup>

<sup>1</sup>Population Council, New York, NY, USA;

<sup>2</sup>Department of Public Health & Family Medicine, University of Cape Town; <sup>3</sup>Women's Health Research Unit (WHRU) Department of Public Health & Family Medicine, University of Cape Town; <sup>4</sup>University of Cape Town

**BACKGROUND:** Participants enrolled in clinical trials for vaginal microbicides are women of childbearing age. Trials endeavor to offer comprehensive services for women who seroconvert, including services for sexual and reproductive health. However, much is still unknown about the needs of women affected by the virus. This study explores the fertility intentions of newly HIV-infected women, their experiences in public and private sector clinics, and their changing reproductive health needs.

**METHODS:** In-depth interviews (IDIs) were conducted with 39 women recently diagnosed with HIV. Respondents had been enrolled in one of two clinical studies in South Africa sponsored by the Population Council at the time they tested positive for HIV. This qualitative study was conducted at the University of Cape Town (UCT) Empilisweni Centre for Wellness Studies and the University of Limpopo/Medunsa campus (Medunsa), Setshaba Research Centre.

**RESULTS:** HIV diagnosis produced a major shift in sexual and reproductive desires, with the respondents almost unanimously reporting that they no longer wished for children. This can be attributed to five reoccurring themes: fear of orphaning their children, blame, guilt, concerns that their own health would be jeopardized by a pregnancy, and fear of community stigmatization. Less than half of the sample was aware of PMTCT programs. Difficulties negotiating safer sexual practices combined with depression and anxiety resulted in a loss of sexual desire. Respondents reported a number of challenges in accessing services, including long queues, insensitive practitioners, and a fear of loss of confidentiality. The presence of ongoing support group at the Medunsa site provided women with immediate care, which they credited with an easier acceptance and ability to disclose.

**CONCLUSIONS:** Meeting the unique reproductive and sexual needs and rights of newly diagnosed women requires effective referral systems to ensure continuity of care and support. In addition to HIV services, HIV+ women require counselling and information on effective contraceptive options, including emergency contraception and access to abortion, as well as PMTCT programs for those wishing to conceive. Ongoing care in HIV prevention trials must include family planning counseling and methods. Ultimately, a lack of continuity of care can lead to missed opportunities to provide health care and endangers the realization of sexual and reproductive rights.

## 412

### Comparison of Socio-Behavioral Formative Data at Two Sites in Advance of the FEM-PrEP Clinical Trial

L. Johnson<sup>1</sup>, K. Agot<sup>2</sup>, V. Mtimkulu<sup>3</sup>, J. Murphy<sup>1</sup>, C. Parker<sup>1</sup>, K. McKenna<sup>1</sup>, C. Wong<sup>1</sup>, M. Lanham<sup>1</sup>, K. Ahmed<sup>3</sup>, A. Cornelius<sup>1</sup>

<sup>1</sup>Family Health International, Research Triangle Park, NC, USA; <sup>2</sup>IMPACT Research & Development Organization, Bondo, Kenya; <sup>3</sup>Setshaba Research Centre, Soshanguve, South Africa

**BACKGROUND:** Family Health International (FHI) and local investigators are conducting FEM-PrEP, a phase 3, randomized, placebo-controlled trial of the safety and effectiveness of daily, oral Truvada as pre-exposure prophylaxis (PrEP) to prevent HIV acquisition in women.

FEM-PrEP is using a comprehensive approach, including collection of socio-behavioral and community formative data before the start of the trial to inform clinical trial procedures at each site.

**METHODS:** A total of 191 in-depth interviews (IDIs) and 36 focus group discussions (FGDs) were conducted in Bondo, Kenya, and Pretoria, South Africa, with potential trial participants, sexually active men, and community members. Topics included study acceptability, retention, and product adherence.

**RESULTS:** The trial was found acceptable at both sites, especially among potential participants, almost all of whom said they would want to participate. More than half of respondents in Bondo (60%) mentioned that there may be misperceptions about the trial in the community, such as that trial participants are HIV positive. These misperceptions were mentioned by less than half of respondents in Pretoria (47%).

The majority of potential participants at both sites said that daily pill adherence would be easy and offered similar reasons, including dedication to the trial and the hope that the pill could prevent HIV. However, potential participants also described possible challenges to pill adherence, including lack of support from others, mentioned only in Bondo, and alcohol use, mentioned primarily in Pretoria.

More than 90% of potential participants said it would be easy to attend monthly clinic visits. About two-thirds mentioned possible challenges, including transportation, illness, and work obstacles. Travel outside the study catchment area was mentioned as an obstacle for coming to follow-up visits by more than two-thirds of respondents at Bondo and by less than one-half of respondents at Pretoria.

**CONCLUSIONS:** The comparison of preparedness data at these sites indicates that while there are some commonalities, there are also notable differences. These data helped to inform FEM-PrEP trial procedures and messages and tailor them for each site.

## 414

### Attitudes About Research Participation in Microbicide Studies

S.L. Rosenthal<sup>\*1</sup>, C. Radecki-Breitkopf<sup>2</sup>, M.L. Loza<sup>2</sup>, L.R. Stanberry<sup>1</sup>, K.L. Vincent<sup>2</sup>

<sup>1</sup>Columbia University Medical Center, New York, NY, USA; <sup>2</sup>University of Texas Medical Branch, Galveston, TX, USA

**BACKGROUND:** Clinical trials require individuals to be willing to participate and to adhere to study demands. Otherwise, trials can be expensive, yield inconclusive results, or damage community relationships. Participants in clinical trials can provide insights about their experience that can be used for future planning.

**METHODS:** U.S. women (18–45 years of age) who are in a clinical trial evaluating the use of a new imaging technique (optical coherence tomography) for assessment of vaginal products completed qualitative interviews during the study to assess reasons for participation, adherence to study demands, and overall experience of trial participation. Basic requirements of the study are attendance at 4 visits with pelvic exams (screening, baseline, after use of 5.5 days of gel use, and 1 week later). The participants must remain abstinent and refrain from douching/ tampon use for approximately 3 weeks. Total reimbursement is \$475.

**RESULTS:** Twenty-six of 30 women have enrolled of which qualitative data has been analyzed for 15. Complete data will be available at the meeting. The 26 women have a mean age of 29 years, with 10 being Hispanic, 10 white, 4 African-American, and 2 Asian. Societal contribution, knowledge, free Pap test, reimbursement and convenience were reasons for participation. Women also noted the absence of a negative (e.g. blood draw, muscle biopsy, experimental product). All women adhered to study demands of visits and product use; most adhered to abstinence. Abstinence was managed by obtaining partner agreement, engaging in other activities (e.g. oral sex), avoiding early intimacy, or scheduling participation when the partner was away. Women continued to use the product despite minor side effects because of their commitment to the study. They reported that those same side effects would lead to discontinuation outside a trial.

**CONCLUSIONS:** Women participate in microbicide clinical studies for a variety of reasons. They have a strong commitment to the study requirements, but helping them to plan how to meet those expectations may foster adherence. Abstinence is obtainable but can be challenging.

## SESSION 48

### Late Breaker Session

**Moderators:** Sharon Hillier, Timothy Farley

Tuesday, May 25, 3:00pm–4:00pm

Ballroom

## LB1

### A Prospective Randomized Double Blind Placebo-Controlled Phase 1 Pharmacokinetic and Safety Study of a Vaginal Microbicide Gel Containing Three Potent Broadly Neutralizing Monoclonal Antibodies (2F5, 2G12, 4E10) (Mabgel)

G. Morris<sup>\*1</sup>, S. Chindove<sup>1</sup>, S. Woodhall<sup>1</sup>, R. Wiggins<sup>1</sup>, B. Vcelar<sup>2</sup>, C. Lacey<sup>1</sup>

<sup>1</sup>Hull York Medical School and Centre for Immunology and Infection, University of York, York, UK; <sup>2</sup>Polymun Scientific, Vienna, Austria

**BACKGROUND:** In the light of the recent disappointing results in phase 3 trials with polyanionic compounds, alternative, HIV specific agents are under evaluation as potential microbicides. Monoclonal antibodies offer a valuable alternative to antiretroviral compounds and may avoid problems with potential drug resistance. The antibodies 2F5, 2G12 and 4E10 are human recombinant monoclonal antibodies (MAbs) which potently, broadly and synergistically neutralize divergent HIV-1 subtypes. They have previously been administered intravenously to human subjects in high doses with demonstrated safety. When administered intravenously they have also protected adult female macaques against vaginal challenge with SHIV 89.6PD. Therefore this combination of monoclonal antibodies could potentially represent an extremely promising vaginal microbicide for use in women.

**METHODS:** The primary objective of the study is to assess the pharmacokinetics of the specified monoclonal antibody combination when applied vaginally. Specifically: 1) to assess the retention of the MAbs in the vagina after administration; 2) to investigate whether there is any systemic absorption of the MAbs. The secondary objective of the study is: to assess the safety of the specified monoclonal antibody combination when applied vaginally. Target recruitment n = 30. Healthy volunteers aged 18 to 45 years were enrolled after screening if they fulfilled eligibility criteria. Participants were low risk, HIV seronegative females who were sexually abstinent during the dosing period. They were randomized to receive either high dose Mabgel (20mg/g of each Mab), low dose Mabgel (10mg/g of each Mab) or placebo. 12 doses of gel were self-applied by participants once daily over 12 consecutive days. Cervico-vaginal examination and pharmacokinetic sampling was performed at 1 hour, 8 hours and 24 hours post the first dose and at 12 hours and 36 hours post the 12th dose. Additional safety evaluations took place at day 5 to 7 of dosing and in the subsequent menstrual cycle.

**RESULTS:** To date (April 15, 2010), 37 volunteers have been screened and 19 have been enrolled in the dosing phase of the study. A scheduled blinded interim analysis of data from the first 11 participants for presentation to the independent Data and Safety Monitoring Board for the study has showed the following: Ten of the 11 participants reported at least 1 adverse event. 3 participants accounted for 50 % of all the adverse events. Overall, 31 individual adverse events have been observed, 29 of these were identified to be at least possibly related to the study gel. Among these 27 were mild and 2 were moderate in severity. Both moderate AEs related to non-menstrual vaginal bleeding. 14 AEs were genitourinary (bleeding, itching, discharge, 1 abrasion < 5mm diameter) 3 were gastrointestinal (nausea, indigestion), 5 were headaches, and 5 were haematological. No serious AEs have been reported to date.

Mab levels in vaginal Weck-Cel samples, cervico-vaginal lavage and serum samples are being analysed currently by Polymun Scientific, Vienna using individual ELISA systems for each antibody with internal purified 2F5, 2G12 and 4E10 monoclonal antibodies as a standard. Growth and decay curves will be estimated for each dose. Doses will be compared by repeated measures analysis with log antibody level as the outcome. Pharmacokinetic data will be available from all time points for presentation at the conference. Results from vaginal flora and acceptability analyses will be presented elsewhere.

**CONCLUSIONS:** This is the first phase 1 trial assessing the topical application of a microbicide containing a combination of potent, broadly neutralizing monoclonal antibodies against HIV-1. These blinded results from a planned interim analysis of the first 11 participants are encouraging and suggest, thus far, that the product is safe. However we await the completion and final analysis of the trial before drawing any firm conclusions.

## LB2

### A Phase I Study of the Safety and Acceptability of 3% w/w SPL7013 (VivaGel) Applied Vaginally in Sexually Active Young Women (MTN-004)

I. McGowan<sup>\*1</sup>, K. Gomez<sup>2</sup>, P. Emmanuel<sup>3</sup>, I. Febo<sup>4</sup>, B.A. Chen<sup>1</sup>, B. Richardson<sup>5,6</sup>, M. Husnik<sup>6</sup>, E. Livant<sup>7</sup>, J. Piper<sup>8</sup>, C. Price<sup>9</sup>, and the MTN-004 Protocol Team

<sup>1</sup>University of Pittsburgh School of Medicine; <sup>2</sup>Family Health International; <sup>3</sup>University of South Florida; <sup>4</sup>University of Puerto Rico, <sup>5</sup>University of Washington; <sup>6</sup>Fred Hutchinson Cancer Research Center; <sup>7</sup>Microbicides Trial Network, Magee-Womens Research Institute; <sup>8</sup>Division of AIDS, NIAID, NIH; <sup>9</sup>Starpharma Pty Ltd.

**BACKGROUND:** The study was designed to assess the safety, adherence, acceptability, and effect on vaginal microflora of VivaGel, a novel dendrimer topical microbicide that inhibits HIV and HSV-2 *in vitro*.

**METHODS:** Sixty-one women aged 18–24 were recruited from three sites in San Juan (PR), Tampa (FL) and Pittsburgh (PA). Participants were randomized 1:1:1 to receive VivaGel (V), VivaGel placebo (VP), or the HEC placebo (HEC) twice daily for 14 consecutive days. Safety endpoints included genitourinary (GU) and/or other adverse events (AEs). Acceptability and adherence were determined by interviewer-administered questionnaires. Changes in vaginal flora were determined from Gram-stained vaginal smears and quantitative vaginal culture.

**RESULTS:** A total of 22, 21, and 18 women were enrolled in the V, VP, and HEC groups respectively. No Grade 3 or 4 AEs, serious AEs, or withdrawals due to AEs were reported. GU symptoms (1 or more attributed to product use) were reported as follows: V (n=14; 63.6%), VP (n=11; 52.4%) and HEC (n=7; 38.9%) (NS, p=0.3). The prevalence of abnormal pelvic exam findings attributed to product use was similar across all arms of the study. Using pair-wise comparison, women in the V arm had a significantly higher incidence of related GU AEs than the HEC gel (0.197 versus 0.083 per 100 person years respectively; p=0.04). Adherence rates including time on product hold were 77% (V), 95% (VP), and 94% (HEC). Thirty-six percent of women in the V arm reported that they would be very likely to use the gel in the future compared to 48% (VP) and 61% (HEC). Exposure to V and VP resulted in shifts in the vaginal microflora but there was no overall impact on BV as assessed by Nugent score.

**CONCLUSIONS:** VivaGel was generally well tolerated and comparable with the VivaGel placebo, although there was lower adherence and acceptability and a higher incidence of related genital AEs compared to the HEC placebo gel.

# ORAL SESSIONS

## LB3

### Safety and Systemic Absorption of Topical UC781: Results from a Phase I Randomized, Placebo-Controlled Trial

S.J. Whitehead<sup>\*1,2</sup>, C. Utaipiboon<sup>2</sup>, S. Thepamnuay<sup>2</sup>, P. Kantipong<sup>3</sup>, W. Leelawiwat<sup>2</sup>, P. Wasinrappee<sup>2</sup>, J. McNicholl<sup>1,2</sup>, W. Sukwicha<sup>2</sup>, P. Mock<sup>2</sup>, C. Mauck<sup>4</sup>, L. Grohskopf<sup>1</sup>

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**BACKGROUND:** The non-nucleoside reverse transcriptase inhibitor UC781 is under development as a topical microbicide. We conducted a single-center randomized, placebo-controlled trial in Chiang Rai, Thailand to evaluate safety and systemic absorption of UC 781 gel.

**Methods:** Forty-five HIV-1 uninfected, healthy sexually active women at low risk of sexually transmitted infections were randomly assigned to apply UC781 0.1% gel, UC781 0.25% gel, or hydroxyethylcellulose placebo intravaginally twice daily for 14 days, and to use condoms during the study period and for an additional 7 days. Women were evaluated at Day 0, day 7, and day 14 for genital findings by colposcopic examination, vaginal microflora, markers of inflammation in cervicovaginal lavage, and self-reported symptoms. UC781 plasma levels and laboratory tests for systemic effects were assessed at baseline and day 14.

**RESULTS:** One woman in the UC781 0.1% group and 1 in the placebo group developed genital epithelial disruption, 1 woman in the UC781 0.1% group discontinued gel use because of genital itching and burning, and 1 woman in the UC781 0.1% group discontinued gel use because of genital itching associated with new onset candidiasis. There were no statistically significant differences between groups in genital symptoms or findings, inflammatory markers, or vaginal microflora. Plasma UC781 levels were detectable ( $>0.05$  ng/ml) in 5 (33%) women in the UC781 0.1% group (mean and range 0.075 (0.060–0.105) ng/ml) and 9 (60%) women in the UC781 0.25% group (mean and range 0.100 (0.056–0.220) ng/mL).

**CONCLUSIONS:** UC781 0.25% was well tolerated when used twice daily for 14 days; one woman discontinued use of the 0.1% concentration due to product-associated genital symptoms. The product is minimally absorbed at both 0.1% and 0.25% doses, without observable toxicity in this small safety study. The significance of low levels of UC781 absorption both for systemic toxicity and potential effect on development of NNRTI-resistance in case of HIV exposure will need to be evaluated in larger trials.

## LB4

### Safety and Acceptability of Vaginal Ring as Microbicide Delivery Method in African Women

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**BACKGROUND:** Vaginal rings are being explored as a potential delivery method for microbicides to prevent HIV infection. Rings offer the advantage of monthly instead of daily use, which may increase product adherence and therefore effectiveness. Since vaginal rings are not widely used in African countries, safety and acceptability of this method were assessed.

**METHODS:** A safety study of a placebo silicone elastomer vaginal ring was conducted among healthy, sexually active women 18–35 years of age at 5 sites in Africa. 3 sites in South Africa and 1 in Tanzania completed the study. Women were randomized to 3 months of ring use and 3 months of observational no-product use, in a crossover design. Women responded to an acceptability and adherence questionnaire at 5 points during the study. Safety was assessed by pelvic/speculum examination, colposcopy and adverse event monitoring.

**RESULTS:** 152 of the 170 women enrolled at 4 sites completed. No ring-related SAEs were reported; 22 women reported possibly/probably related AEs. Genital AEs were equally distributed between ring and no treatment arm. No safety concerns were identified.

More than 90% women reported liking the ring, felt it was comfortable and easy to insert; and liked that it did not alter sexual experience. Awareness of the presence of the ring decreased over time. A minority of women experienced either spontaneous ring expulsion or removal for cleaning during the trial which decreased over time.

**CONCLUSIONS:** Use of a silicone vaginal ring for 3 months was safe and acceptable to African women. The observed rate of expulsion was less than reported with other vaginal rings, but participant removal of the ring for cleaning may indicate a need for enhanced adherence counseling.

**369**
**Managing Regulatory and Administrative Documentation and Compliance to GCP for Multiple Clinical Trials—Experience from Durban, South Africa**

A. Guptar, S. Naidoo, G. Ramjee  
*HIV Prevention Research Unit, Medical Research Council*

**BACKGROUND:** Ethical issues are of paramount importance in a clinical trial, however little attention is focused on the management of regulatory correspondence. Documentation management of clinical trials must be efficient and compliant with GCP and sponsor requirements. The HIV Prevention Research Unit (HPRU) has been conducting various trials including MDP 301, HPTN 035, MTN and behavioral studies between 2004 and 2009. The aim of this study is to present an overview of systems implemented at HPRU to manage the regulatory correspondence for multiple clinical trials and experiences communicating with regulatory bodies.

**METHODS:** Systems implemented to regulate documentation included development of SOPs for corresponding with regulatory bodies, a specified filing system, e-storage of documentation, regulatory information and tracking logs. Types of correspondence included regulatory approval and recertification applications, and responses to regulatory queries, and acknowledgements from regulatory bodies for all trials.

**RESULTS:** Approximately 1500 MDP 301 and 600 HPTN 035 regulatory communiqués were dealt with by HPRU. Ongoing studies including MTN 003 are similarly laden. Adherence to GCP was maintained by direct liaison with regulatory coordinators of each study to ensure confidentiality of regulatory documentation. Communication via e-versions and hardcopy distribution of incoming and outgoing documents ensured timely responses to regulatory queries. Regulatory logs included expiration and submission dates of reports, ethics recertification and Federalwide Assurance which aided in timely submission of documents thus preventing undue delays. Establishing good relations with regulatory bodies resulted in identifying appropriate resources for assistance. Challenges with regulatory bodies included poor turn around time, duplicate correspondence received and incorrect information on recertification notifications.

**CONCLUSIONS:** It is imperative that all administrative personnel undergo Good Clinical Practice and study specific protocol training to ensure adequate adherence to protocol requirements. Document management and monitoring systems should be well structured and controlled for efficient handling of information.

**370**
**How Much Is Enough?: Standards of Prevention in HIV Prevention Trials**

S. Philpott<sup>1,5</sup>, E. McGroarty<sup>\*2</sup>, C. Hankins<sup>3</sup>, L. Paxton<sup>4</sup>, L. Heise<sup>1,6</sup>, the Participants in the 2009 GCM/CDC/UNAIDS Consultation on Standards of Prevention in HIV Prevention Trials

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**BACKGROUND:** As effective new HIV prevention tools are developed, researchers face ethical and logistical questions about how and when to include them in the standard prevention package of ongoing and future HIV prevention trials. Current UNAIDS/WHO guidance recommends that decisions about adding new tools to the prevention package be made in consultation with "all relevant stakeholders" but leaves open questions of process and implementation. In March 2009, GCM, UNAIDS, and CDC convened an international consultation in Uganda to develop practical approaches to these questions.

**METHODS:** Through presentations, participatory exercises and debate, 59 diverse participants (including researchers, ethicists, advocates and policymakers) worked to reach agreement on key criteria to guide decision-making on whether and when to include new HIV prevention tools in future trials, and whether ongoing trials should be stopped or modified when another trial shows a new method to be effective.

**RESULTS:** Participants developed a set of specific criteria to inform decision-making, including:

- whether the method has been recommended by international bodies or adopted at a national level
- size of the effect and the weight of evidence
- relevance to the trial population
- whether the tool has been approved or introduced in the trial country
- feasibility
- whether adding the tool to a current trial would lead to futility of trial continuation
- the extent to which adding it might compromise ability to evaluate future methods
- outstanding safety issues
- status of the trial

Researchers and sponsors must clearly justify and communicate to all stakeholders any decision not to include a new method in a prevention package. While no consensus emerged regarding how researchers should balance their opportunity and responsibility to provide state of the art prevention and care and concerns about providing a method that cannot be sustained in that setting after the trial, the consultation made significant progress in developing practical criteria to help researchers, sponsors and other stakeholders address some critical trial issues facing the HIV prevention field.

**CONCLUSIONS:** It will be important to widen the discussion and continue to adapt the criteria and points of agreement with different stakeholders and as new data emerge. Additional work is also needed to develop, implement, and evaluate approaches to ensuring stakeholder participation in decision making.

**371**
**Cervical Barriers for Use in Macaque Model Assessments**

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<sup>1</sup>University of Washington, Seattle, WA, USA;  
<sup>2</sup>ReProtect, Inc., Baltimore, MD, USA

**BACKGROUND:** Cervical barrier devices are approved for contraception and are used safely and effectively by women all around the world. In order to conduct preclinical studies assessing cervical barriers alone and in combination with topical microbicide gel formulations, we attempted to create miniature cervical barriers appropriate for use in macaque studies.

**METHODS:** Initially O-rings of four different outer diameters, ranging from 11/16 to 7/8 inch, were situated over the cervix in various *Macaca nemestrina* monkeys to establish an appropriate size for further barrier development. Next, nine prototype cervical barriers were made from latex domes sealed with silicone adhesive to semi-flexible O-rings. The size, depth and rigidity of each barrier device was assessed for optimal fit and retention in the macaque vagina. Finally, optimized macaque sized cervical barriers have been prepared and provided for an initial preclinical efficacy evaluation in the macaque model for chlamydial infection (ongoing).

**RESULTS:** The initial O-ring sizing study determined that the 3/4-inch (approximately 20-mm) sized O-ring could best accommodate the majority of female macaques in our study. Further testing of barrier devices noted that the slightly less rigid O-ring (70 vs. 90 durometer) was easier to position appropriately in the macaque vagina, and that 13/16 inch outer diameter was optimal. In order to hold test gel formulations to be assessed in preclinical studies, the latex dome is designed to have a height of 1/2 inch measured from the top of the O-ring rim.

**CONCLUSIONS:** An optimized cervical barrier of defined proportions and materials has been developed for use in preclinical macaque studies.

# TUESDAY POSTERS

## 372

### **Improving Microbicide Trial Participants' Understanding of Randomisation, Double-blinding and Placebo Use: A Pilot Intervention in Malawi**

P. Ndebele, D. Wasenaar

**BACKGROUND:** Several authors have reported some difficulties that research participants face in understanding the three trial procedures of randomisation, double blinding and placebo use as well as their personal implications. Other studies have also revealed that trial participants have problems understanding the differences between research and routine care. Understanding of these concepts is critical to informed consent and limited understanding of trial procedures is a clear sign that decisions to enroll in a trial are not based on adequate information. A study was conducted in Malawi to assess microbicide trial participants' understanding of the three study procedures.

**METHODS:** The study was conducted within the context of the HPTN035 microbicide trial which was being conducted in Blantyre and Lilongwe in Malawi. The study used a combination of qualitative and quantitative methods which included review of study documents, in-depth interviews with study staff, structured interviews with a random sample of 203 participants.

**RESULTS:** The study established that about one third of the participants had low levels of understanding of the three concepts. The study also established that the majority of participants had negative attitudes towards the three procedures. Based on these findings, a pilot intervention was designed aimed at improving understanding. The pilot intervention was delivered using a sample of 36 participants who were randomly assigned to the intervention and non-intervention arms. An assessment after the intervention established that the intervention was successful in improving understanding of the three procedures.

**CONCLUSIONS:** The study concluded that some participants had lower levels of understanding of the three procedures and that the majority of participants had negative attitudes towards the three procedures. The study also established that the majority of participants were not aware of the personal implications associated with the three procedures. Based on the findings from the intervention study, it was confirmed that research participants can understand the 3 procedures if they are explained in user-friendly terms and if information concerning their justification and personal implications is provided.

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### **Kar Geno, Place of Hope: Designing Referral Systems for Use during the Conduct of a Microbicide Trial in Kisumu, Kenya**

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Centre for Microbiology Research (CMR), Kenya Medical Research Institute

**BACKGROUND:** HIV prevention trials, including microbicide trials, are usually conducted in resource-limited settings in which available health care falls very often short of the recommended standards of care. In addition, due to the limited availability of resources for conducting these trials, it is not always possible to completely upgrade the existing health care services and maintain the upgrades after clinical trial completion. We therefore sought to establish various referral systems and support networks to be used during the conduct of a phase I/II microbicide trial in Kisumu, Kenya.

**METHODS:** As part of the preparatory phase of the trial, we listed the conditions that would require us to make referrals. We then mapped out health facilities within the community that could serve as potential referral sites. We approached these facilities and sought to obtain either written or verbal agreements on the referral process. An SOP for referring participants was created, and the staff was trained on all referral procedures.

**RESULTS:** We determined that we would refer participants for continued HIV care, contraceptive counseling and provision, professional counseling, treatment of other general medical conditions and participation in other research studies, for those who failed to meet our entry criteria. We identified 7 health facilities that provide free HIV care including ART; 5 centres that offer contraceptive services; 3 professional counseling centres and 3 main referral centres for general medical, surgical and gynecological services. For each of these facilities we noted working hours, special clinic days and requirements for admission to enable us to better inform our participants during referral. We established agreements with these facilities after a formal introduction of our group and the planned microbicide trial.

**CONCLUSIONS:** In a resource-limited setting where access to medical care is difficult, setting up referral systems is a challenge. Despite the agreements made with the referral centres, we cannot ascertain the access to and the quality of care that participants will receive at these centres. Furthermore, we cannot ensure that there would be continuity of care for former participants after study closure. However, from our experience the establishment of referral systems is key for improving local standards of health care and should be encouraged during the conduct of clinical trials.

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### **Addressing Coercion in the Conduct of Clinical Trials on Microbicides**

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<sup>1</sup>Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria

<sup>2</sup>New HIV Vaccine Microbicide Advocacy Society

**BACKGROUND:** There has been a lot of ethical debate around the concept of undue incentives and coercion in the field of microbicide research. Many consider the probability of compensation acting as undue inducement for enrolment in such clinical studies while a few differ on the subject matter. However, there have been few discussions on other possible forms of subtle coercion in the field of clinical trial practice in Africa. This abstract reviews some of these forms of ethically acceptable subtle coercions and argues for the consideration of compensation of research participants' time and effort expended in microbicide research as not being considered as undue inducement.

**METHODS:** A review of the literature in the field on the subject matter. Reflection of events on microbicide clinical trials on the field in Africa

**RESULTS:** A few articles in the literature notes that when there is emphasis on ensuring risk minimization in trials, compensation should no longer be perceived as an undue inducement for risk taking. In the conduct of microbicide clinical trials in Africa, forms of subtle coercion do exist in the field. This include the provision of high standards of health care within trials that can otherwise not be obtained outside the trial setting; provision of gifts and services as a form of compensation for retention in trials. Also, the pressure for meeting recruitment and retention targets on time also makes the use of some subtle coercion to ensure that trial participants continue to stay on in the trial.

**CONCLUSIONS:** 'Ethically acceptable' subtle coercion goes on in the conduct of microbicide clinical trials in Africa. There is however the need to address disparity in access to health services as a form of coercion in microbicide trials. There is also the need to re-evaluate the current ethical definition of compensation of trial participants' time and effort invested in the research process as undue incentive as such compensation are economic empowerment tools rather than undue inducement in trials where risk is considerably minimized. Finally, it would be advocated that compensation should not be viewed as undue inducements as IRBs are saddled with the responsibility of minimizing risks in such trials more so that true voluntarism is the guiding philosophy behind trial compensation in clinical research.

**375****Male Circumcision as a HIV Risk Reduction Prevention Strategy**P.M. Mutiga, G.W. Nganga  
*Ambassadors Of Change NGO, Nakuru, Kenya***BACKGROUND:** The underside of the foreskin of the penis is vulnerable to HIV infection and circumcision reduces the risk of HIV. Research findings confirm certain HIV target cells are much more common on the underside of the foreskin than on the shaft of the penis increasing vulnerability to HIV infection in uncircumcised men.**METHODS:** Most HIV prevention strategies are accessible to fewer than 1 in 5 people in Sub-Saharan Africa. Existing data indicates that circumcised men who practice penile-vaginal intercourse have approximately 50% less chance of acquiring HIV than non-circumcised men. Male circumcision is practiced among different communities for a diverse range of reasons which include cultures, hygienic concern and religious beliefs.**RESULTS:** In Luo, Nyanza, Kenya, male circumcision has been opposed due to cultural beliefs, practices, myths and taboos, resulting in high prevalence rate of HIV 14% as compared to 7.4% of the national prevalence level hence the need for an alternative HIV prevention strategy to strengthen the existing prevention methods e.g. condom use.**METHODS:** The purpose of this study was identify the level of acceptability of male circumcision among men and women in the Luo as a risk reduction strategy where circumcision has encountered increased resistance based on cultural dynamics.**RESULTS:** The study engaged 154 women of reproductive ages (14–49) yrs in group discussions, interviews and questionnaires observing ethical issues on confidentiality and carried out in a central location site. 79 men who had undergone circumcision were interviewed, Literature review.**RESULTS:**

- 26% of the females interviewed expressed acceptance of male circumcision while 49% of male had fears that circumcision would allow women of their communities to have partners outside and promote inconsistent use of condoms through misconceptions.
- Idea of circumcision was more acceptable among the youth and adolescents(14–35 yrs) than the older generation who play the key role as gate keepers of the cultural values
- The elite are more prepared to accept circumcision as preventive strategy than the rural, hard-rich populations
- Circumcision is often carried out secretly in public health clinics for fear of community isolation.
- Social mixing with other communities in learning institution, intermarriages plays a predominant role in promoting circumcision initiatives.
- Policy makers from the community have indicated political will, sensitized the community, and provided financial resources in support of circumcision as a preventive strategy.

**CONCLUSION:**

- Increased advocacy for universal access to existing prevention methods
- Added community awareness on risks involved while prioritizing male circumcision as a HIV risk reduction prevention strategy through outreaches, media campaigns and engagement of the community in Microbicides research.
- Challenge harmful cultural practices (e.g., wife inheritance, forced early marriages) that contribute to HIV transmission.

**376****Promoting Female Condoms in Sex Work Establishments in Southern China: A Multi-level Community Intervention**M.R. Weeks<sup>\*1</sup>, S.S. Liao<sup>2</sup>, J.H. Li<sup>1</sup>, F. Li<sup>2</sup>, J. Dunn<sup>1</sup>, Q.N. Zhang<sup>2</sup>, Y.H. Wang<sup>2</sup>, B. He<sup>3</sup>, Y.J. Zhou<sup>4</sup>, Y.X.M. Zeng<sup>4</sup><sup>1</sup>Institute for Community Research, Hartford, CT, USA; <sup>2</sup>Peking Union Medical College and Chinese Academy of Medical Sciences, Department of Public Health and Epidemiology, Beijing, China; <sup>3</sup>Hainan Centers for Disease Prevention and Control, HIV/AIDS Division, Haikou, Hainan Province, China; <sup>4</sup>Guangxi Center for Disease Prevention and Control, HIV/AIDS Division, Nanning, Guangxi Province, China**BACKGROUND:** Uneven economic growth, population migration, and changing social mores in China have contributed to a rise in the sex industry, leading to rapidly increasing rates of HIV and STI.**METHODS:** In a collaboration between U.S. and Chinese researchers and Chinese provincial and local CDC and health workers, we developed and implemented a multi-level HIV/STI prevention program to promote female condoms (FC) for HIV prevention among women working in establishments in which sex work takes place. These included massage/beauty parlors, boarding houses, roadside restaurants and hotels. Formative ethnography in the communities and in the sex work establishments, intensive process evaluation of the intervention, and pre-post risk attitude/behavioral assessments of sex workers provided data on the context and implementation of the FC promotional intervention that was primarily based within sex work establishments, but which integrated community-level, establishment-level, and individual-level components.**RESULTS:** The first full cycle of research and intervention was conducted in two rural towns in Hainan Province (2007–2008) and in small city in Guangxi Province (2009–2010). Social relationships between establishment "bosses" and the sex workers, among the women themselves, and between both bosses and women and the local health care workers who delivered the intervention affected responses to the intervention and initial and continued FC use. Other influential factors included type of sex work establishment, women's experiences trying the FC, cultural and other differences by ethnicity (e.g., Han majority vs. Li minority), prior use of other prevention options (including male condoms as well as popular "remedies" like douching and antibiotics), and town-level social dynamics.**CONCLUSIONS:** Community research collaborations have significant potential for success in designing, implementing, and testing multi-level interventions to increase availability and use of woman-initiated prevention options like FC, and potentially microbicides, to increase these options for high-risk populations like female sex workers.

# TUESDAY POSTERS

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### The Role of MRCZ in Empowering Communities in Zimbabwe in HIV/AIDS Prevention Studies

S. Ruzario<sup>\*1</sup>, R. Chekera<sup>1</sup>, P. Ndebele<sup>3</sup>, E E. Gomo<sup>4</sup>, M. Phiri-Shana<sup>1</sup>, F. Tsumbirani<sup>1</sup>, R. Gunda<sup>1</sup>, O. Zenda<sup>1</sup>, L. Gomo<sup>1</sup>, S. Munyati<sup>1,2</sup>, R.B.L. Zinyama-Gutsire<sup>1</sup>

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**BACKGROUND:** The assumption in research is that if there is meaningful and active community participation in any research, research benefits will be maximised and potential harm and risks are minimised. The Medical Research Council of Zimbabwe, which houses the National Ethics Committee, took it upon itself to educate Community Advisory Board members (CABs) from various HIV/AIDS prevention studies so that they can effectively mobilise their communities. Simplified modules on research ethics were developed specifically for the CABs.

**METHODS:** With assistance from the EDCTP grant, the National Ethics Committee trained CABs from various studies on issues on confidentiality, collective versus individual consent, protocol development, the informed consent process, prevention and treatment studies and results dissemination. Annual CAB forum was introduced where researchers and CABs meet annually to discuss issues on community involvement in research

**RESULTS:** Through this empowerment, the CAB members were able to come up with queries and questions that they wish to be addressed by regulators and researchers. More than 50 enquiries and 20 complaints were received in 2009 as compared to the previous year. Complaints received ranged from photos having been taken without consent, late bus fare reimbursements, rushed consent process and not being enrolled in a study after having been screened and no adequate explanation given.

**CONCLUSIONS:** Through this empowerment, the CAB members were able to come up with queries and questions that they wish to be addressed by regulators and researchers. More than 50 enquiries and 20 complaints were received in 2009 as compared to the previous year. Complaints received ranged from photos having been taken without consent, late bus fare reimbursements, rushed consent process and not being enrolled in a study after having been screened and no adequate explanation given.

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### Attitudes Towards Female and Male Condoms Among Nigerian Female University Students: Predictive Factors and Implications for Future Microbicide Acceptability

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**BACKGROUND:** Predictors of positive attitudes toward female (FC) and male condoms (MC) may be critically valuable in enhancing microbicide acceptability. Objective was to assess attitudes of Nigerian female university students towards FC and MC with a view to determine their implications for microbicide acceptability.

**METHODS:** The survey involved 121 interviews with sexually active female undergraduate students from a premier university in southwest Nigeria. We examined if experience with vaginal products and practices, condoms efficacy beliefs, and communicating about sex with a partner were associated with positive attitudes towards condoms. SPSS 11.5 was used for analysis. P value <0.05 was significant.

**RESULTS:** Mean age of participants was  $20 \pm 1.6$ ; mean age at first sex was  $16 \pm 1.7$ ; 29% had never been pregnant; and 48% had had  $\geq 2$  partners before. Use of contraceptives in the last 2 months prior interview was 19% and 78% with MC and FC respectively. 27% used MC at last sex while 0.9% ever used FC (only 6% ever seen FC). 23% use tampons regularly and 11% used various vaginal practices. Only 19% of participants felt they were at risk of HIV. Attitude about condom use was somewhat or extremely positive (MC: 61%; FC: 24%). Condom efficacy belief to protect against STI/HIV was MC:72%; FC:79%. 77% of participants agreed or strongly agreed that it was easy for them to communicate to their partners what they liked during sex. Belief in condom efficacy to prevent STI/HIV was associated with a positive attitude toward that condom type. Greater comfort in communicating with partners what was liked during sex was negatively associated with positive attitude toward FC (OR:0.52;p=0.012) but not associated with attitude toward MC (OR:0.81; p=0.2).

**CONCLUSIONS:** Study shows that perception of product efficacy affects attitude toward it and that women who're not comfortable discussing sex with partners may exhibit more positive attitude toward female-controlled preventive methods such as microbicides.

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### Comparative Acceptability of K-Y Jelly Versus Female Condoms and Their Impacts on Sexual Pleasure, Sex Partner Multiplicity and Sexually Transmitted Diseases among a Cohort of Sexually Active Women in Lagos, Nigeria

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**BACKGROUND:** Female condom (FC) and microbicides have globally been credited as potential female-controlled mechanisms for reducing the burden of STIs, vaginosis and HIV/AIDS in sexually active women (SAW). However, data on the level of their acceptability coupled with impacts on sexual pleasure, sex partner multiplicity and sexually-transmitted diseases in developing countries are grossly inadequate. This study determined the acceptability of K-Y Jelly and female condoms as anti-sexually transmitted disease tools among sexually active Nigerian women.

**METHODS:** A total of 104 K-Y Jelly and female condom-naïve SAW aged 18–35 years residing in shanties and suburbs of Victoria Island, Lagos, currently being characterized for HIV/AIDS prevention projects were longitudinally studied over a period of 3 months (Feb–April, 2009) after obtaining their informed consent. Fifty-two women each were randomized into two intervention arms (K-Y Jelly vs. FC) of 52 each after giving orientation on their use, personal hygiene and awareness of STI/HIV. Blood samples and vaginal swabs collected from the women were screened for HIV-1 seropositivity, candidiasis and vaginosis respectively before and after interventions using standard serological and microbiological methods. The use of female condoms was monitored by condom count technique, pre-intervention cases of candidiasis and vaginosis were treated appropriately and HIV cases were referred. Data obtained were statistically analyzed.

**RESULTS:** Pre-intervention analyses revealed overall prevalence rates of 7.7% for HIV-1 infection. Overall cases of candidiasis (18.3%), vaginosis (10.8%) and polyclonal infections (5.8%) ( $p < 0.05$ ) were also found with *Candida albicans*, *Trichomonas vaginalis* and *Gardnerella vaginalis* as the commonest aetiological agents. K-Y Jelly usage was associated with greater sexual pleasure (100 vs. 73.1%;  $P < 0.05$ ) by both partners, risk of multiple sex partners (OR = 2.8; 95%CI, 1.2–6.6) and acceptability (92.3 vs. 71.2%;  $P < 0.05$ ) by men but higher incidence of candidiasis (10.2 vs. 4.3%;  $P < 0.05$ ) and vaginosis (8.2 vs. 2.1%;  $P < 0.05$ ) when compared with FC.

**CONCLUSIONS:** Our findings indicate greater potential of female condom as STI/vaginosis preventing tool and suggests availability of lubricant microbicides superior to K-Y Jelly in the context of acceptability and male preference for use by sexually active Nigerian women. Advocacy for improved acceptability of female condom is strongly recommended in Nigeria.

# TUESDAY POSTERS

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Tuesday, May 25, 2010

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### Community Monitoring of the Rollout of Male Circumcision for HIV Prevention and its Implications for Women

C. Feuer<sup>\*1</sup>, C. Odada<sup>2</sup>, A. Kuteesa<sup>3</sup>, M. Katana<sup>1</sup>, J. Kehler<sup>4</sup>, J. Gatsi<sup>5</sup>, C. Dlamini<sup>6</sup>, M. Natukunda<sup>7</sup>, T. Crone<sup>8</sup>, M. Chatani<sup>1</sup>

<sup>1</sup>AVAC; <sup>2</sup>Women Fighting AIDS in Kenya; <sup>3</sup>Women's Treatment Action Group; <sup>4</sup>AIDS Legal Network;

<sup>5</sup>Namibia Women's Health Network; <sup>6</sup>Swaziland for Positive Living; <sup>7</sup>Mama's Club; <sup>8</sup>ATHENA Network

**BACKGROUND:** Pending the rollout of male circumcision for HIV prevention, women in affected communities have identified the potential for increased rates of infection from newly circumcised partners who do not abstain prior to wound healing as well as from a possible increase in men's risk behaviors. Women have also expressed concern that spending on women-focused prevention might decrease and that greater stigma and blame could be directed at HIV-positive women. In recognizing the need to monitor male circumcision for HIV prevention and to amplify women's concerns and inform its scale up to ensure that the intervention is beneficial to men and women, AVAC and ATHENA launched the Women's HIV Prevention Tracking Project (WHiPT) in 2009.

**METHODS:** To inform policies and programs related to male circumcision, WHiPT country teams were formed in Kenya, Namibia, South Africa, Swaziland and Uganda out of networks of women living with HIV who work predominantly at the community level. Each team developed a work-plan tailored to their context and trained women in qualitative data collection to capture local women's impressions of male circumcision. WHiPT teams developed a standard interview questionnaire and focus-group template to be adapted to local contexts. Teams met to evaluate data across countries for common and context-specific themes. An advocacy agenda was shaped according to the data and implemented at a global, country and local level.

**RESULTS:** The central outcome of WHiPT is to build the capacity of women stakeholders to understand and engage with HIV prevention research. WHiPT members gained skills in research literacy with a focus on male circumcision, data collection and analysis, grant writing, report writing and advocacy. The women have built linkages between themselves and researchers/policymakers; strengthened their HIV prevention research literacy; and learned how research translates into policies and programs.

**CONCLUSIONS:** The WHiPT project is a thriving mechanism by which community-based women, who often stand outside of research and policy circles, are able to engage. Further, the project is a vehicle by which a particular cohort of HIV affected communities—HIV-positive women and their networks—participate in the AIDS response, specifically in HIV prevention research, implementation and advocacy.

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### Targeting At-Risk US Women for Trials of Cervical Barriers

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**BACKGROUND:** Women are in need of new, women-initiated, approaches to protect against HIV/STI.

**METHODS:** Review of user acceptability data on cervical barrier methods worldwide and historical family planning experience with these methods.

**RESULTS:** Cervical barrier research for microbicide delivery and STI/HIV prevention has been conducted mainly outside of the US and has found the diaphragm to be a safe and acceptable method. User challenges included practicality of covert use, potential violent partner reprisals, compatibility with vaginal cleaning practices, portability and disposability, and problems of coitus-related insertion. Except for one hypothetical acceptability study among college women, US studies are mainly on low-risk populations of diaphragm users. With the exception of two intervention trials promoting a hierarchy of methods (including diaphragm/cap comprehensive education and fitting) minority US populations, including drug-using women, are to date, not well represented. Promotion of cervical barriers for contraception to minority women has historically been poor, resulting in less frequent use. The cervical cap or varied diaphragm approaches, may represent the 'better barrier' for STI prevention and microbicide delivery; the cap in particular, has longer wear (48 h), is less coitally dependent, more discrete, may protect better due to suction seal, and may not interfere with vaginal cleaning practices while staying secure on the cervix. Because studies have been conducted outside of the US, there is a gap in scientific knowledge on acceptability and efficacy among at-risk US populations, where HIV/STI rates are highest among women, injection drug users, and minorities. Prior studies of cervical barriers among HIV-positive women living in Harlem, and active crack users in Philadelphia demonstrated high interest, citing esthetics, ability for weekend wear, discrete use, continual 'back-up' protection to complement condom use, and contraceptive properties as positive aspects. The numbers of women fitted were small, however. A new pilot study underway in Miami targets Haitian-American women to collect user feedback about cervical caps.

**CONCLUSIONS:** Cervical barrier research should target US at-risk women to develop user preference data.

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### Dual Method Use for HIV/AIDS and Pregnancy Prevention Among Young Couples in Nakasongola District

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**BACKGROUND:** This paper is based on a community study on family planning and sexual behavior in the era of HIV/AIDS carried out in Nakasongola District, Uganda. The study was carried out to understand individuals perceptions of the risk of AIDS and other STDs especially in relation to unwanted pregnancies, strategies considered by sexually active individuals to be appropriate; practical and effective way to cope with these risks; and constraints and opportunities on changing behavior, particularly with reference to partner communication in high HIV/AIDS prevalence areas.

**METHODS:** Both qualitative and quantitative methods were utilized to collect data. Twelve focus group discussions, and a survey composed of 577 men and 620 women were conducted. In addition thirty in-depth interviews were conducted with selected respondents who had participated in the survey to understand factors that increase vulnerability to HIV/AIDS and unwanted pregnancy.

**RESULTS:** The study revealed that dual method use for HIV/AIDS and pregnancy prevention among couples in Nakasongola district is minimal. Only 35.2% of men and 27.7% of women reported using condoms with partner together with another family planning method. When asked to consider the possibility of using a condom together with other methods of family planning in order to avoid STI, some discussants explained that people are already doing so and they need sensitization on the advantage of using both. Women groups however mentioned that there are other better methods of contraception such as pills and injectables than using condoms for pregnancy prevention. In situations where many women use contraception secretly, it would be difficult them to use condoms. Men also prefer using condoms to irregular partners than to regular partner.

**CONCLUSIONS:** It is recommended that all stakeholders in the delivery of youth-friendly reproductive health services and HIV/AIDS prevention need to address the dual use of use of contraceptives and condom for prevention. There are still information gaps and these can be bridged if the stakeholders are involved.

# TUESDAY POSTERS

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### **Standards-of-Care in Microbicide Trials: Do Research Participants have Special Entitlements?**

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**BACKGROUND:** Whether or not researchers are obligated to provide participants in HIV prevention trials with antiretrovirals (ARV) has been the subject of intense international debate for more than a decade. The current consensus of the universal treatment access movement is that, where possible, participants in efficacy trials should be provided with ARV, but the ethical basis of this obligation is contested. It is not clear, for example, whether participants have a special entitlement to receive scarce goods due to their status as research participants or as part of a community-wide benefit predicated on a universal right to health. This study maps trends in the provision of ARV in HIV prevention studies and examines the moral justification for current practice.

**METHODS:**

- Systematic review of standard-of-care and ancillary care provisions in microbicide trials from 2000 – 2009, through analysis of trial protocols, research publications and related 'grey' literature describing the benefits of trial participation to participants.
- Qualitative analysis of statements made by researchers about their decisions regarding provision of ARV to trial participants

**RESULTS:** Eight microbicide studies (phases IIb and III) were included in the review. Access to ancillary care such as antiretrovirals for participants who seroconvert on microbicide trials has changed markedly since 2000, with a shift from no provision of ARV to full access (through linkages; not necessarily provided by sponsor). Reported provision of free condoms and contraception varied between trials. There is conflicting evidence as to how and why researchers recognise specific obligations towards research participants, with variation occurring between and even within trials.

**CONCLUSIONS:** Provision of ARV to trial participants is now the norm, but the cost is not born solely by research sponsors, and may be more a function of improvements in the care of people with HIV than any shift in research practice. Research participants do however have exclusive access to some ancillary care in some circumstances. This suggests that conflicts between the putative universal right of all people to life-saving treatment, the particular obligations of researchers to participants and the doctor-patient dynamic force researchers and research communities into high-stakes moral negotiation regarding benefits.

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### **Promotion and Distribution of Female Condom 2 Among Women and Men of Sexually Reproductive Age in a High-Density Population Community Using Interpersonal Communication (IPC) Conductors**

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*The Good Neighbor*

**BACKGROUND:** Female condom was first distributed in Nigeria in the '90s by federal ministry of health, this first attempt by the ministry to introduce female condom as a family planning method faced a lot of challenges which includes; social, cultural and religious barriers resulting in very low uptake of the product and programmatic challenges that resulted to myths and misconceptions among the population concerning female condom. National Reproductive Health Survey 2005 (NARHS 2005) shows that only 13.4% men 20.6% women of Nigeria population know about female condom inclusion into family planning method.

Mushin community is among the site in Nigeria selected for the implementation of FC2 project. Mushin is a densely populated area in Lagos with a 2006 population figure of 4,973,709 people. The objectives of this project are to; Decrease the incidence of STIs and HIV/AIDS; Decrease unwanted pregnancy; extend the choice of contraceptive methods available.

**METHODS:** The Good Neighbour (TGN) in partnership with The Society for Family Health (SFH) trained twenty-five (25) interpersonal communication conductors for the purpose of promoting and distributing female condom 2 in the community. The IPC conductors were resident members of the community involved in different enterprises like primary health care services (community health promoters and community health educators), nurses, community women leaders, market women, religious women leaders, male out of school, female out of school and female sex workers.

**RESULTS:** Retention percentage of IPC Conductors was 83%. The number of males reached with the female condom message was high in the first month and then decreased gradually in the subsequent months from 1,150 to 1,020. The number of females reached with the female condom message increased from 5,560 in the first month to 7,426 in the third month. A total of 10,000 female condoms were distributed.

**CONCLUSIONS:** Awareness of female condom 2 have been raised in several hot spots in the community, also many primary health care centers and major markets in the community were venues for information dissemination and distribution of female condom.

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### **Monitoring Clinical Trials for ICH-GCP Compliance and Protocol Adherence: Experiences from the Medical Research Council of Zimbabwe**

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**BACKGROUND:** The Medical Research Council of Zimbabwe (MRCZ) is the National Ethics Committee in Zimbabwe. It is made up of a board whose members are nominated by relevant institutions and appointed by the Minister of Health, Zimbabwe. The main mandate of MRCZ is the scientific review, ethics approval and oversight of all ongoing medical research studies in Zimbabwe. The MRCZ secretariat is the functional arm of this Council and carries out inspections and monitoring on behalf of the board using an established Human Protection System. MRCZ is registered with OHRP as an international ethics committee, it adheres and subscribes to all international ethics guidelines on the conduct of human research.

**METHODS:** During the inspection process the following aspects are thoroughly reviewed: protocol version at study site, initial MRCZ approval letters, National Regulatory Body approval in case of drug clinical trials, valid continuing review approvals, staff qualifications and experience, evidence of research ethics and GCP training, data storage, signed and dated participant consent forms, approved study data collection tools, reception areas and procedure rooms. Interviews are carried out with respective study participants. Routine inspections and 'for cause' inspections are carried out to ensure utmost protection of research participants and to ensure protocol adherence and ICH-GCP compliance.

**RESULTS:** The first microbicide clinical trial carried out and completed in Zimbabwe was the Phase II/IIb Safety and Effectiveness Study of the Vaginal Microbicides BufferGel and 0.5% PRO 2000/5 Gel (P) for the Prevention of HIV Infection in Women. A microbicide study that is currently recruiting in Zimbabwe is the Phase 2B Safety and Effectiveness Study of Tenofovir 1% Gel, Tenofovir Disoproxil Fumarate Tablet and Emtricitabine/Tenofovir Disoproxil Fumarate Tablet for the Prevention of HIV Infection in Women. Clinical trials, like these two mentioned above, receive more detailed attention during inspections as they are more delicate studies.

**CONCLUSIONS:** The MRCZ wishes to share its inspection methods and experiences of inspecting microbicide and other research studies.

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### Common Decimals on Community Perspectives on the Ethical Conduct of Clinical Trials: A Review of the Oral Tenovofir Controversy

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**BACKGROUND:** There has been lots of effort to promote community engagement in biomedical HIV research over the last decade. As such, there is an increasing recognition of the benefits of engaging the communities as research evolve far beyond the sole purpose of preventing trial disruption. Despite this growing interest and support for communities to engage in biomedical HIV research, the pace has remained slow. This paper shall review the events that resulted in community agitations and controversies around some oral tenofovir pre-prophylaxis studies in the past. It will share insights into the multiple community interest and concerns about the trial across several countries.

**METHODS:** A review of anecdotal reports of key players involved with raising concerns around the planned oral tenofovir trials in Cameroon, Cambodia, Thailand and Nigeria. A review of the literature on the subject matter. Concerns that were common to all the countries are highlighted.

**RESULTS:** Communities were primarily interested in reviewing the trial oral tenofovir research protocols in order to ensure that trial participants' health related issues were addressed. Health related concerns include issues of standard of care and the standard of prevention. In addition, despite the fact that the research team conducted community consultations in the form of FGDs in these countries, these did not translate to community consultation from the perspectives of advocates. Advocates will rather have a consultative process that focused on facilitating community inputs into trial design (their main desire) rather than consultations which primarily aimed at achieving support for trial implementation. The conduct of a phase II trial with no Phase I data was also a source of concern. The use of the word 'unethical' did take on a different meaning for the community; different from the working definition by the research team.

**CONCLUSIONS:** It appears that health concerns are of paramount interest to communities when it comes to trial design and implementation. The communities also have mechanisms for consultation on issues which facilitates self education. When the rule and norm of science needs to change, extensive community discussions and consultations may help prior to its utilization in research design as communities equally keep themselves updated on these issues.

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### Role of The Medical Research Council of Zimbabwe (MRCZ) in Protection of Research Participants in Zimbabwe

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**BACKGROUND:** The MRCZ houses the National Ethics Committee (NEC) whose main mandate is the scientific review and ethical approval of all medical research. In Zimbabwe, MRCZ offers research ethics and GCP training to all researchers including those currently involved in HIV microbicide clinical research studies. The role of research ethics in biomedical research cannot be over-emphasized. The research community as a whole suffers when even a few investigators ignore the basic principles of ethics. The consent process should go beyond the written consent form. Researchers need to understand that there should be follow-up and continuous education of the participants throughout the research. There is need for researchers to not over emphasize potential benefits to participants. It is worthwhile for researchers to find innovative ways of describing research to participants. MRCZ is therefore taking a leading role in creating research ethics awareness among researchers.

**METHODS:** Researchers, IRB members, medical students and research teams were trained in research ethics and good clinical practice (GCP). After the training activities, MRCZ carried out routine inspection of all ongoing studies to ensure that researchers are adhering to their protocols and complying with ICH-GCP and other international guidelines.

**RESULTS:** In year 2009, 700 researchers were trained in Research Ethics and Good Clinical Practice (GCP). Those trained were made aware of the current versions of international guidelines on the ethical conduct of research. These guidelines include ICH-GCP, CIOMS and Helsinki Declaration. The training workshops that have been carried out in the last three years have greatly increased awareness of research ethics amongst researchers

**CONCLUSIONS:** There has been significant improvement in the informed consent process as researchers in Zimbabwe are increasingly becoming aware of the importance of protecting the rights and welfare of research participants. In research design of protocols, researchers have the responsibility to ensure that priority is given to the rights and welfare of research participants.

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### Uptake of Referrals to HIV Care and Treatment Services in Mwanza, Tanzania during the MDP 301 Phase III Clinical Trial of PRO 2000 Microbicide Gel

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**BACKGROUND:** During the Microbicides Development Programme (MDP) clinical trial to assess the efficacy and safety of PRO 2000/5 gel participants who were diagnosed HIV positive at their screening visit and those who seroconverted during the clinical trial were referred to the government HIV Care and Treatment services (CTC). This study investigated the uptake of the CTC services by trial participants and using qualitative research methods, explored the enablers and barriers to uptake of these services.

**METHODS:** Staff at the CTC clinics were asked to collect MDP referral slips at the first visit. Trial clinicians visited the clinics regularly to collect slips. Qualitative research involved in-depth interviews (IDIs) with HIV positive women (2 screening/CTC attenders, 4 seroconverters/CTC attenders, 3 seroconverters/CTC non-attenders), focus groups (FGDs) with HIV negative trial participants (2) and participatory learning activities with participant representatives.

**RESULTS:** From September 2006 to February 2008, all women diagnosed HIV positive during screening (317) and all women who were positive at baseline or seroconverted during the trial (19 out of 1146 women enrolled) were referred to HIV CTC services. Women who seroconverted were given intensive support to attend the CTC centre. Of these, 42 (13%) women positive at screening attended CTC services and 7 (37%) seroconverters attended CTC services ( $p=0.005$ ). IDIs and FGDs revealed a good understanding of the cause and treatment of AIDS by HIV positive and negative women. Barriers to attending services were identified as disbelief of diagnosis, stigma, fear of disclosure to partners, disbelief in treatment effectiveness, or belief that God would provide a cure. Enablers to attending were good knowledge, supporting partner or family, or positive perceptions about the CTC clinic.

**CONCLUSION:** Referral was more effective with seroconverters due to the intensive support provided by the team, including follow up if they did not attend. Poor uptake of CTC services should be addressed during HIV prevention trials by offering support for HIV positive participants to attend, such as escorting the participants to the clinic or offering follow up counselling. Further investigation into community barriers to the uptake of CTC in Tanzania is also needed.

# TUESDAY POSTERS

## 389

### A Comparison of Peer-mediated HIV Prevention Interventions Under Different Environments for Female Sex Workers in Kenya

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*Bar Hostess Empowerment and Support Programme*

**BACKGROUND:** Many salonists (hair dressers and beauticians) double up as part time sex workers in Kenya. Peer-mediated HIV prevention interventions among female sex workers (FSW) have been the cornerstone of most sex worker organizing programs in Kenya. A comparison of different role of the environment under which the interventions are carried out was evaluated

**METHODS:** A pre-intervention survey in Nov.2007, recruited 342 FSW using snowball sampling. 16 peer educators were trained (8 full time sex workers, 8 salonists/part time sex workers). Thereafter, the peer educators provided STI/HIV education, condoms, and facilitated HIV testing, treatment and care services. In Nov. 2009, data were collected using identical survey methods, allowing comparison with historical controls, and between FSW who had received peer education from salonists, sex workers or had not received peer interventions

**RESULTS:** Over the two years consistent condom use with clients increased from 52.0% (178/342) to 82.4% (285/346) as well as the likelihood of refusing clients who were unwilling to use condoms. In Nov.2009, FSW who received peer interventions from salonists (35.1%, 120/342), had more consistent condom use with clients compared with FSW who received peer education from other sex workers and those unexposed FSW (90.1% versus 87.5%; 58.4%). HIV prevalence was 10.6% (8/85) in FSW attending salon peer education sessions compared with 10.9% (11/101) in those attending sex worker (streets/ bars) peer education, and 18.3% (13/71) for FSW not attending peer education sessions. FSW who attended salon sessions indicated they spent more time with their peer educators compared with FSW who were attended to by other sex workers in the streets/ bars on average (35 versus 20 minutes). They were more comfortable to read IEC materials and collect condoms from the salons than from other sex workers on the streets

**CONCLUSIONS:** Peer-mediated interventions were associated with an increase in protected sex. Though peer-mediated interventions remain important, sex workers feel more comfortable receiving information/ prevention materials in a free and secure environment. Drop in centres in selected salons are an effective intervention in HIV prevention for sex workers

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### Nurses' and Doctors' Knowledge and Opinions About Microbicides in Southern Africa

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**BACKGROUND:** Primary care providers will be a critical resource for information and supplies when an effective microbicide becomes available. Provider knowledge and opinions about microbicides and their ideas about patients' needs for pregnancy and HIV prevention are important inputs for developing an acceptable microbicide that women can use; as community leaders their knowledge and opinions during product development can also influence public and political support for trials and funding.

**METHODS:** Based on formative qualitative work, as part of a larger study on HIV/pregnancy prevention counseling and practices, we surveyed nationally representative samples of nurses and doctors in Zimbabwe (n=846) and South Africa (n=624). We asked providers about their clients' needs for HIV/STI prevention and contraception, current counseling and service provision practices, knowledge about microbicides and opinions about whether they would recommend and whether their clients would use a microbicide once it became available. We compared responses across country and provider type; final results will include analysis of responses by gender and other demographic variables (rural vs. urban, education, age, etc.).

**RESULTS:** Preliminary results show that more than 75% of providers in South Africa and 47% of providers in Zimbabwe believe it is highly important for their patients to have access to HIV/STI prevention methods without their male partner knowing (>=6 on a scale of 1 to 10). Providers in South Africa were more than twice as likely to report their patients needed a contraceptive they could use discreetly (42% v 16%). 5% of providers in Zimbabwe and 11% of providers in South Africa reported they were familiar with microbicides. 69% of providers in Zimbabwe and 59% of providers in South Africa said they would recommend a 33% effective microbicide. In both countries fewer providers were comfortable recommending microbicides to teenagers and most reported their clients would use a microbicide sometimes or usually.

**CONCLUSIONS:** Providers are a critical constituency and few were familiar with microbicides. Their keen awareness of their patients' needs for discreet HIV/STI and pregnancy prevention options and their support for the idea of a microbicide mean there are many opportunities to partner to raise awareness as products are tested and once they are proven effective and are ready to be introduced.

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### Predictors of Delayed Sexual Debut Among Youth in the Nyanza Province, Kenya

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**BACKGROUND:** Describe the distribution of age at sexual debut by gender and identify predictors of delayed sexual debut (after 16 years of age) among 15- to 24-year-old youth in the general population within Kisumu, Kenya.

**METHODS:** We conducted a cross-sectional study in which every fourth household from 40 clusters throughout Kisumu District was systematically sampled. Everyone aged 15–49 who slept in the house the night before the interview was eligible to participate. Informed Consent was obtained and/or assent and then collected data on socio-economic and demographic attributes, and behavioral and cultural factors. Cox proportional hazards model was used to analyze data.

**RESULTS:** Of the 1655 participants in the study, 910 (55%) were youth aged 15–24 years. Of these youth, 55% were female and 53% had at least a primary level education. The average age of sexual debut was 16 years. The median abstinence time before first sex was the same (18 years) for both males and females. However, after age 18 the proportion of females abstaining from first sex was consistently higher than for males. None of the variables considered were independently associated with delayed sexual debut.

**CONCLUSIONS:** In this population with high risk sexual behavior and STI prevalence, none of the socio-economic and demographic factors considered could independently predict delayed sexual debut. This could be an important finding for programs aimed at delaying sexual debut to focus on all the youth regardless of their socio-economic and demographic attributes.

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### Sexual Networking in a Nigerian University: Implication for HIV Transmission

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**BACKGROUND:** Tertiary institutions world over provide a platform for young people to get educated, enhance their personalities and build skills that enable success in their chosen career paths. However, these institutions have also been identified as "hotspots" for the transmission of sexually transmitted infections including HIV/AIDS. This study aims to identify the social and environmental factors involved in sexual networking with a view to informing evidence-based policies and programs targeted at HIV prevention amongst youth.

**METHODS:** The study population employed a two-stage sampling method aimed at selecting eligible people.

Study was cross sectional in design, and involved 480 randomly selected unmarried undergraduates. Data was collected using self-administered questionnaire. Data was analyzed using bivariate and multivariate analysis.

**RESULTS:** Males constituted 51.5%, and 64% of respondents were between 18–24 years. A higher proportion of males (69.6%) were sexually experienced compared to females (57.2%) ( $p=0.006$ ). While the majority of sexually experienced people did not have first sexual experience till late adolescence, 21.8% of males and 8.8% of females had their first sexual experience before the age of 15. The first sexual partner was older than the respondent in 36.1% of sexually experienced males and 94.3% of females. 8.6% of male and 9.4% of female respondents indulged in homosexuality. 60.1% of males and 51.4% of females used condom consistently in the six month prior to the survey. Significantly higher proportion of males were engaged in sexual networking (70.6%) compared to females (47.1%). Binary logistic regression showed only sex as a significant factor for multiple sexual engagement ( $OR=2.98$ ; 95% C.I. = 1.49–5.95). Factors significantly associated with sexual networking included sex ( $p=<0.001$ ) age of first sexual exposure ( $p=<0.001$ ), level of income ( $p=0.03$ ) and off-campus residence ( $p=0.017$ ). Knowledge of HIV/AIDS was not significantly associated with networking.

**CONCLUSIONS:** There is high level of sexual networking among undergraduates in tertiary institutions, and implies that campus environment could be high-risk locations for HIV transmission. Thus, there is an urgent need to intensify HIV behavior change communication programs in campuses.

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### Unmet Need for Safe Sex Practice and Opportunities for Vaginal Microbicides Among HIV-Uninfected Female Sex Workers (FSW) in India

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**BACKGROUND:** Prevention of HIV transmission in core groups such as Female Sex Workers (FSW) is likely to reduce further transmission to bridge populations and the general population. Possible HIV prevention options among FSW include vaginal microbicides. Objective of this paper is to explore the vulnerability and safe sex practices among HIV uninfected FSWs in India.

**METHODS:** Data are drawn from the first round of Integrated Biological and Behavioural Assessment (IBBA) study that aimed at evaluating "Avahan, the India AIDS initiative," a large HIV prevention programme among high risk populations. District-wide probability sampling methods such as conventional cluster sampling or time-location sampling were used to recruit FSWs from 29 of 83 Avahan districts in 4 states of India in 2007. The paper reports on data from over 8000 HIV uninfected FSWs among those surveyed.

**RESULTS:** 60% of the FSWs were illiterate and they were practicing commercial sex work for over 6 years with at least 2 different types of partners. Types of sexual partners included occasional clients, regular paying clients and regular non-paying clients. Consistent condom usage with regular paying and regular non paying partners was 66% and 14% respectively. Anal sex was also reported by 13% and 69% had used condom at the last anal sex. Nearly 20–25% FSWs reported condom breakage or inability to convince clients to use condoms in the previous month. Of the one in three FSWs who had awareness and risk perception about HIV transmission; half had their HIV test done. Symptoms of Sexually Transmitted Infections (STI) were reported by 49% in the last one year and currently 29% were symptomatic. Symptoms of Sexually Transmitted Infections (STI) were reported by 49% in the last one year and 29% were symptomatic at the time of the survey. Of these HIV negative FSWs, 2300 were examined for four STI pathogens. 66% tested positive for at least one; 9% tested positive for syphilis by RPR, 2.7% for Gonococci by GC NAT, 5.1% for Chlamydia trachomatis by CT NAT and 63% for HSV2.

**CONCLUSIONS:** Data clearly highlight huge gaps in safe-sex practices among FSWs in India, predisposing them to HIV and STI. This unmet need for protection can be fulfilled by vaginal microbicides.

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### Are Women Ready for Change?: Gender Power Relations in North Western Tanzania: Implications for Gender Empowerment and Sexual and Reproductive Health Programmes

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**BACKGROUND:** Unequal gender power relations in families and sexual relations have been partly responsible for women's sexual and reproductive health problems. Benefit from women-initiated prevention methods such as microbicides require that women are sufficiently autonomous in making decisions concerning their sexuality. This study explores women's views about culturally prescribed norms of behaviour of subordination and decision making in families and sexual relationships and how they would like the power balance to be.

**METHODOLOGY:** This study employed an ethnographic research design. Data collection involved eight weeks of participant observation, 17 focus group discussions and 46 in-depth interviews conducted with young people aged 14–24 years and parents of young people in this age group. Thematic analysis was conducted with the aid of NVIVO 7 software.

**RESULTS:** Themes that emerged indicate that men are still the overall decision makers in most families and in sexual relationships. Women seem happy with the status quo for several reasons—social security (reputation) and economic support. Family members including mothers and sisters socialize male family members as superior to the females. Although young women have some power in making decision at the start of a relationship (e.g. concerning sexual exchange and whether or not to have sex with a condom), this power seems to diminish once they consent to sex. After one or several sexual encounters, men take control. A good sexual reputation is something that most women aspire to have and maintain and young women may do this by having one secret sexual partner that they are submissive to always (may not want to challenge or question). Women, both married and single, referred all issues that required decision making to men (e.g., discussion of daughter's marriage, sale of property). The few women who were able to exercise some agency were sometimes stigmatized and labeled as behaving like men.

**CONCLUSIONS:** Most women seem to be content with the status quo of men being in control in decision making on their lives and young people are socialized to obey and perpetuate this. These findings have implications for sexual and reproductive health interventions especially those interested in empowering women in decision making in their families and in sexual relationships. There is need for reflective dialogue or feedback with the community so as to come up with ways through which norms around socialization can be tackled.

# TUESDAY POSTERS

## 395

### **Microbicides in Africa—The Importance of Involving Both Men and Women in Their Usage**

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**BACKGROUND:** HIV/AIDS pandemic in Africa has always had a female face. The reasons given are varied, from their anatomy to socialization of the women and girls especially in Africa.

The escalation of HIV infection among women is largely because women's position in society is inferior to men's and they often are unable to make informed choices about their sexual health. The inferiority complex is made worse by most of our cultures where a woman who negotiates on Condom use is seen as promiscuous.

**METHODS:** Randomly selected and Interviewed 15 men and 20 Women, The method used was individual interviews with 2 Focus group discussions with 7 young Men aged between 20 and 25 years, and Women 5 Women over 35 years. Mostly who are students in universities and colleges in Nairobi.

**RESULTS:** More than 75% of the interviewees agreed that Vaginal microbicides would be a major step in the reduction of HIV/AIDS new infections since they give women bargaining power as far as sex is concerned.

The majority of the men interviewed 82% expressed that they would like their partner to use a microbicide but they strongly stressed that they would like to be involved in the decision to use a microbicide. On the other hand 90% of women interviewed agreed that microbicides use was an idea whose time has come. Out of that 73% (mostly single) Women felt that they did not need to inform their partners if they were using any form of microbicides. On the other hand married women expressed the opinion that they would need consent of their spouses before using any form of microbicides since they would not want to create suspicion in their relationships. 23% of the participants expressed suspicion that microbicides would be used to finish the African population.

In addition majority of the participants (82%) felt that users need to know the side effects of the products in advance before using any of them. In addition 87% expressed their fear that if made too available, it would encourage promiscuity amongst young people.

#### **CONCLUSIONS:**

- Information on the use of vaginal microbicides, although meant for women, should be given to men since most women interviewed (especially married women) felt that they need their partners permission to use them.
- In order to make the microbicides acceptable, the role of the African researchers and Scientists should be strongly stressed so that there will be no suspicion that the microbicides are "tools of finishing the African population."
- The options of contraceptive-based microbicides vis-a-vis non-contraceptive microbicides should be available.
- Microbicides use by women should not be seen as a way of reducing Condom use by Men, ideally both partners should use them, and there is need to package information on Microbicides in a way that will cause Men to become interested and positive in their introduction.
- Microbicides are welcome to both men and Women since they give everyone bargaining power on sex, although Women need more assurance that their use would also avoid other Sexually transmitted diseases.

## 396

### **Participation Diversity in HIV Prevention Studies: An Urgent Need Among Most at Risk Populations in Malawi**

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**BACKGROUND:** Research and media reports show that there about 10,000 men who have sex with men (MSM) in Malawi. HIV prevalence amongst MSM is higher than the generalized rate of 12%. Studies have demonstrated a high prevalence of infection among men who have sex with men (MSM) being 21%. While a number of studies have examined the acceptability of HIV prevention research such as microbicides and HIV Vaccine clinical trials among heterosexual subjects, little has been done on MSM in Africa. There's also high level of stigma against homosexuality in Malawi.

The aim of the study was to assess MSM interests, knowledge, awareness and willingness to and concerns regarding participation in trials of microbicides and HIV Vaccine in 5 districts in Malawi.

**METHODS:** 300 MSM were included in the study from December 2008 to October 2009. 180 were interviewed using focus group discussion, 120 responded to a questionnaire loaded into a Personal Data Assistant (PDA) to assess knowledge on HIV vaccines and microbicides trials. A Geographical Position System was used to locate geographical location of the respondents.

**RESULTS:** The response rate on sample was (98%; median age 25 years) completed the study questionnaire. 80% were not aware of any clinical trial going on. When informed of the ongoing research, 67% of MSM indicated willingness to participate in microbicides and HIV Vaccine research. A total of 93% indicated willingness to disclose that they were MSM to a health professional in order to obtain the vaccine and microbicides.

**CONCLUSIONS:** Diversifying participation in HIV prevention clinical trials to all most at risk populations would help accelerate equitable and fair results of HIV prevention products among high risk populations in HIV and AIDS. MSM in Malawi appear willing and interested in participating in HIV prevention studies, despite high levels of stigma prevailing.

**397****Peer Education Training Outcomes as Part of HIV Prevention Clinical Trials in Communities**

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**BACKGROUND:** Peer education involves training and supporting members of a given group and is often used to effect change at individual or societal levels; this can result in the modification of attitudes, beliefs or behaviours. Peer education draws on the assumption that certain members of a given peer group can be influential in eliciting individual behavior change among their peers.

The primary focus of the HPRU Peer Education programme initiated in 2008 was to educate clinical trial participants who assisted with awareness of the trial in their community. Peer educator training sessions included training on the transmission and prevention of HIV and other sexually transmitted infections. Refresher sessions included feedback from peer educators on implementation of their role. Here we review the outcomes of the trainings, as reported by the participants.

**METHODS:** 28 peer educators from six MRC clinical trial sites were recruited in 2009, to participate in in-depth interviews which aimed to evaluate the effectiveness of the peer programme. 22 peer educators participated in in-depth interviews conducted by an individual not linked to any of the clinical research sites. The data gathered in the in-depth interviews was analyzed through thematic analysis.

**RESULTS:** Peer educators reported that through their training they were able to conduct community education about condom use, importance of testing for HIV, and health promotion. The training gave the peer educators confidence to expand their community work with some reporting initiatives such as soup kitchens, being viewed as lay counselors in their community, providing health information and involvement in home based care. The peer educators training feedback has resulted in iterative changes to the training sessions to accommodate the training needs reported by the peer educators such as ARV training.

**CONCLUSIONS:** The HPRU peer training programme has improved community HIV education, skills development and increased community involvement. The peer programme has addressed misperceptions and rumors about trials and results through feedback from the peer educators. It has also shown the importance of continuously updating the knowledge and skills of peer educators to increase their value to the group, and highlighted an increasing demand for training and education.

**398****Exploring Adolescent Girls' Protection Strategies against HIV in Lusaka, Zambia**

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**BACKGROUND:** For microbicides to deliver on their promise of preventing a significant number of HIV infections, they need to be adopted by young women, among whom incidence rates are higher than men. Yet what do we know about how this target group perceives risk and strategies for protecting themselves? Both before and within marriage, young women are disproportionately infected and affected by HIV/AIDS in Zambia. Prevalence is 5.7% among 15- to 19-year-old females (vs. 3.6% for males), and 11.8 % among 20- to 24-year-old females (vs. 5% for males). Like the adult female population, young female youth are twice as likely to be infected as male youth.

**METHODS:** A cross-sectional survey of 821 young women ages 15–24 was conducted in four urban slums of Lusaka, Zambia to explore their notions of risk and safety, perceptions of HIV risk in particular. The survey sample was randomly selected from four compounds using WHO cluster survey methodology. The survey was supplemented by qualitative data from focus groups and in-depth interviews with a subset of girls in two of the study communities.

**RESULTS:** Overall knowledge levels about HIV/AIDS was high; 86% of respondents correctly knew measures to reduce risk of HIV, 73% knew where to go for HIV testing. Risk perception of HIV/AIDS was high; 38% believed they were at risk, and 33% had been tested at some point. 66% had heard of the female condom, however only 2% had ever used them. Lack of safety in the home, school, and community arose as a significant hazard for girls in this study. 37% reported being threatened by physical violence, 21% reported having been forced, pressured, coerced or tricked into having sex. The vast majority (79%) reported doing something to protect themselves. Key "protection strategies" included: not walking alone, avoiding certain places, having only one partner (24%), abstinence (67%), and condom use (17%).

**CONCLUSIONS:** Sexual violence and coercion, lack of physical safety, and economic dependence on men leave many young women vulnerable to unsafe sex, and given the high HIV prevalence, to HIV. Delivering effective HIV prevention programs and products (i.e. microbicides) to this target group requires attention to these contextual variables and broader structural interventions.

**399****Awareness of University Teachers on Microbicides and HIV/AIDS in Muslim-dominated Northern Nigeria**

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**BACKGROUND:** Awareness of microbicides is still at low ebb among the general public in sub-Saharan Africa. This is likely to be worse in settings where women are largely uneducated and forced by culture and religion to be kept in puddah as we have in Northern Nigeria. Objective was to assess awareness of university teachers on microbicides and HIV/AIDS in muslim-dominated Northern Nigeria

**METHODS:** Level of awareness of 106 consented teachers(60 males, 46 females) were assessed through a pre-tested questionnaire that contained 36 items/questions to be marked either true (T) or false (F). All the participants had postgraduate degrees and were teaching sciences. SPSS 11.5 was used for analysis. P value <0.05 was significant.

**RESULTS:** Mean age of participants was  $43 \pm 6.7$  (males) and  $39 \pm 5.8$  (females). Only 4% of participants had heard of microbicides before with about a quarter of them knowing what microbicides would be used for when they become available. Mean correct answers for questions on sexual transmission of HIV  $49.2 \pm 18.4$  (males) and  $38.1 \pm 19.7$  (females). Mean correct answers on questions on prevention were  $32.8 \pm 22.6$  (males) and  $33.5 \pm 19.4$  (females). The 2 questions most frequently answered wrong by participants were: (1) that it was false that female condom can prevent vaginal transmission of HIV (86%); and (2) that it was true that methylated spirit can destroy HIV (71%).

**CONCLUSIONS:** The level of awareness of well-educated science lecturers on microbicides and HIV/AIDS was abysmally low. There's an urgent need for measures to educate the general public on microbicides and other female-controlled preventive methods.

# TUESDAY POSTERS

## 400

### Does Knowledge Influence Attitude to Condom Use? Observations from a Nationally Representative Sample of Nigerian Youths

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**BACKGROUND:** A good understanding of the patterns of young people's sexual behaviour and associated factors is needed for designing effective policies that can address their reproductive health problems. While HIV/AIDS and STIs still remain a major public health challenge in sub Saharan Africa, it is clear that both risks of HIV and unwanted pregnancy are real among young people because of their low use of preventive services. While studies have been conducted on condom use among adults in Nigeria, research gap exists with regards to young people's knowledge and attitude to condom use.

**Research Hypothesis:** based on the proposition that knowledge of condom can influence the attitudes and practice of consistent condom use and are useful for planning service delivery and condom promotion, this study aims to examine young people's knowledge and attitude to condom use from a unique set of data collected in 2007 from a nationally representative survey of "never married" young people in Nigeria.

**METHODS:** The study population comprises a nationally representative of "never married" young people who participated in the 2007 National HIV/AIDS and reproductive Health Survey (NARHS) in Nigeria. In all, a total number of 3423 respondents participated in the survey. The participants were selected from all 36 states of Nigeria through a multi-stage probability sampling method. A structured interviewer administered questionnaire was used in conducting the study. Univariate and bivariate analysis was done using SPSS

**RESULTS:** 63.4% of the respondents were male while 36.6% were female. The level education was good as over 65% of the respondents have attained secondary school education. Greater than 30% of the respondents have had their sexual debut before age 20. Over 30% of the respondents have never heard about the male condom. Level of sexual activity in the last 12 months is high especially among males. However, only 12.7% of the respondents used a condom at their last sex. When asked about condom efficacy, most of the respondent agreed that that condom protect against unwanted pregnancy however about 40% of the respondent disagreed that it can protect against HIV/STIs. Overall knowledge of condom efficacy is good. Similarly, males and females who have stronger belief in the efficacy of condoms and more positive attitudes to family planning were more likely to initiate sex early.

**CONCLUSIONS:** The special risks of HIV infection and unwanted pregnancy in sub-Saharan Africa make youths an especially vulnerable population. Hence, the need to ensure effective programmes that will reach them. Among other strategies, health education and behaviour change communication programmes targeting young people in Nigeria need to consider how the issues of myths, wrong information, and poor attitude to such simple but critical interventions such as condom use impact behaviour.

## 401

### Integrated Campaign as a Platform for Community Microbicides Mobilisation and Advocacy: Lessons Learnt From Cross River State (CRS) Integrated Measles Campaign (IMC)

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**BACKGROUND:** Community mobilisation and advocacy, is often associated with challenges which need to be solved for efficient future interventions, irrespective of the area. Other child-health intervention programs (Polio, Vitamin A & ITNs) utilized the acceptance and success of measles campaign to reach hitherto difficult communities in CRS. Lessons learnt from that campaign could be applied for successful Community Microbicides trials. Microbicides as a new component of HIV & AIDS prevention strategy is alien to most individuals and communities, therefore devising an effective means of community outreach is crucial for a successful microbicide mobilization and advocacy.

**METHODS:** 935 Participants; 35 supervisors and 900 volunteers drawn from 18 LGAs, were trained by 7 Stakeholders to implement the IMC. The researcher set to verify how elements of communication for community mobilization will impact outcome of IMC. These elements included shared vision, roles delineation and message integration, among the stakeholders and the impact these elements will have on participants. It studied how the stakeholders shared critical information by asking others about the same information one of them already has. It also asked each stake holder the roles they are expected to play during the campaign and compared it actual roles during implementation. Finally, it studied various messages sent by each stakeholder with a communication role to see how the messages converged towards a common goal.

**RESULTS:** 1 of the 7 stakeholders remained focused with shared vision throughout the campaign, others were irregular and infrequent in coordination meetings. 4 committees; Technical, Logistics, Social Mobilisation, Monitoring & Evaluation were created with mixed representation from Stakeholders; however, conflict of interest with each stakeholder conducting separate training for virtually the same set of volunteers marred the committees. 2 out of the 7 consistently sent conflicting messages that were contrary to the common goal instead of coming back to the common forum to have a harmonized message. Despite these challenges, 13 LGAs were covered and 5 recorded low coverage due to poor Communication and difficult terrain.

**CONCLUSIONS:** The first IMC recorded 60% success, which would have been better with harmonized community mobilization efforts. Going by lessons learnt from the IMC, effective and efficient community microbicide mobilization and advocacy must have stakeholders vision clearly defined and shared, duties properly assigned and communication messages converged. This way, microbicides trials would ride on the acceptance and success of other HIV/AIDS intervention programs to mobilize communities for its trials.

## 402

### The Social Construction of Masculinity, Male Sexuality and Acceptability of Vaginal Microbicides Gel: Insights from a Microbicides Trial Setting in Mazabuka, Zambia

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**BACKGROUND:** Understanding masculinity and male sexuality in microbicides research is important because they have a significant role in HIV epidemiology. This study focused on understanding the social construction of masculinity and male sexuality and their implications on the acceptability of vaginal microbicides gel in Mazabuka, Zambia.

**METHODS:** Ten-month ethnographic fieldwork was conducted with men and women through observations, informal conversations; 10 key informant and 12 in-depth interviews; 9 focus group discussions; and documentary review. Data was analysed thematically using QSR NVIVO.

**RESULTS:** Both men and women argued that man should marry, have children and is the head of the house and should provide basic needs. They argued that his sexual desires are strong; hence, initiates sex and should be in charge of sexual encounters and expected to satisfy his partner. They further indicated that man as head of the house should be informed and authorize gel use. Some men indicated that consistent gel use is impossible because sex is sometimes spontaneous. Some women stopped using gel when their partners found it to be cold, slippery and questioned their effect on fertility. Some women liked the gel because it increased sexual pleasure and made the vagina tight, which is an attribute men are perceived to like. Some men liked the gel and saw it as a means for satisfying their partner.

**CONCLUSIONS:** Acceptability of gel use by women reflects the social constructs on masculinity and male sexuality. Masculinity ideals challenge the idea of a woman-controlled HIV prevention product, secret use, and consistency use.

**403****Correlates of Willingness to Participate in Vaginal Microbicide Trials Among Female Medical Students in Ibadan, Nigeria**

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**BACKGROUND:** A Phase 3 trial of a vaginal microbicide was recently terminated at the University of Ibadan, Nigeria with attendant bad press. This study was conducted to assess the willingness to participate as subjects in future trials of vaginal microbicides among female medical students of the University of Ibadan, Nigeria.

**METHODS:** Self administered questionnaires were completed by 151 respondents with age ranging from 19 to 25 years. SPSS version 10 data editor was used to analyze data. Univariate odds ratios and 95% confidence intervals (95 % CI) were used to evaluate the correlates of willingness to participate (WTP) in future trials.

**RESULTS:** A total of 76 (51%) of the respondents reported that they will be willing to participate in future vaginal microbicide trials. Higher willingness was associated with previous involvement in high risk sexual behaviour (OR = 1.44, 95%CI: 1.08–1.22), better knowledge about microbicides (OR = 1.37, 95% CI: 1.14–1.45) and availability of incentives (OR = 1.79, 95% CI: 1.01–1.48). Decreased WTP was associated with concern about the discontinuation of the previous trial (OR = 0.62, 95% CI: 0.21–0.54), social stigmatization (OR = 0.51, 95% CI: 0.52–0.78) and coloured microbicides (OR = 0.71, 95% CI: 0.36–0.83).

**CONCLUSION:** The level of WTP recorded indicates that despite the bad press occasioned by the discontinuation of the previous trial, medical students were still had a reasonable WTP. Further work has to be done to educate this community about vaginal microbicides and the reason why some trials may be discontinued mid-way.

**404****Sexual Behavior of Part-time and Transactional Sex Workers in Nepal**

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**BACKGROUND:** Studies on sex work in general are common giving substantive findings on the importance of HIV prevention. Understanding the needs for the part-time and transactional sex workers is a new area of intervention. Unlike characteristics of the part-time sex workers similar in nature with the general sex workers who take this profession as their only means of livelihood, transactional sex workers are difficult to identify. They are further reluctant to be named as sex workers. Studies on part-time and transactional sex-workers are very few in South Asia, particularly in Nepal. This study aims to investigate the sexual behaviours of part-time and transactional sex workers in Nepal

**METHODS:** This was a cross-sectional study. Two hundred and forty part-time and transactional sex workers from urban areas were interviewed using semi-structured interview guidelines. Descriptive analysis of variables was done using SPSS to identify the extent of sexual behaviours of the sex workers.

**RESULTS:** The mean age of the sex workers was 32.5 years. About 66% of the sex workers were born in rural areas, but 75% were currently residing in urban areas. Identified sex workers had mixed ethnic groups, two-fifth were reported to be illiterate, and one-third was unmarried. The mean age at which they first entered into sex trade was 20.3 years with the main reasons for meeting personal (73%) as well as family (67%) needs. The main reason for continuing this activity was for meeting basic needs (86%). On an average they had sexual relations with 3 persons in a week. Three in every 10-sex workers reported not using condoms regularly. About 24% had reported experiencing STI related symptoms. Government employees, security personnel and businesspersons were their main clients.

**CONCLUSIONS:** Since many of the sex workers had not used condoms consistently promotional activities on condom use should be further strengthened. Sex workers need improved access to STI services. As the main reason for entering and continuing sexual activity was to fulfil the family as well as personal needs the program thus should identify alternatives to sex work such as income generation.

**405****Online Survey to Identify and Analyze Factors Related to the Acceptability and Willingness to Use Microbicides among the MSM Population in Greater Buenos Aires, Argentina**

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**BACKGROUND:** Between 2001 and 2007, it was estimated that unprotected homosexual male intercourse accounted for 31% of new HIV diagnoses in Argentina. New infections in men who have sex with men (MSM) occur predominantly among well-educated men and young men. Homosexual male transmission has not changed significantly its percentage between 2001 and 2007 (31.1% and 32.9%). Despite the lack of change in the means of transmission, we have failed to provide an answer to decrease the infection risk.

**METHODS:** The objective of the study is to identify and analyze factors related to the acceptability and willingness to use new prevention methods such as microbicides among the MSM. During the study 200 MSM with different HIV status living in Greater Buenos Aires will be surveyed through an online questionnaire. The stages of development included, firstly, the modification of a previous microbicides acceptability ACASI survey used with heterosexual women. Secondly, interviews with MSM participants will be conducted in order to obtain key information to modify the questionnaire regarding sexual practice, language, and culture. The survey applied in the first stage of the study includes variables such as: socio-economical level, age, education, HIV status, perception of risk, presence of couple violence, condom use, lubricant use, rectal douching, use of rectal products, use of prep and post-profilaxis, ARV adherence, sexual practices and psychological aspects such as: gender-roles, attitudes towards health, HIV-related knowledge and use of prevention methods.

**RESULTS:** The conducted pilot study among 27 MSM determined appropriateness of the survey and revealed that 42, 9% always use condom. 50% would definitely like to use a microbicides in the future and 36, 7% would possibly like to use one. Results also indicated a low level of use of prep and post-profilaxis, 96, 7% never use it. The survey is in the collect process and final results are expected by mid year.

**CONCLUSIONS:** New alternatives for HIV and STI prevention are necessary for this group, such as microbicides and prep and post profilaxis. New microbicides safety studies have began but few acceptability or behavioral studies have addressed what types of products MSM may be willing and able to use

# TUESDAY POSTERS

## 406

### Attitudinal Barriers to HIV/AIDS Prevention Among Youths

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**BACKGROUND:** The issue under investigation was a Comparative Analysis of The Influence of Attitude on The Use of HIV/AIDS Prevention Methods amongst Youths.

**METHODS:** For This Research, Two Groups of Respondents were used: University youths and Out-of-School Youths. Five Northern States were purposively sampled. 50 university students and 50 out-of-school youths were administered questionnaires and interviewed (in the event they were illiterate). SPSS ANOVA one-way test was used to test the significance of their use of preventive methods of HIV.

**RESULTS:** The results from the field indicated that there was a significant difference between the users and non-users of preventive methods in all the states (for the out-of-school) youths sampled. This was attributed to the deep traditional and religious beliefs despite being exposed to the radio jingles and other advocacy channels. They believed that their life and death is "destined" hence, no need to take extra precaution as they believe death is certain and is determined by "fate." In other words, there were substantially less users of preventive methods than users.

For the university students, four (4) out of five (5) did not have any significant difference between users and non-users. It is noteworthy to state here that respondents who did not use protection or prevention methods always were categorised as non-users. The results here were attributed to various reasons: the sexual activity not being "planned," that is "seizing an opportunity," sex partners often co-habiting thus leading to mutual trust—further encouraging unprotected sex. The last states experienced significantly more students using preventing methods as even female respondents who were in "relationships" carry male condoms on them "just-in-case."

**CONCLUSIONS:** Factors such as "destiny," "unplanned" sexual activity should be incorporated into advocacy sessions to help curb the present trend. The "just-in-case" factor as observed in one of the universities should be promoted for youths. This will increase knowledge and the use of HIV/ADIS preventive techniques.

## 407

### Family-Related Challenges Among HIV-Positive Women

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**BACKGROUND:** Experiences leading to and following awareness of HIV status can be very challenging for women. Such challenges needs to be well documented and used as guide for support facilities/intervention put in place for women. This study documents such familial challenges experienced by HIV-positive women.

**METHODS:** This was a cross-sectional study of 396 HIV-positive women accessing care at the PEPFAR clinic University College hospital, Ibadan. They were selected by systematic random sampling technique. Data were collected on socio-demographic characteristics and familial challenges emanating from knowing HIV status, using semi-structured interview. Descriptive statistics and chi-square were used for analysis.

**RESULTS:** The mean age of the participants was  $34.8 \pm 9.0$  years. Three hundred and fifty four (89.4%) respondents had formal education. Seventy-six percent were employed. A majority, 207 (52.3%) were married, 13 (3.3%) widowed but remarried, 14 (3.5%) separated but remarried, 13 (3.3%) married but living separately, 54 (13.6%) widows, 5 (1.3%) widow in steady relationship, 34 (8.6%) separated, 4 (1.0%) divorced, 21 (5.3%) single and 31 (7.8%) single in steady relationship.

Among the respondents, 27(6.8%) separated because of HIV, 41 (10.4%) were widowed from HIV, 15 (3.8%) did not know if their husbands died of AIDS or not. Forty one (10.4%) of the women had at least one HIV positive child. Factors that prompted respondents to access HIV screening included ill health (50%), pregnant status (27.3%), partner's ill health (6.8%), voluntary screening (5.6%), partner's death (4.5%), child's sickness (4.3%) and accident and blood transfusion (1.5%).

Among the respondents, 314 (79.3%) were reportedly sexually active and they reported their partners' disposition towards condom use. Eighty one (25.8%) reported that their partners do not like using condom at all, 80 (25.5%) reported that their partners only endure it, 73 (23.2%) reported that their partners enjoy it, 67 (21.3%) reported that their partners complain about it, 10 (3.2%) reported that it annoys them, 3 (1.0%) reported that their partners are fed up.

Majority of the 262 respondents whose partners were aware of their HIV status were married 172 (65.7%), ( $p < 0.05$ ). Other marital challenges reported among the women included difficulty in getting choice partner 33 (8.3%); concerns about spousal disclosure and partners' negative HIV status 33 (8.3%).

**CONCLUSIONS:** Voluntary testing is not yet practiced among women and the familial challenges following awareness of HIV status is burdensome. There is the need to put in place support facilities that will specifically address the unique needs of HIV positive women.

## 408

### Behavioral Recall Accuracy in a Microbicide Trial: Effects of Psychological Stress, Serostatus and Timing of Interview

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**BACKGROUND:** Nested case control studies comparing seroconverters and nonseroconverters may provide an efficient way to investigate relationships between adherence, exposure, and product effectiveness in microbicide trials. However delays in timing of interviews and differential stress may lead to biased recall if participants are interviewed after receiving positive or negative HIV test results. We assessed recall accuracy as part of a case control study nested with the CAPRISA 004 microbicide trial evaluating safety and effectiveness of tenofovir gel.

**METHODS:** Trial participants with indeterminate or positive rapid HIV test results at any study visit ( $n=80$ ) and a randomly selected comparison group with negative rapid results ( $n=216$ ) were recruited to participate in an in-depth behavioral assessment. 120 participants were recruited prior to post-test counseling (107 negative controls, 13 indeterminate/positive cases) and 176 after counseling (109 controls, 67 cases). All participants completed a Perceived Stress Scale (PSS), an Impact of Event Stress Scale (IES), and questions assessing accuracy of recall of procedures conducted at the most recent previous study visit (RPS). Responses were evaluated using two-sample T-tests.

**RESULTS:** No significantly different results were observed in total PSS, total IES or total RPS scores for cases and controls interviewed prior to counseling. For those interviewed after counseling all scores differed significantly by serostatus (total PSS  $p=0.031$ , total IES  $p=0.007$ , total RPS  $p<0.001$ ); there was also a greater delay between testing and timing of the interview for cases (mean=17 days) compared to controls (mean = 3 days).

**CONCLUSIONS:** Knowledge of non-negative HIV test results is likely to lead to delays in the timing of interviews and to induce psychological stress; both may impact recall accuracy for self-report data. The resulting potential for bias needs to be addressed in the design and implementation of nested case control studies assessing relationships between adherence, exposure, and product effectiveness in microbicide trials.

**409****Predictors of Intravaginal Cleansing During a 4-arm Prospective, Randomized Study of the Diaphragm with Candidate Microbicide**

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**BACKGROUND:** Women who cleanse intravaginally may disrupt their vaginal environments in ways that predispose them to adverse health outcomes. Relatively little has been written regarding the potential effect of microbicides and other barrier contraceptive methods on women's intravaginal practices.

**METHODS:** In a 4-week prospective, randomized study of Malagasy sex workers, we evaluated associations between self-reported intravaginal cleansing and randomization to use diaphragm with candidate microbicide gel (Acidform), diaphragm with placebo gel hydroxyethylcellulose (HEC), Acidform alone, or HEC alone. We evaluated changes in self-reported intravaginal cleansing across the study and the effects of treatment assignment and key covariates on frequent (more than once daily) intravaginal cleansing. Predictor variables that were significant in domain-specific models of frequent intravaginal cleansing were evaluated in an all-domain multiple regression model.

**RESULTS:** The proportion of women reporting intravaginal cleansing decreased from baseline (97%) to Week 1 (82%) ( $p < 0.0001$ ). Self-reported frequent intravaginal cleansing decreased from baseline 87% to 56% during the same time period ( $p < 0.0001$ ). The Acidform-diaphragm group had 60% lower odds of frequent intravaginal cleansing (odds ratio: 0.4, 95% confidence interval: 0.2, 0.9), compared to the control group (HEC-only). HEC-diaphragm and Acidform-only users did not differ from HEC-only users. Study site, marital/cohabitation status, having a main partner, gravidity, and adherence to use of diaphragm/gel during sex were significant predictors of frequent intravaginal cleansing.

**CONCLUSIONS:** As compared to other treatment assignments, assignment to Acidform-diaphragm was most conducive to avoiding frequent intravaginal cleansing.

**410****Refining Recruitment in HIV Prevention Trials Through Analysis of DHS Data**

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**BACKGROUND:** One of the greatest challenges faced by microbicide and other HIV prevention effectiveness trials has been their ability to recruit sufficiently high-risk but HIV-negative individuals. We set out to determine whether use of biological and behavioral data routinely collected through Demographic and Health Surveys (DHS) could assist in identifying sub-populations within a given country at greater risk of HIV infection in order to refine recruitment approaches for future trials. Specifically, we hypothesized that population sub-groups (or, latent classes) could be characterized by different levels of HIV knowledge, attitudes, risk perception and self efficacy; that membership in these latent classes would vary by socio-demographic and geographic variables and be associated with different levels of HIV risk.

**METHODS:** Using data from the Tanzania 2003 HIV/AIDS Indicator Survey (AIS), we identified or developed psychosocial, socio-demographic and geographic measures based on an a priori conceptual model. We fit latent class models based on six psychosocial variables from a sample of 4,568 sexually active women for whom an HIV test result existed and were either HIV negative or did not report a previous positive HIV test result. We examined relationships between class and socio-demographic and geographic variables and then conducted logistic regression to identify predictors of HIV risk. Finally, we replicated our approach with male data from the same dataset and with female data from the Côte d'Ivoire 2005 DHS.

**RESULTS:** Four classes emerged from the latent class modeling based on women's responses to the six psychosocial variables. All six variables showed significant differences between classes. Socio-demographic and other variables also varied by class. Latent classes significantly varied by HIV status. The largest or "typical" group had the highest HIV prevalence rate, followed by a rural, traditional group. The urban traditional and urban empowered groups had the lowest rates. (Similar patterns were observed in the male data from Tanzania and the female data from Côte d'Ivoire.) Geographic variations in HIV prevalence and proportions of latent classes were also evident.

**CONCLUSIONS:** Given that most trial site identification activities currently rely on personal networks and/or intensive, but limited, primary data collection efforts, this approach has the potential to provide a more systematic, inexpensive and relatively rapid approach to identifying countries, regions and sub-districts for HIV prevention trials. By comparing the models developed from different country or regional-level DHS data, it may also provide new insights into the role that culture plays in shaping HIV risk, leading to better tailoring of recruitment strategies to the local contexts in which a trial is situated.

**411****I Am Forever Scared: The Sexual and Reproductive Health Needs and Rights of Women Newly Diagnosed with HIV**

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**BACKGROUND:** Participants enrolled in clinical trials for vaginal microbicides are women of childbearing age. Trials endeavor to offer comprehensive services for women who seroconvert, including services for sexual and reproductive health. However, much is still unknown about the needs of women affected by the virus. This study explores the fertility intentions of newly HIV-infected women, their experiences in public and private sector clinics, and their changing reproductive health needs.

**METHODS:** In-depth interviews (IDIs) were conducted with 39 women recently diagnosed with HIV. Respondents had been enrolled in one of two clinical studies in South Africa sponsored by the Population Council at the time they tested positive for HIV. This qualitative study was conducted at the University of Cape Town (UCT) Emplisweni Centre for Wellness Studies and the University of Limpopo/Medunsa campus (Medunsa), Setshaba Research Centre.

**RESULTS:** HIV diagnosis produced a major shift in sexual and reproductive desires, with the respondents almost unanimously reporting that they no longer wished for children. This can be attributed to five reoccurring themes: fear of orphaning their children, blame, guilt, concerns that their own health would be jeopardized by a pregnancy, and fear of community stigmatization. Less than half of the sample was aware of PMTCT programs. Difficulties negotiating safer sexual practices combined with depression and anxiety resulted in a loss of sexual desire. Respondents reported a number of challenges in accessing services, including long queues, insensitive practitioners, and a fear of loss of confidentiality. The presence of ongoing support group at the Medunsa site provided women with immediate care, which they credited with an easier acceptance and ability to disclose.

**CONCLUSIONS:** Meeting the unique reproductive and sexual needs and rights of newly diagnosed women requires effective referral systems to ensure continuity of care and support. In addition to HIV services, HIV+ women require counselling and information on effective contraceptive options, including emergency contraception and access to abortion, as well as PMTCT programs for those wishing to conceive. Ongoing care in HIV prevention trials must include family planning counseling and methods. Ultimately, a lack of continuity of care can lead to missed opportunities to provide health care and endangers the realization of sexual and reproductive rights.

## TUESDAY POSTERS

**412**
**Comparison of Socio-Behavioral Formative Data at Two Sites in Advance of the FEM-PrEP Clinical Trial**

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**BACKGROUND:** Family Health International (FHI) and local investigators are conducting FEM-PrEP, a phase 3, randomized, placebo-controlled trial of the safety and effectiveness of daily, oral Truvada as pre-exposure prophylaxis (PrEP) to prevent HIV acquisition in women.

FEM-PrEP is using a comprehensive approach, including collection of socio-behavioral and community formative data before the start of the trial to inform clinical trial procedures at each site.

**METHODS:** A total of 191 in-depth interviews (IDIs) and 36 focus group discussions (FGDs) were conducted in Bondo, Kenya, and Pretoria, South Africa, with potential trial participants, sexually active men, and community members. Topics included study acceptability, retention, and product adherence.

**RESULTS:** The trial was found acceptable at both sites, especially among potential participants, almost all of whom said they would want to participate. More than half of respondents in Bondo (60%) mentioned that there may be misperceptions about the trial in the community, such as that trial participants are HIV positive. These misperceptions were mentioned by less than half of respondents in Pretoria (47%).

The majority of potential participants at both sites said that daily pill adherence would be easy and offered similar reasons, including dedication to the trial and the hope that the pill could prevent HIV. However, potential participants also described possible challenges to pill adherence, including lack of support from others, mentioned only in Bondo, and alcohol use, mentioned primarily in Pretoria.

More than 90% of potential participants said it would be easy to attend monthly clinic visits. About two-thirds mentioned possible challenges, including transportation, illness, and work obstacles. Travel outside the study catchment area was mentioned as an obstacle for coming to follow-up visits by more than two-thirds of respondents at Bondo and by less than one-half of respondents at Pretoria.

**CONCLUSIONS:** The comparison of preparedness data at these sites indicates that while there are some commonalities, there are also notable differences. These data helped to inform FEM-PrEP trial procedures and messages and tailor them for each site.

**414**
**Attitudes about Research Participation in Microbicide Studies**

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**BACKGROUND:** Clinical trials require individuals to be willing to participate and to adhere to study demands. Otherwise, trials can be expensive, yield inconclusive results, or damage community relationships. Participants in clinical trials can provide insights about their experience that can be used for future planning.

**METHODS:** U.S. women (18–45 years of age) who are in a clinical trial evaluating the use of a new imaging technique (optical coherence tomography) for assessment of vaginal products completed qualitative interviews during the study to assess reasons for participation, adherence to study demands, and overall experience of trial participation. Basic requirements of the study are attendance at 4 visits with pelvic exams (screening, baseline, after use of 5.5 days of gel use, and 1 week later). The participants must remain abstinent and refrain from douching/ tampon use for approximately 3 weeks. Total reimbursement is \$475.

**RESULTS:** Twenty-six of 30 women have enrolled of which qualitative data has been analyzed for 15. Complete data will be available at the meeting. The 26 women have a mean age of 29 years, with 10 being Hispanic, 10 white, 4 African-American, and 2 Asian. Societal contribution, knowledge, free Pap test, reimbursement and convenience were reasons for participation. Women also noted the absence of a negative (e.g. blood draw, muscle biopsy, experimental product). All women adhered to study demands of visits and product use; most adhered to abstinence. Abstinence was managed by obtaining partner agreement, engaging in other activities (e.g. oral sex), avoiding early intimacy, or scheduling participation when the partner was away. Women continued to use the product despite minor side effects because of their commitment to the study. They reported that those same side effects would lead to discontinuation outside a trial.

**CONCLUSIONS:** Women participate in microbicide clinical studies for a variety of reasons. They have a strong commitment to the study requirements, but helping them to plan how to meet those expectations may foster adherence. Abstinence is obtainable but can be challenging.

**415****Effects of Partnership Change on Gel Adherence in HPTN 035**

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**BACKGROUND:** Use of HIV prevention methods may be particularly challenging for women with new sexual partners. The effect of partnership change on women's reported gel was analyzed in a microbicide safety and effectiveness trial (HPTN 035).

**METHODS:** 1,757 women in HPTN 035 gel arms completed the Follow-up Partner Status (FPS) questionnaire at their last study visit. Women married at baseline were asked if they had the same husband, new husband or new partner; unmarried women if a partner had changed or they married. Gel adherence was compared between women with ongoing partners versus new partners. Adherence was assessed as cumulatively across all quarterly visits as percent of last vaginal sex with gel use comparing medians for bivariate analyses and as high gel use (85–100% of last vaginal sex acts) and lower gel use (<85% of last vaginal sex acts) in multivariate models assessing associations with partner change.

**RESULTS:** A new partner was reported by 7% of women (n=123), and of those, 42% (51) had a new husband, and 3% (49) reported no longer having a partner. The cumulative proportion of last vaginal sex with gel use (with or without a condom) was higher for women with ongoing partners than for women with new partners (median: 100 versus 75%; p<0.001), the proportion of last sex with a condom only was higher for those with a new partner than for those with an ongoing partner (median: 12.5 versus 0%; p<0.001); and the proportion using gel and condom was lower for those with a new partner (median: 50 versus 71.4%, p<0.001). In logistic regression women with an ongoing partner were significantly more likely to report high gel adherence than those with a new partner after controlling for age, site, education level, and sexual frequency (AOR: 2.5, 95% CI: 1.6, 3.9).

**CONCLUSIONS:** Women new partners in this a microbicide trial reported using a microbicide gel less than women with ongoing partners, suggesting specific counseling for some HIV prevention methods may be needed women who experience partner change.

**416****Association between HIV Risk Perception and Product Use Among Microbicides Development Programme (MDP) 301 Phase III Trial Participants in KZN, South Africa**

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**BACKGROUND:** An individual's belief in his or her personal susceptibility to illness or disease is an important element in health behaviour and influences whether someone adopts risk-reducing behaviour and/or preventive strategies. According to the AIDS risk reduction model, knowledge of AIDS is a prerequisite to recognizing risky behaviour and taking action to change it, but findings regarding the relationship between knowledge and behaviour have been inconsistent. The relationship between HIV risk perception and sexual behaviour is complex and poorly understood and this may impact on use of HIV prevention methods.

How a woman decides to use a novel HIV prevention technology such as microbicides is influenced by several factors: a balance of her perception of risk, understanding product use, partner acceptability and consideration of the relationship's balance of power.

As part of the MDP301 Phase III trial, social science researchers explored the relationship between HIV risk perception and product and condom use in HIV prevention trials.

**METHOD:** Between January 2006 and August 2009, 327 in-depth interviews were conducted with 124 consenting women. Data was collected on risk perception, gel and condom use different time points during the trial. Data was coded thematically for understanding of risk perception and product adherence.

**RESULTS:** At week 4, 118 interviews were conducted. Of these 80 women perceived themselves not at risk. Reasons reported: using condoms consistently, trust their partners, beliefs that the gel works and being faithful. Of the 83 women, 40% reported using gel and condoms for all sex acts. 28 women perceived themselves at risk, reasons reported: condoms might burst; partners are unfaithful and not using condoms consistently whilst 10 women did not know if they were at risk. At week 52, 104 interviews were conducted. Of these 75 women perceived themselves not at risk. 24 women perceived themselves at risk. Reasons reported at week 4 were similar to week 52. Of the 104 women, 50% reported using gel and condoms for all sex acts.

**CONCLUSIONS:** Risk perception of women may determine use of HIV prevention methods such as microbicides and condoms. Women's understanding of risk increased over time and this could be related to trial procedures such counseling, risk reduction strategies and HIV education. Impact of HIV risk perception on product use becomes increasingly important with respect to PrEP trials such as VOICE.

**417****Evaluation of Participant's Sexual and Health Seeking Post SAVVY Microbicide (C31G) Trial in Lagos, Nigeria**

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**BACKGROUND:** Phase 3 SAVVY microbicide (C31G) gel trial was conducted to determine its effectiveness in preventing male to female vaginal transmission of HIV infection among women at high risk. During the trial, participants were educated on safe sex practices and risk reduction strategies. However, there had been doubts on the sustainability of this learnt behavior after the trial. The aim of this study was to evaluate the sexual and health seeking behavior of participants of SAVVY trial one year after the trial was stopped.

**METHODS:** A semi-structured questionnaire used for data collection was administrated to consented participants that went through 12 monthly follow-up of gel usage and health education/counseling during the trial.

**RESULTS:** Of 80 participants interviewed, 100% believed in reality of HIV post-trial compared to 35% before trial. They acknowledged being at risk of HIV infection. For sexually transmitted infection treatment and abortion, 42% visited hospital; 25% visited herbal practitioners and 32% visited local pharmacy shops before trial. On the contrary 83% and 17% reported at hospitals and herbal practitioner in the post trial. Fifty-four (68%) reported using condom during sex, with willingness to negotiate unprotected sex for right high price. After SAVVY 71(89%) reported consistent use of condom and their unwillingness to negotiate unprotected sex, while 9(11%) were still not using condom during sex particularly with their boyfriends. Prior to trial 57(71.5%) has STIs at least once in a month and only 13(23%) of these sought treatment at the hospital, while 27(47.5%) visited herbalists. In contrast, only 15(18.5%) reported STIs once in every 2 months and sought treatment at the hospital post trial. Pregnancy rate before SAVVY was (27%) per women, per month but this dropped to (8.3%) post trial. Factors such as educational status, income and belief significantly influence the participants' sexual and health seeking behavior (p<0.05).

**CONCLUSIONS:** Result showed that counseling and health education component of SAVVY trial had significant positive effect on the sexual and health seeking behavior of the participants.

## TUESDAY POSTERS

**418**
**Seeking Risk Factors for Recent HIV Infection Among Four Potential Populations for a Microbicides Trial in Kenya**
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**BACKGROUND:** While seeking baseline information for the development and testing of appropriate prevention and intervention strategies for human immunodeficiency virus (HIV) we sought to identify any factors associated with recent HIV infection.

**METHODS:** We chose four study sites based on HIV prevalence, availability of stable populations of women and/or a cohort of high-risk women. Enrollment efforts were focused on women mobilized in the community and who presented themselves to selected VCT centers. We conducted interviews and collected blood samples. We tested confirmed HIV+ samples, using BED capture enzyme immunoassay to identify recent infections. We analyzed associations between recent infections, socio demographic and behavioral risk factors using a logistic model. We also estimated incidence rates with an epidemiologic model using age-specific prevalence.

**RESULTS:** We enrolled 4000 women. Of the 488 with HIV infections, 96 (19%) were identified as recent infections. Overall incidence rates were: Nandi Hills 6.72 (95% CI, 4.39- 9.05), Naivasha, 2.86(95% CI, 1.46-4.26), Thika 5.67(95% CI, 3.53-7.80) and Meru 3.91 (95% CI, 2.20-5.63). In logistic regression analyses, the site of enrollment (Nandi Hills compared to Meru [odd ratio (OR) 1.7; 95% confidence interval (CI) 0.6-5.2]), marital status (never-married women compared to those married (OR, 2.4 95% CI 0.7-7.9) or education (primary education compared to no education at all (OR, 0.9 95% CI 0.1-5.4) did not influence the likelihood of a recent infection, but prevalence of HIV infection was lower in women with any secondary education. The risk of incident HIV infection for women with steady, or occasional other sexual partners was also similar to that of women who had one partner only (OR, 1.0 95% CI 0.3-2.8). Epidemiologic estimates of annual incidence rates by age group and site were lower, ranging from 1.6 to 3.2% for 18- to 22-year-old women, and from 2.1 to 4.5% for 23- to 27-year-old women. Both methods found higher incidence rates in older women.

**CONCLUSIONS:** This analysis did not identify any single risk factor that was strongly associated with risk of recent infection. Having any secondary education was protective in terms of prevalent infections. Surprisingly the older age group had a higher incidence rate than younger women. This may reflect the maturity of the epidemic in Kenya and changing behavior among younger adults. This is consistent with a maturing, generalized epidemic.

**419**
**Vaginal Hygiene Practices and Intravaginal Products: Implications for Microbicides Acceptability Among Nigerian Women**
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**BACKGROUND:** The use of vaginal products and vaginal hygiene practices has been linked to the presence of bacterial vaginosis, PID and acquisition of HIV. This study assessed vaginal practices and its implications on the acceptability of microbicides amongst Nigerian women. These practices include washing the vagina with soap, lime juice, water and other chemicals

**METHODS:** Three hundred and seventy women who are sexually active and of reproductive age were randomly selected and interviewed using semi-structured questionnaire. Their ages ranged between 19 to 45 years with mean age of 26+. Each participant completed a questionnaire in order to provide biographical data. Other information that was sort included: knowledge and use of male/female condom, vaginal hygiene practices and use of intra-vaginal products. Knowledge and willingness to use microbicides when available were also assessed. This information was collated and analyse with Epi info 2.5 statistical package 2003.

**RESULTS:** Of the 370 respondents, 51.6% were married, 39.5% single, and 61.4% had tertiary education. 91.9% had knowledge of male condom, 60% partners use male condom, 45.7% of women receive co-operation from their partners when they insist on condom use while 21.1% uses condom consistently. Similarly, 70.3% have knowledge of female condom, while 9.5% uses it consistently. 19.2% accepted their partners use other lubricants apart from condom. 12.4% inserts tightening substances to increase sexual pleasure. 62.2% have knowledge of microbicides, 81.0% indicated willingness to use when it is available. On hygiene practices, On hygiene practices, 64% douche actively, 91.7% bath twice daily during menstrual period. 19.7% who reported having itching after menstrual period uses trosyd or canesten insertion creams. 49.5% cleans up with water and soap after sexual act, 31.9% uses water only. 19.7% who reported having itching after menstrual period uses trosyd or canesten insertion creams. To prevent pregnancy, 24.9% uses local contraceptives, 16.2% uses natural method, 34.1% family planning while 19.5% uses condom.

**CONCLUSIONS:** Nigerian women frequently practice a certain level of lubricated inserts beside male and female condoms. This is indicating that they would accept lubricated microbicides in exchange for protection from HIV, and genital hygiene products. Those who use local contraceptives may be willing to accept contraceptive microbicides.

**420**
**Knowledge, Attitudes and Perceptions About Microbicides Among Healthcare Providers in Tashkent, Uzbekistan**

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Reproductive Rights

**BACKGROUND:** Health care providers play a key role in introducing and promoting new and emerging HIV/STD and pregnancy prevention technologies. The aim of this study was to explore knowledge, attitudes and perceptions of microbicides among health care service providers and examines their willingness to recommend microbicide use.

**METHODS:** Semi-structured interviews were conducted with 54 health care providers from 12 public and 4 private obstetric/gynecological clinics and AIDS service organizations in Tashkent, capital of Uzbekistan. Since the majority of Uzbek population access services through the public health service due to financial reasons, most health care providers were purposely sampled from public sector. The interview addressed knowledge, attitudes, obstacles to acceptability and strategies for promotion of female-initiated barriers methods. Interviews were recorded and transcribed.

**RESULTS:** 61.5% of respondents had heard of microbicides, while 38.5% did not have any knowledge of the product. The depth of knowledge varied. Whereas some had a good understanding of what microbicides are and their mechanisms of protection, others possessed some basic knowledge. Sources of information on microbicides among the respondents include conferences (24.8%), colleagues (29.8%), articles in the journals (9.6%) and other sources (36.3%). There was an implicit expectation that a new product will be highly effective. The overwhelming majority of health care providers was enthusiastic about microbicides and was willing to counsel users regarding potential use. Correlation between knowledge, perception and acceptability of microbicides and willingness to recommend microbicides was significant. Respondents said that microbicides should be made available to everyone, regardless of age, HIV status, and history of STI. Health care providers also believed that the challenges facing the introduction of microbicides are extensive.

**CONCLUSIONS:** This study shows the urgent need for training health care providers prior to making the product accessible, as well as the importance of addressing the potential barriers to use of the product by women. The study also indicated that education of potential users will be necessary even prior to microbicides becoming available. Although health care facilities may be perceived as adequately prepared to handle the introduction of microbicides, additional staff resources will be required to address foreseeable shortages.

**421**

### **Sexual Communication Among Married Couples in the Context of a Microbicide Clinical Trial and Acceptability Study in Pune, India**

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**BACKGROUND:** Previous research in India indicates that there is little communication within marriage about sex. Lack of communication about safe sexual behaviors may increase couples' vulnerability to HIV. This study explores couple level sexual communication and socio-cultural norms that influence couples' communication about sex and its implications for HIV prevention.

**METHODS:** Data are from in-depth interviews at two points in time with 10 couples participating in a prospective study to examine the acceptability and use of vaginal microbicides. Secondary qualitative analyses of couples' interviews were conducted using inductive and deductive coding techniques. Qualitative analyses focused on couples' sexual communication patterns and socio-cultural norms influencing communication about sex. Further qualitative analyses examined whether or how participation in a clinical trial and/or acceptability study affected couples' sexual communication patterns.

**RESULTS:** Factors limiting open communication about sex included: women's and men's lack of knowledge about sexuality, sexual power imbalances within relationships and social norms that reinforced these imbalances, and physical environments that limited privacy needed to have sex and to discuss sexual matters. Half of the couples described improved communication about sex and HIV/AIDS after participation in the clinical trial and/or acceptability study, as well as increased sexual activity, improved relationships by alleviating doubts about their partner's fidelity and forgiving their partners.

**CONCLUSIONS:** This study reveals that creating socially and culturally acceptable safe spaces where men and women can ask frank questions about HIV/AIDS, sex and sexuality potentially can improve couples' communication about sex. Couples interventions addressing the ability to refuse sex in marriage, gender stereotypes about sex and sexuality and suspicions of infidelity within marriage could serve to improve married Indian couples' communication about sex and consequently, reduce their risk for HIV infection.

**422**

### **Sustainable Personal Development of Peer Educators in a Peer Education Programme Nested Within HIV Prevention Research Clinical Trials in South Africa**

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**BACKGROUND:** Research has documented the benefits of implementing a peer programme in communities but has not necessarily delved into the positive outcomes for the peer educators. The HIV Prevention Research Unit (HPRU) peer education programme which was nested within HIV prevention clinical trials began in 2005. The aim of the peer education study was to plan, implement and evaluate the effectiveness of a peer education programme in communities. This abstract explores the perceptions of personal growth and development experienced by peer educators.

**METHODS:** 53 peer educators who were trial participants were recruited from clinical trials in line with peer study inclusion criteria. 28 peer educators were identified as active and were contacted in 2009 to participate in the in-depth interviews where data was collected on the experiences of being a peer educator. 22 peer educators were interviewed in total, manual coding was done and thematic analysis approach was used to analyze data. The data was collected by a research member who was not part of the clinical trial team.

**RESULTS:** Peer educators reported that the peer programme contributed to their skills and development in terms of communications, knowledge and awareness with regards to HIV/AIDS related issues. This included: increased self-confidence, better public speaking skills, ability to communicate about sex with partner, increased knowledge about sexual health and increased self esteem in terms of being able to assist their community.

**CONCLUSIONS:** The implementation of the HPRU peer education programme within an HIV prevention clinical trial setting has functioned as a means of allowing peer educators to be more active in their communities. The programme improved knowledge and awareness on HIV and HIV-related issues but also enhanced communication skills. Peer educators grew to be more self confident in terms of public speaking and felt more comfortable speaking to their male partners. The experiences and training of trial participants who engaged in the peer programme made them a sustainable resource in their respective communities.

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### **HIV Prevalence Risk on Women and Girls who Indulge in Unsafe Anal Sex in Malawi**

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**BACKGROUND:** 1,100,000 women are sexually active every year in Malawi. Vaginal sex is mostly and widely well accepted while anal sex and homosexuality are considered taboo, not accepted, and punishable by law in Malawi. Media reports indicate that most women indulge in unsafe anal sex. Primary health care plays a vital role in HIV prevention. HIV prevalence among women practicing anal sex in Malawi exceeds 20% compared to the normal country prevalence of 12%. Accessibility of health services is a problem in rural areas where women may indulge in casual anal sex. This research aimed at finding out more information on the existence of anal sex among these women.

**METHODS:** Global Hope Mobilization conducted the research in the following areas as well as to promote microbicides in Lilongwe, Mzimba, Mangochi, Mchinji and Ntchewa districts rural setting of Malawi. Ethical considerations were considered. Households with women and girls who have been sexually active were randomly sampled and a total of 1000 representing 200 women from each district were included in the exercise. Local meetings, counseling sessions, home visits, ANC data were collected and used from the nearest health facility, one to one interviews, focus group discussion, and questionnaires. They were all referred for Voluntary HIV testing and counseling.

**RESULTS:** Out of the women and girls interviewed, 80% consented to practicing anal sex for the past nine months. The exercise revealed reasons being child spacing, pleasure for dry sex, fear of breaking traditions beliefs such as virginity and unwanted pregnancies among girls of age 12-25. Among those tested and counseled for HIV, 25% were found to be with HIV and 55% were found with STIs. 80% consented not having using condoms due to lack of information and negligence.

**CONCLUSIONS:** Development of rectal microbicides for women and girls practicing anal sex may be a tool to reducing HIV among this population.

# TUESDAY POSTERS

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## A Survey on the Knowledge of Microbicides Amongst the Members of the National Youth Network on HIV and AIDS, Population and Development

A. Olalekan

**INTRODUCTION:** The National Youth Network on HIV AND AIDS, Population and Development is a network of youth-based and youth-focused Non Governmental Organization (NGO), Faith Based Organization (FBO) and Community Based Organization (CBO) working on HIV and AIDS Prevention and Control in Nigeria. The Lagos State Chapter has over 400 organizations has its intervention implementing partners. With recent development on New Prevention Technology (NPT) especially microbicides, understanding member's perception about microbicides would assist in creating awareness and sensitization programs and interventions about microbicides.

A descriptive survey was carried out amongst members of the network to establish their current knowledge, attitudes and acceptance of microbicides. The study was conducted as a baseline in setting up community awareness and education intervention and mobilizes for the acceptance and use of microbicides.

**METHOD:** A total of three hundred (300) structured questionnaires were administered by volunteers to members attending the monthly meeting within the age of 25–35 years for three (3) months after a sensitization talk. Epi-info 2002 was used to analyse the data.

**RESULTS:** All respondents knew that HIV is transmitted sexually, 72% (216) have heard about microbicides, 46.5% (139.5) know what microbicides are, 92% (276) believe that microbicides are a better prevention option since they're women-controlled, 73% (219) want the gel to be one-use disposable, 55% (165) want the civil society to be actively involved in the marking and distribution of microbicides.

**CONCLUSIONS:** The data shows that knowledge on microbicides was high amongst members of the network and more efforts should be put into sensitization and community education.

**425**

## Knowledge, Perception, and Acceptability of Microbicides among Female Sex Workers in Nigeria: Combining Quantitative and Participatory Methodologies

O. Tolulope

**BACKGROUND:** Female Sex Workers (FSWs) are the most vulnerable groups for the contraction and transmission of HIV/AIDS and other STDs, due to the nature of their work, stigmatization and marginalization in their countries. Vaginal microbicides are being developed to reduce HIV infection in women for whom correct and consistent condom use is impossible or undesirable. If matters related to the development of microbicides and its eventual introduction are to be addressed, it is important first to know the extent of its knowledge, perception as well as its acceptability. This article therefore aimed at assessing the knowledge, perception and acceptability of microbicides among FSWs in Ekiti State, Nigeria.

**METHODS:** The study was cross-sectional and descriptive, using both quantitative and qualitative method of data collection. A convenience sample of 123 FSWs in Ekiti State, Nigeria was interviewed using an interviewer administered structured questionnaire. For the qualitative phase, individual interviews and focus group discussions (FGD) were conducted among respondents. The objective was to facilitate behavioral and attitudinal changes for accepting microbicides and microbicide trial programs.

A new tool called PMAS—Participatory Microbicides Acceptability and Support was developed for stimulating discussion, learning and adoption of microbicides among respondents. It consists of awareness and learning activities that facilitators do with respondents of up to 25 people in a group which enabled them make action plan for microbicides awareness, support and education of potential trial participants. The data collected were subjected to basic analysis with the STATISTICA software version 8.

**RESULTS:** Out of the 123 respondents, only 28 (22.7%) had ever heard about microbicides while the respondents that had never heard accounted for 77.3%. There were large differences in level of knowledge in people from different ethnic groups within the State with respondents from Edo having the highest level of knowledge of microbicides. Majority of the respondents (64%) believed that microbicides could reduce but not prevent transmission totally, 24% believed it could prevent transmission totally while the remaining 12% believed it could neither reduce nor prevent transmission. However, 48% of the respondents were ready to use the microbicide products if approved and with no side effects, because they perceived that they were at risk of HIV/STI infections due to violence experienced on the job, on streets or in their personal lives, which increases their vulnerability to HIV and other health concerns. Multivariate logistic regression analysis revealed that some selected socio-demographic characteristics were significantly associated with knowledge, perception and acceptability of microbicides ( $p<0.05$ ).

**CONCLUSIONS:** Microbicides knowledge, perception and acceptability studies are highly important and relevant among high-risk and marginalized groups such as FSWs because these will ultimately determine use. Educating the public about microbicides is a community level strategy that should be integrated into the national program in order to maintain and expand its success. More organizations may wish to field test PMAS.

**426**

## HIV-1 Risk Characteristics of HIV-1 Serodiscordant Couples Enrolled into an Efficacy Trial of Pre-Exposure Prophylaxis

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**BACKGROUND:** Pre-exposure prophylaxis (PrEP) is a novel biomedical strategy being investigated for the prevention of HIV-1 acquisition in ongoing clinical trials. If efficacious, PrEP may be targeted to high-risk subpopulations, including HIV-1 serodiscordant couples.

**METHODS:** The Partners PrEP Study is a phase III, randomized, placebo-controlled efficacy trial of daily tenofovir and combination emtricitabine-tenofovir pre-exposure prophylaxis for reducing HIV-1 acquisition risk among heterosexual African HIV-1 serodiscordant couples. Enrollment commenced in July 2008 and target sample size is 3900 couples, recruited from 9 trial sites in Kenya and Uganda.

**RESULTS:** From July 2008 to November 2009, 4433 HIV serodiscordant couples were screened and 2723 enrolled. For 970 enrolled couples (36%), the HIV-1 uninfected partner was female. Median age was 35 years (IQR 29,42) for HIV-1 uninfected partners and 34 years (IQR 28,40) for HIV-1 infected partners. Most couples (97%) were married, with a median duration of partnership of 8.5 years (IQR 3.3,15); however, most had only recently learned of their serodiscordant status [median time since learning status 0.7 years (IQR 0.1,2.2)]. Couples reported a median of 4 sex acts (IQR 2,7) during the month prior to enrollment, and 27% reported sex unprotected by condoms. 14% of male and <1% of female HIV-1 uninfected partners reported sex with non-study partners. 51% of HIV-1 uninfected male partners were circumcised. The median CD4 count of HIV-1 infected partners was 487 cells/ $\mu$ L (IQR 367,652), and the majority (61%) had WHO stage 1 HIV-1 disease.

**CONCLUSIONS:** Couples at high risk of HIV-1 transmission can successfully be recruited into an efficacy trial of PrEP. HIV-1 serodiscordant couples should be a priority population for the evaluation of new HIV-1 prevention strategies.

**427****Polymorphisms in HLA:  
A Barrier in Synthesis of  
Potent Microbicides?**

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**BACKGROUND:** The HLA (human leukocyte antigen) system codes for a family of recognition molecules that discriminates self from non-self and signal the immune system. This immune response may be a major cause of limited action of any microbicides, while HIV exploits this system to enter and multiply. Scientists had anticipated the rapid development of effective vaccines or microbicides to prevent this rather small virus. However attempts have not been taken to evaluate the role of HLA while designing vaccine or microbicides leading to restricted success. The present study objective is to highlight the HLA distribution in the population of Mumbai, India, that may be taken into consideration while formulating effective microbicides or vaccine. Mumbai, is affected by HIV pandemic and being considered for clinical trials of microbicides or vaccine against HIV.

**METHODS:** Study subjects were from the Gynecology OPD and Integrated Counseling and Testing Centre of Microbiology Department of a Municipality Hospital. Enrollment and HLA analysis using PCR-SSOP was done with their consent. Two hundred thirty five unrelated individuals belonging to one ethnic group (Maratha, 30–40 years) were studied for HLA analysis.

**RESULTS:** Results revealed wide polymorphisms in both class I and II alleles. HLA A\*24 (33.9%), HLA A\*11(31.2%), HLA A\*02(22.3%), HLA A\*33(21.9%) were observed more frequently than other HLA A alleles. There were 23 alleles identified in HLA B, of which HLA B\*40(18.9%) was present in significantly high frequency, followed with HLA B\*07(11.4%), HLA B\*15(8%), HLA A\*35(12.4%), HLA B\*44(9.2%). Among HLA DRB alleles, DRB1\*15 was present in significantly high proportion (28.6%) compared to other 14 identified alleles. Among the 6 identified DQB1alleles, DQB1\*15 was the most observed (48.9%) allele. There was also variation in expression of these alleles in population from Northern, Southern and Eastern India. In serodiscordant couples, few alleles were observed exclusively either in HIV positive or negative spouse.

**CONCLUSIONS:** This information on HLA polymorphism is critical in design of T-cell epitope vaccine in the identification of peptides with proven ability to bind to many HLA alleles. Reactions to candidate microbicides reflects polymorphic action of vaginal or rectal cells due to variation in HLA and highlight the strategies to be considered while synthesizing a potent microbicide that can withstand the host rejection leading to HIV prevention.

**428****Home-Based HIV Counseling  
and Testing a Key Strategy  
of HIV Prevention. What  
Factors Hinder Testing for  
HIV by Men at Home? A Case  
of Kumi District in Uganda**

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**BACKGROUND:** Voluntary HIV Counseling and Testing (VCT) is a key strategy for the prevention and control of HIV/AIDS. However, despite the introduction of Home based delivery of HIV counseling and testing (HBCT) the uptake of VCT services particularly among men is still low as noted in Kumi district. This study was conducted to determine the factors hindering uptake of VCT among men in old Kumi district.

**METHODS:** A population-based cross-sectional study conducted in 2008 using both quantitative and qualitative methods in Kumi district. A sample of 240 men aged 15–60 years, and resident in old Kumi district were interviewed and key informant interviews (23). The odds ratios and their 95% confidence intervals (CI) of uptake of HBCT by the independent variables were estimated.

**RESULTS:** Overall, the uptake of HBCT by men in Kumi district was 66.9% and the key factors found to hinder uptake of Home based HIV counseling and testing being married (OR 0.11, 95% CI (0.04–0.28)), lack of knowledge and awareness regarding HBCT implementation (OR 8.07, 95% CI (1.26–51.48)), not involving men when appointments are being made for HBCT visits (OR 6.50 95% CI (1.45–29.16)), absence of men at home during the time of home visits (OR 0.39, 95% CI (0.17–0.89)). Stigma and fear for HIV results though not significantly associated (OR 1.32 95% CI (0.63–2.76)) was raised by Key informants as a contributing factor to low uptake of HBCT.

**CONCLUSION:** Programs that implement Home based HIV Counseling and testing need to enhance community participation through involvement of men in making appointments for the home visits and also carry out sensitization campaigns pointing out HBCT program benefits and addressing the issue of fear of taking a test, stigma and discrimination in order to increase uptake of VCT among men.

**429****Identifying Target  
Populations for a Future  
Potential Antiretroviral (ARV)  
Pre-Exposure Prophylaxis  
(PrEP) Program**

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**BACKGROUND:** As governments, researchers, and stakeholders begin to plan for rollout of ARV PrEP if found safe and effective, one important question is which populations should be targeted with this new prevention tool. This question was addressed in ongoing socio-behavioral research to prepare for future potential PrEP rollout conducted as part of the FEM-PrEP clinical trial in Kenya.

**METHODS:** In-depth interviews were conducted and recorded with public health stakeholders and civil society leaders/gatekeepers in Bondo and Rarieda districts, Kenya. Both groups were asked to identify the best target populations for initial and expanded phases of a pilot PrEP program and to explain why the groups were appropriate.

**RESULTS:** Public health stakeholders (n=10) recommended that for the first two years, a pilot PrEP program should target male and female "youth" both married and unmarried from their teens to their 30s/40s, specifically sex workers and barmaids, at the beaches and in Bondo town. Respondents said these populations are where most new infections are occurring because people are engaging in a high degree of sexual activity due to lifestyle and are motivated by money to have sex. For a later phase, respondents said a program should target the same people as the initial phase, as well as the unemployed, people working at night, truck drivers, farmers, and bicycle taxi drivers, because they have less information about HIV and are highly sexually active.

Civil society leaders/gatekeepers (n=15) most often recommended unmarried and newly married male and female youth 18–35 for the first two years of a program. This was because they still have their lives ahead of them and are the most sexually active groups. Respondents also mentioned "middle-aged" (30s-50s) women, sex workers, and women at the beaches who exchange sex for fish. For an expanded program phase they most often recommended married people of middle age because they are still at risk of HIV infection. Widows, widowers, youth, factory workers, and polygamists were also mentioned. They most often said youth would be eager to take the pill, whereas middle-aged and older adults would not want to take it.

**CONCLUSIONS:** Both sets of respondents agreed that the first phase of a pilot PrEP program should target married and unmarried young adults. Planned interviews with target populations, community members, and clinical trial participants will establish key sub-groups and provide potential PrEP users' perspectives.

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### Combining Antiretroviral (ARV) Pre-exposure Prophylaxis (PrEP) with Behavioral Risk Reduction

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**BACKGROUND:** Designing programs to roll out ARV PrEP if found safe and effective requires 1) decisions about how long people should take daily ARV PrEP and 2) consideration of how to combine PrEP with behavioral risk-reduction methods. These issues were addressed in ongoing socio-behavioral research to prepare for potential PrEP rollout as part of the FEM-PrEP clinical trial in Kenya.

**METHODS:** In-depth interviews were conducted with public health stakeholders (n=10) and civil society leaders (n=15) in Bondo and Rarieda districts, Kenya. Interviews were analyzed using qualitative methods. Public health stakeholders were asked how long people should be able to take PrEP and why. Civil society leaders were asked which populations were ready to commit to different behavioral risk-reduction methods and why.

**RESULTS:** The majority of public health stakeholders recommended that program clients be able to take ARV pills as long as they feel they are at risk of HIV infection or are sexually active. Some people classified this as "long-term," along with taking it for years and for life, whereas others called it "short-term," which also included a few months or for periods in life. Still others said duration depended on safety and resistance data, or side effects.

Civil society leaders reported that "youth" (teens to 30s/40s) were willing to use condoms to reduce their risk of HIV infection, but older and married people were not. Male youth were also most frequently reported as prepared to get circumcised, as opposed to elderly and "adult" men. Married people in general were described as able to be monogamous, whereas youth and people in unstable/unhappy marriages were not. Youth and married people, as well as women, adults, and the elderly, were reported as ready to reduce their number of sexual partners, but youth were also reported not to be ready, along with fishermen.

**CONCLUSIONS:** If program clients were eligible to take PrEP as long as they are at risk of HIV infection, as advised by public health stakeholders, risk-reduction counseling would be needed to minimize that time and therefore limit program costs and potential side effects of the drug. If youth become a target population for PrEP, they may be disposed to reduce their risk over the long term through condom use and male circumcision. However, more extensive counseling would be needed with youth on the need for a combination of preventive methods including monogamy and reducing the number of sexual partners.

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### Taking it to the Streets: Engaging In-Country Stakeholders to Plan for Possible PrEP Implementation

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**BACKGROUND:** As the experience with male circumcision has shown, engaging key stakeholders is essential for the implementation of any new HIV prevention intervention. Having an informed and engaged group of national-level stakeholders aware of and prepared for a research result increases the chances that the transition from research to rollout will be swift and thoughtful. There are a range of preparatory activities that can take place before a research result is known. Initiating these steps early may help reduce time to implementation after a positive result is released. Additionally, if stakeholders feel they have been uninformed or underinvolved while research is ongoing, they may be less receptive to implementing the results. Furthermore, if the result shows no effect or presents a safety concern, having an informed group of national stakeholders can help to minimize any misinformation that may emerge.

**METHODS:** Beginning in 2008, AVAC has worked with in-country partners in Kenya and Uganda to implement a systematic engagement process to help prepare key community and national stakeholders for PrEP results. Key steps include: identifying national partners in leadership roles to take ownership of preparing for PrEP results; identifying and bringing together community leaders, policy makers, health care implementers, researchers and other vital stakeholders; providing stakeholders with up-to-date information on PrEP; working with stakeholders to identify the national and local needs for preparation of PrEP results; and working with stakeholders over time to accomplish the tasks identified.

**RESULTS:** The initial consultations have identified short- and long-term needs as each country prepares for results, including gaps in general PrEP knowledge, consideration of implementation issues, cost effectiveness modeling, and need for national policies.

**CONCLUSIONS:** These in-country consultations have highlighted the fact that engaging national-level stakeholders prior to the release of research results is essential to uncovering potential impediments to implementation. Supporting in-country stakeholders and processes to prepare for trial results and gain ownership of the research agenda and results may greatly improve effective roll out of prevention strategies that are shown to be effective. This approach must be replicated in other countries where PrEP trials are ongoing and planned.

## 432

### Group Therapy Promotes Prevention with Positives among Discordant Couples, TASO Masindi Experience

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**BACKGROUND:** Discordance is a serious emerging issue in HIV/AIDS and most couples are unknowingly living with HIV positive partners. In Uganda over 60% of couples living with HIV are in discordant relationships.

**METHODS:** In 2008, The AIDS Support Organization (TASO), Masindi center had total cumulative clientele of 5440, 2735 being couples of which 503 were in discordant relationship. This posed critical challenges in HIV/AIDS prevention among such couples as the negative partners were at a very high risk of acquiring HIV. Early 2009, discordant couple groups were formed at center, outreaches, and at community levels

Group therapy (a form of psychotherapy in which a small group of individuals meet regularly with a therapist and share their mutual struggles and feelings) and prevention with positives interventions were introduced to help discordant couples to identify coping strategies, risk reduction plans and seek support while acknowledging those living with HIV as part of the solution and not part of the problem. This offered safe environment where group members worked to establish a level of trust that allowed them to talk personally, honestly and support one another in emotional growth and personal problem solving. The psychosocial safety of the groups allowed expression of those feelings that were often difficult to express outside the group.

With the help of counselors, members regularly acted and discussed scenarios affecting them: having children, domestic violence, negotiation skills, disclosure, substance abuse, safer sex, ART, STI management, couple RCT, gender and social discrimination among others.

**RESULTS:** Comparing 2008 and 2009, we noted that there was an increase of 63% in condoms distributed, 35% increase in utilization of family planning services, and 90% of discordant couples on ART have adherence level of greater than 95%. There was an increase in uptake of PMTCT services in 2009, as compared to 2008; a decrease in reported cases of gender based violence; and an increase in disclosure of HIV positive sero status to children, family members and the significant others of 47% and an increase in STI/STD re-screening by over 100%.

**CONCLUSIONS:** Discordance requires behavioral change interventions such as group therapy that provides opportunity for couples to improve their ability to relate to each other and mutually adopt and sustain desired practices of prevention with positives.

## 433

### Cloning of HIV-1C Genes into SFV Vector for Development of Recombinant Vaccine

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**BACKGROUND:** Efforts are being made worldwide to develop recombinant vaccines for HIV using different vectors. However no effective recombinant vaccine is available to-date. Semliki Forest virus (SFV) vector has proved to elicit both cell-mediated and humoral immune responses. The present study demonstrates cloning of *gag*, *env* and *RTpol* genes of HIV-1 C into SFV vector for development of recombinant vaccine.

**METHOD:** HIV-1C *gag*, *env* and *RTpol* genes were PCR amplified using gene specific primers from proviral DNA extracted from seropositive individuals and cloned individually into the SFV2gen expression vector. RNA transcripts were produced from the three linearized recombinant SFV2gen plasmids and pSFV Helper-2 DNA by *in vitro* transcription. Both the recombinant RNA and helper RNA were cotransfected into BHK-21 cells by lipofection to produce recombinant SFV particles. The recombinant viral particles were activated with alpha chymotrypsin and used to infect BHK-21 cells for analysis of protein expression.

**RESULTS:** The restriction digestion of *gag* and *env* inserts at BamHI and Xhol sites resulted in a band of approximately 1.4 kb and 2.5 kb respectively while that for *RTPOL* at Xma I and Xhol sites produced a band of approximately 1.6 kb. Sequencing of these inserts using gene specific primers showed the identity with the respective genes. The Western blot analysis of the expressed proteins following transfection of these recombinant constructs into BHK-21 cells showed the reactivity with antibodies to gp120, p24 and RTase for *env*, *gag* and *RTpol* respectively.

**CONCLUSIONS:** The HIV-1 C *env*, *gag* and *RTpol* genes were successfully cloned into SFV 2gen vector and showed the expression of respective proteins *in vitro* suggesting its potential as an effective vaccine.

## 434

### Prevention with Positive (PWP)—“A Light at the End of the HIV Tunnel”

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**BACKGROUND:** Until recently, HIV prevention efforts mainly targeted individuals at risk of HIV infection. However, HIV-positive persons require knowledge and support to protect self and others from re-infection and infection respectively. This information improves wellness and reduces illness related to HIV disease among those infected. PWP helps health providers maintain dialogue about prevention, knowledge and assist tested couples access support to practice prevention irrespective of being concordant or discordant. The process encourages discordant couples reinforce prevention and join support groups.

**METHOD:** All HIV-positive clients above the age of 15 and sexually experienced at the MCH clinic, are counseled and provided with information on the PWP strategy (Partner testing, disclosure, abstinence, partner reduction, faithfulness, condom use, adherence to medication, treatment of STI and use of family planning method). Attention is paid to those who report not knowing their partners HIV status. They are encouraged to bring them for HIV counseling and testing with weekly reminders.

**RESULTS:** Since September 2008, 831 index HIV-positive individuals who do not know their spouses HIV status have been educated on PWP strategy. 222 are on sex break and have no current sex partners. 152 are yet to bring their sexual partners for counseling and testing. Of 457 index patients whose sex partners have been screened for HIV, 145(31.7%) are discordant couples while 312(68.3%) are concordant. Of those in the discordant relationships, 98 males are HIV negative while 47 are females. 25.6% of those in the concordant group had never been tested before and are now accessing care at our facility.

**CONCLUSIONS:** The PWP strategy is feasible and should be adopted by all programs. Our 145 discordant couples are now living “positively” with their HIV infected partners. This special group is now ready for ongoing microbicides trials and/or studies on correlates of HIV protection.

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### Prevention with Positives; Using Multiple Strategies to Involve Persons Living with HIV in Prevention. TASO Masindi Experience, Uganda

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**BACKGROUND:** Efforts to prevent new HIV infections have expanded from a focus on HIV-negative individuals to also include interventions with people who are living with HIV/AIDS. Adopting strategies to involve persons living with HIV in prevention is key to reducing new infections.

**METHODS:** The AIDS Support Organization (TASO) is a national non governmental organization in Uganda, involved in HIV prevention, care and support both at facility and community. TASO Masindi one of the service centers started implementing HIV prevention strategies in 2007 with a focus on involving HIV-positive people in prevention. Some of the strategies included;

- Counseling on prevention of HIV to positive persons this involved discussion of behavioral intervention in medical and counseling visits
- Promoting the use, and provision of condoms to sexually active HIV positive individuals accessing services from TASO
- Promoting adherence to antiretroviral drugs, by counseling, use of pill boxes, community peer support groups and medicine companions
- Encouraging HIV-positive persons to disclose HIV status to sex partners either by self or counselor mediated during home visits or at the nearest opportunity
- Home-based HIV counseling and testing targeting partners and children born to all individuals living with HIV/AIDS
- Involvement of HIV-positive persons in HIV sensitization through Music, Dance and Drama (MDD) and also formation of peer support groups in the community of HIV-positive persons hence integrating prevention with positive in the community

**RESULTS:** Most of the persons living with HIV realize that they have a role to play in prevention of HIV.

There is 8.5% increase in the uptake of condoms by HIV-positive persons between the year 2008 and 2009.

Adherence levels on antiretroviral drugs is at 92% of the clients on ART have adherence >95%.

There is a remarkable increase of 50% in disclosure of HIV status by HIV-positive individuals between 2008 and 2009.

Community awareness of HIV has increased in the area served by the organization.

**CONCLUSIONS:** HIV-positive persons are very important partners in HIV prevention therefore their involvement should be prioritized in all HIV prevention activities.

# TUESDAY POSTERS

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### Target Population for Early Access to Pre-Exposure Prophylaxis in India

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**BACKGROUND:** There are 2.5 million PLHAs, 4 million High risk groups and 12 million highly vulnerable populations in India. Under the national programme, prevention of new HIV infections has been identified as the foremost strategy to halt and reverse the HIV/AIDS epidemic. If the results of the ongoing efficacy trials on PrEP are positive, at risk population in India could be the largest beneficiary. Who within the large at risk population should qualify for early access to PrEP is important to prioritize. Providing access to PrEP drugs would raise ethical, regulatory and economic challenges that also need to be addressed.

**METHODS:** In order to assess the suitable target population for the promising new prevention option of PrEP we reviewed latest information from National AIDS Control Organization and the National Institutes' (NARI, NIMS, and YRG CARE) publications on HIV epidemiology in India. As per NACO HIV Sentinel Surveillance data the epidemic remains heterogeneous in terms of geographic spread and routes of transmission. Impact of program is noted in Southern states where HIV was predominant and interventions were started earlier. Rising trends among antenatal women- a proxy for general population are observed in northern states of Gujarat, Rajasthan, Orissa and West Bengal. New pockets of IDU and MSM are identified in metropolitan cities of Delhi & Mumbai. Published data on new infections is scarce. However, a review of literature indicates an incidence of 6.52/100 person yrs among heterosexual discordant couples in Chennai; an overall incidence of 10.2/100 person yrs in a cohort of High-risk patients attending STD clinics in Pune. Analysis of recent trends in HIV incidence in coastal South India shows total prevalence and incidence in Mangalore to be increasing among high risk groups, among females and those attending VCT centers & STD clinics. Incidence in urban areas is greater as compared to rural.

**RESULTS:** It would be appropriate to initiate new intervention with PrEP among the high-risk groups of emerging IDU, MSM populations & serodiscordant couples in metropolitan cities of Delhi & Mumbai, antenatal clinics in selected north Indian states and VCT center & STD clinic attendees in coastal South India. Initial data on acceptability & feasibility of introducing PrEP would emerge from these settings and guide the Public Health system. Cost effectiveness analysis should be built into these small-scale demonstration projects with different populations-targeting scenarios considering risk behavior, rate of incidence, efficacy and cost of ARV PrEP drugs. Challenges to be addressed include development of resistance to ARVs posed by over the counter availability of drugs in India, commitment of resources to meet the infrastructure and delivery costs and regulatory approval for this new indication.

**CONCLUSIONS:** Choosing the right population for early access to PrEP in India should be a well-informed decision. PrEP should be integrated within consolidation plan for targeted interventions for HRGs, in order to halt and reverse the epidemic.

## 437

### Injection Safety in Nairobi City Health Facilities—Kenya

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**BACKGROUND:** Injections given in formal and informal health care settings are probably the most common percutaneous procedures worldwide. W.H.O suspects that unsafe injections occur routinely in developing countries, which could lead to substantial morbidity and mortality, particularly from Hepatitis B and C and HIV. We conducted a representative survey of public health facilities in Nairobi city in order to assess injection safety practices. Nairobi city has a high population density and high number of health facilities.

**METHODS:** Sixty-eight health facilities (representing 80 percent of all health facilities) public health facilities were randomly selected. Data collection included observation of available injection equipment and injection administration practices. Injection providers and health cleaners were randomly selected and interviewed for history of exposure to accidental prick injuries, vaccination status, knowledge and practices on injection safety.

**RESULTS:** All facilities were using disposable syringes and auto disable syringes for therapeutic and immunization injections respectively. Ninety-nine percent of facilities had disposable needles and syringes at the time of the study. About 12.3 percent lacked safety boxes in the injection rooms, and 8.8 percent had expired syringes. Burning sharps and injection equipment in a pit or in an enclosure was the most common method of injection waste disposal in 38 percent of the facilities. Offsite treatment, burning sharps on an open ground was observed in 32 percent and 16.2 percent of health facilities respectively. Only 11.8 percent of the facilities had an incinerator while 1.5 percent throws sharps in a pit latrine. Inappropriately disposed sharps in the compound were observed in 58 percent of the health facilities. Sixteen percent of the injection providers and 15.2 percent of health cleaners reported accidental prick injury in the year preceding the study. Only 18 percent of health workers and 22 percent of health facility cleaners, who had needle stick injuries, went for HIV prophylaxis. Only 5 percent of health workers and cleaners had had a hepatitis B vaccination.

**CONCLUSIONS:** Health care workers, cleaners and the community are at risk of blood borne infections resulting from unsafe injections. Inappropriate disposal of injection related waste from health facilities rather than unsterile injections is the most common danger to blood borne transmissions in the setting.

**438**

**Prevention Strategies to Control HIV Through the Mobile HIV Peer Counselling and Testing System in 5 LGAs, Nassarawa State, Nigeria**

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**BACKGROUND:** Nigeria is 2nd globally going by its HIV/AIDS population. In Nigeria, North Central(NC) top 2nd in the national prevalence rate(PR). Nasarawa state within the (NC), has the 2nd- highest (10%) HIV PR nationally. Akwanga, Karu, Keffi, Kokona and Wamba Local Government Areas(LGAs) within Nasarawa top in the state HIV PR. (2008 National HIV Sero-Prevalence Sentinel Survey)

In these 5LGAs, many myths and misconceptions about the AIDS pandemic were been spread and 70% dwellers believed in them e.g. HIV is caused by spiritual forces, not through sex, thereby they engaged in casual and unprotected sex. The people held unfounded beliefs about the degree to which HIV is communicable. Secondly, there are dilapidated road networks making the available HIV services inaccessible for those willing to.

**METHODS:** 5 peer educators were trained from each of the 5LGAs with mandate to train five others each from their LGAs for this project. They were trained on community HIV counseling, specific strategies to carry out the HCTmobile outreaches in the selected communities.

Initial outreaches were carried out in July 2008. First follow-up outreach in September 2008 and second-follow up outreach in December 2008. In the follow-ups, the same clients in the initial outreach were targeted.

**RESULTS:**

TABLE 438.1 Initial Outreach July 2008

LGAs	Total tested/ counseled	Females	Males	Negatives	Positives	Percentage Ratio
Akwanga	5,313	3,488	1,825	4,941	372	7%
Karu	5,500	3,390	2,110	5,104	396	7.2%
Keffi	4,800	2,970	1,830	4,560	240	5%
Kokona	3,910	2,660	1,250	3,675	235	6%
Wamba	5,132	2,950	2,182	4,747	385	7.5%
<b>TOTAL</b>	<b>24,655</b>	<b>15,458</b>	<b>9,197</b>	<b>23,027</b>	<b>1,628</b>	<b>6.54%</b>

TABLE 438.2 Second Follow-up Outreach December 2008

LGAs	Total Tested/ Counseled	Failed to Turn Out	Females	Males	Negatives	Positives	% Ratio
Akwanga	4,925	388	3,477	1,448	4,924	1	0.02%
Karu	5,030	470	3,370	1,660	5,025	5	0.99%
Keffi	4,540	260	2,956	1,584	4,540	-	0.00%
Kokona	3670	240	2,655	1,020	3,670	-	0.00%
Wamba	4,737	395	2,944	1,793	4,735	2	0.04%
<b>TOTAL</b>	<b>22,902</b>	<b>1753</b>	<b>15,402</b>	<b>7,505</b>	<b>22,894</b>	<b>8</b>	<b>0.159%</b>

The targeted population was reached except the 1753. On-going counseling among the peer groups brought down prevalence rate from 6.54% to 0.159% within 6 months. With the good tracking system adopted, only 122 clients out of 1636 were lost to follow up.

**CONCLUSIONS:** These findings highlight critical gaps on what was there before and after this project was carried out. Using peer educators and reinforcing strategic measures through consistent support-supervision and training according to each community peculiar HIV challenge.

**439**

**High-Risk Population of Sexual Violence Willingness to Use Pre-Emergency Prophylaxis**

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*Media Concern Initiative for Women and Children*

**BACKGROUND:** MEDIACON's runs a rape crisis response centre since 2005. The influx of calls to the helplines and rape cases from the population of pre-teens (10-12) and teenagers (13-16years old) increased from 1000 in a year to approximately 3000 a year. Pre-Exposure Prophylaxis when widely available can reduce risk of HIV infection to these high-risk population and impact on national and regional development.

**METHODS:** Utilized a random selection of students from two schools in Lagos State. A total of 223 female students in both junior and senior secondary school were candidates. Questionnaires were developed and distributed, collected and analyzed.

**RESULTS:** Of the 223 participants who answered the questionnaires, 171 are willing to make use of pre-exposure prophylaxis agents if available, to reduce risk of HIV, 38 were not sure they will make any attempt to try, and 14 did not respond. Of these, 70% were concerned for more than HIV; issues of fear of pre-teen and teenage pregnancies were expressed while 20% were concerned about consent use. Over 90% were concerned about accessibility.

**CONCLUSIONS:** Availability of Pre-Exposure Prophylaxis to reduce HIV infection will be highly welcomed by this high risk population. Accessibility to Youth Friendly Clinics will increase contact of population with information and facilitate openness to medical and counselors.

# TUESDAY POSTERS

## 440

### Male Partner Involvement in the HPTN 035 Microbicide Trial

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**BACKGROUND:** Clinical trials recruiting women can introduce tension between the autonomy of the woman and a need to solicit buy-in and/or permission from her male partner to take part in a trial and use the investigational product. HPTN 035, a Phase II/Ib trial, engaged 3,099 women at six sites in Africa and one in the United States so as to study two candidate microbicides. The study had a record for timely participant recruitment and high participants' retention. This was however, only possible through timely responses made to identified challenges to participants' recruitment, retention and visit adherence. This abstract reports on one major factor identified in all the six African HPTN 035 study sites as a potential challenge to participants' recruitment, retention and visit adherence and how this challenge was addressed.

**METHODS:** Quarterly site community reports generated by research sites were reviewed. Also, each site's completed questionnaire on community challenges and efforts related to participant accrual, retention and visit adherence at the close of the study, were also reviewed. Factors that facilitated trial participants' accrual, retention and visit adherence were identified.

**RESULTS:** Male partner involvement was one of two factors that affected trial participants' accrual, retention and visit adherence at each of the six African sites. Recruitment strategies were adjusted to include encouraging women to discuss the study details with partners and family prior to making their decision even though each woman was counseled and supported in making their own decision about joining the trial. Informational materials for male partners were developed. On-site clinic-based informational and counseling sessions for male partners were offered (if desired by the woman). It became apparent that male and female partners hardly held open discussions sex related issues. This made difficult for some trial participants to inform their male partners of their participation in this trial. This then posed a challenge to trial product use and clinic visit as the culture expect females to be totally accountable to their male partners.

**CONCLUSIONS:** Male partner support of women's involvement in microbicide studies is of significant importance in African settings. Community education, outreach, recruitment, and retention strategies for future trials should proactively address male involvement.

## 441

### Tapping into the Strength of Faith-Based Organizations: An Effective Strategy in Promoting Microbicides

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**BACKGROUND:** In some resource-poor countries information about new HIV prevention technologies is still not as wide spread as it should be, religious leaders can support enlightened attitudes, opinions, policies and laws, redirect charitable resources for spiritual and social care and raise new funds for HIV prevention, promote action from the grass roots up to the national level, use their pulpits to spread messages about new HIV prevention technologies as well as help disseminate accurate information and influence opinion about microbicide development and research

**METHODS:** A quasi-experimental study was carried out at a yearly retreat for members of clergy in a faith-based organization; people in attendance include those in training seminaries and those who are already working in various parishes. A structured self-administered questionnaire was used to collect information on day 1 of the retreat after which a 30 minute lecture was given on microbicides, on the 5th day of the retreat during the closing ceremony the questionnaire was again readministered.

**RESULTS:** A total of 267 people attended the retreat; all were men, computer literate and had post secondary education. Pre intervention analysis of data revealed that only 5 (2%) had heard of microbicides in the past therefore they could not answer any of the other questions. Post intervention data revealed that 91% could explain what microbicides were, 89% were willing to talk about microbicides when talking about ABC prevention strategies, ways of disseminating information to their congregation suggested include incorporating microbicide information into church HIV/AIDS IEC materials, updating the theological HIV/AIDS curriculum to include lectures on microbicides, talking about microbicides during pulpit sermon on HIV/AIDS. The participants were of the opinion that if they have adequate information about microbicides they will be in a good position to serve as advocates.

**CONCLUSIONS:** Faith-based organizations have a broad reach through numerous channels for social mobilization coupled with creativity in delivering messages, leadership and influence, affiliations with large numbers of people. This opportunity can be tapped into and used to effectively mobilize communities for microbicide research, development and acceptance.

## 442

### Community Involvement in Providing Access for MSM in Nairobi, Kenya

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**BACKGROUND:** HIV/AIDS continue to impact MSM disproportionately. There have been minimal prevention interventions targeting this group. According to the Modes of Transmission Study (MOT, 2008) MSM and IDU's, hitherto overlooked populations in Kenya, maybe contributing a significant percentage of new infections. The data from these communities still remains incomplete, and when the national model (MOT) indicated that MSM and IDUs combined account for about 15% of new infections, the model for Nairobi placed these groups contribution to at 26% almost one third of new infections.

**METHODS:** Ishtar MSM developed a program to provide access to relevant sexual health information to 1000 MSM in Nairobi in order to reduce the rate of spread of HIV/AIDS and STI's among the group through different community engagement strategies including group and interpersonal discussions, production of IEC Material, referrals and Peer education through participative and proximity approaches allowing MSM Peer Educators active involvement in the project design and implementation.

**RESULTS:** 1. There was a hitch in promoting safer sex information because of lack of alternative lubrication. 2. MSM capacities in HIV/AIDS focused prevention are reinforced. 3. IEC tools and messages produced with MSM participation are accepted by MSM population. 3. A space for information and ideas' exchange is created and active listening skills are developed by Ishtar MSM members. 4. There is increased up take of VCT services due to the direct community mobilization. 5. There is increase in numbers for the PTC from 20 to 45. 6. A total of 1646 identified MSM reached.

**CONCLUSIONS:** Focused prevention, designed and implemented through a participatory approach are important to reduce HIV/AIDS vulnerability among MSM population. Involving Peer Educators in this effort multiplies the effect of prevention outreach. There is a need for further research on the alternative locally available sources of lubricants including rectal microbicides to increase the alternatives for safer sex practices.

## 443

### Microbicides in Spain: an Experience of Advocacy for New HIV/AIDS Preventive Tools

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**BACKGROUND:** In a context of pandemic's feminization, microbicides are the first preventive tool designed to address women needs to protect themselves against HIV infection and can have a direct impact in countries in development, where HIV/AIDS extensively affects millions of people.

In coherence with the most innovative cooperation policies, which have a substantial focus on research and development in health, leading civil society groups wanted Spain to support the international efforts aimed at obtaining safe, efficient and accessible microbicides to all people who need them.

For those reasons, an advocating strategy was implemented in order to include microbicides—both vaginal and rectal—in the agenda of key Spanish stakeholders (civil society, policy makers, media, etc.) in order to ultimately achieve political and economical support from the Spanish Government to the R&D of vaginal microbicides as a first step.

**METHODS:** Key audiences were identified: HIV/AIDS and Cooperation for Development NGOs; Community Networks; Members of the Spanish Government with responsibilities in health and in cooperation; Members of the Congress and the Senate (Commissions of International Cooperation for Development and Health); Media and Researchers.

The first stage of the advocacy project focused on raising awareness about microbicides through direct contacts with key audiences: personal interviews; lunches with the press; a workshop on microbicides at the Spanish National AIDS Congress in 2007; toolkits for politicians and media; and official visits to the Parliament.

The second stage was the organization of several activities that could show the specific support to microbicides from each audience. Non-Binding Proposals, amendments to the Spanish General Budget and an Institutional Declaration were promoted in the Spanish Parliament; input was delivered to a wide variety of media; civil society input was also delivered to public policies related to international cooperation and health, such as the Master Plan for Cooperation 2009–2012 and the Strategy on Health in Cooperation.

The third stage (on-going) consists in keeping audiences well-informed and up-dated through continuous communication and educational activities (including online information: website, blogs and social networks, community workshops to improve the knowledge and commitment with microbicides' development; and promotion of Parliamentarian Initiatives like Official Hearings) that help to reinforce the agenda on microbicides.

**RESULTS:** Main key Spanish stakeholders are aware of the need for microbicides as part of a comprehensive response to HIV. Firstly, microbicides issues have been included in the social, media and political agenda, showing explicit support to the development of new preventive tools for HIV. And secondly, the Spanish government decided to support the R&D of microbicides with 1.5 millions of Euros through the International Partnership for Microbicides (IPM) in 2008. This support was renewed in 2009.

#### CONCLUSIONS:

- Access to substantial and specific information about microbicides is indispensable as an advocacy tool, but not enough. A detailed plan of actions and activities is also needed to mobilize wide, active support leading to political and financial support of microbicide R&D.
- It's essential to select the key audiences and what kind of relationship should be established with them in order to pursue the overall goal.
- Advocacy efforts need a strategic framework where the target (in that case, a public good for global health) has to be incorporated. The framework in this project was structured around first, the need for R&D of new biomedical products to strength HIV/AIDS response and second, the relation between health as a human right and women needs.
- Networking is crucial. Political advocacy and lobbying should not be implemented in isolation and require a hard work of collaboration with other NGOs and opinion leaders to obtain their active support to microbicides, including funding to microbicide R&D.

## 444

### Identifying Priorities, Gaps and Opportunities in Prevention Research Through Community Advocacy: How the AVAC-GCM HIV Prevention Research Advocates Fellowship Program Is Nurturing Emerging Leaders

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<sup>1</sup>AVAC: Global Advocacy for HIV Prevention;  
<sup>2</sup>The Global Campaign for Microbicides

**BACKGROUND:** Global advocacy around HIV prevention research is most meaningful and sustainable when rooted in local priorities. Yet there are few resources to support local community advocates and networks to be effective at national and global levels. This identified need guided the development of the "AVAC-GCM HIV Prevention Research Advocacy Fellowship" in 2009. The program goal is to expand the capacity of civil society advocates and organizations, especially in the Global South, to monitor, inform and advocate around HIV prevention research. It also aims to increase the cadre of skilled advocates who can contribute to setting the prevention research advocacy agenda.

**METHODS:** The project was designed to attract individuals interested in HIV prevention research advocacy and/or the rollout of HIV prevention options. Applicants identified a priority area and local host organization, and developed a project that would fill a critical gap. A broad call for applications was launched in May 2009. Applications were reviewed by an independent committee of various stakeholders.

**RESULTS:** Of the 112 applications from Africa, Asia and South America, nine Fellows were selected. Inaugural Fellows are from: Kenya (1), Malawi (1), Rwanda (1), South Africa (2), Uganda (2), and Zimbabwe (2).

The Fellows were selected based on stringent criteria and on projects that explore innovative approaches to address gaps in prevention research advocacy. Project foci include: monitoring broad community engagement during the trial life-cycle, developing media training across technologies, facilitating dialogue with diverse communities in rollout plans, engaging national stakeholders around trials and bolstering the capacity of HIV advocacy campaigns to integrate research literacy.

About half of the applications were not considered as they focused on trial-specific activities such as improving recruitment, increasing adherence and developing advisory boards.

**CONCLUSIONS:** The Fellowship Program has identified emerging leadership in prevention research advocacy, diverse activities that community players consider important, as well as significant gaps in trial-specific activities. Priority should be put on identifying additional resources to support advocacy activities and tools to measure the ongoing impact of them on the nature and quality of research conduct. It is also vital that trial sponsors and advocates seek new mechanisms to address gaps identified in trial-specific activities.

# TUESDAY POSTERS

## 445

### New Prevention Technologies and Sex Workers: Protection for Whom and at What Price?

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Network of Sex Work Projects

**BACKGROUND:** As a group highly vulnerable to HIV infection, sex workers are frequently cited as potential targets for future microbicide and/or PrEP introduction. Social science research among sex workers on microbicide acceptability has produced important data but does not address how access to new prevention HIV tools may interact with the social, economic and physical parameters of sex work. The Asia Pacific Network of Sex Workers (APNSW) 2009 report entitled, "Sex work and the new era of HIV prevention and care," raises fundamental questions on this issue for consideration by social scientists and access planners as we expand the universe of HIV prevention technology.

**METHODS:** A 2006 cross-training held by APNSW, the Network of Sex Work Projects and the Global Campaign for Microbicides led to creation of a joint advocacy agenda of issues requiring additional investigation and attention. GCM is using this agenda to raise awareness of these issues among sex worker advocacy groups and other NGOs. Since sex worker organizations are profoundly stigmatized and have almost no funding with which to mobilize, their issues rarely get attention on the HIV/AIDS research agenda. To date, sex worker marginalization has exceeded even that experienced by MSM, injection drug user communities and other high-risk groups. One illustration of this is that US-imposed regulations policies such as "abstinence-only-until-marriage" sex education, "no promo homo" and the syringe exchange funding ban are dropping away because HIV/AIDS and SRHR advocates have shown their counterproductive effects on HIV prevention. While contested, however, the PEPFAR-mandated "prostitution pledge," remains in force.

**RESULTS:** This presentation will outline the results of this mobilization effort; the challenges confronted, and successful and unsuccessful aspects of our strategies.

**CONCLUSIONS:** Sex workers and their advocates are the experts on the real consequences that the introduction of microbicides and PrEP are likely to have on their working lives. The HIV prevention research field must attend to these issues before product introduction to assure mitigation of foreseeable and potentially harmful effects wherever possible. Generating advocacy for this demand is complicated by the profound stigma against sex work but can be achieved. Once recognized as both a human rights issue and a pragmatic necessity, the specific HIV prevention needs of sex workers can be addressed just as the unique risks and needs of MSM, injection drug users and other high risk populations have been.

## 446

### Using Innovative Participatory Learning Activities to Enhance Understanding of Clinical Trials

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**BACKGROUND:** There is an increasing need for advocates and clinical trial staff to access structured materials and trainings that move beyond the basics of microbicides science to address the complexities of the field as it matures. In response the Global Campaign for Microbicides (GCM) launched Microbicides Essentials, an on-line course designed to respond to the scientific and ethical challenges faced by advocates who work in HIV prevention. In June 2009, GCM began series of in-person microbicides research literacy trainings conducted with advocates and trial staff from Eastern and Southern Africa. GCM collaborated with Family Health International (FHI) to develop a series of participatory learning activities that incorporated content from FHI's 'Introduction to Health Research Workshop,' designed to build capacity of community stakeholders, partners and staff with little or no experience of clinical research. This formed part of a larger Site Identification and Development Initiative (SIDI), funded by USAID to build capacity and partnerships with local institutions in Africa and Asia.

**METHODS:** To date GCM has conducted 5 trainings with participants from Rwanda, Kenya, Uganda, Tanzania, Nigeria, Zambia, Zimbabwe, Botswana and South Africa. These trainings use participatory learning techniques to teach concepts related to clinical research. The use of participatory learning activities help participants understand the complexities of the research process while providing them with methods and tools that they can use in their own communities to explain the process and value of HIV prevention research.

**RESULTS:** This presentation will outline (1) specific activities used, (2) highlight the successes and challenges of training advocates on microbicides research literacy through participatory learning, and (3) demonstrate educational and community outcomes following implementation of these strategies.

**CONCLUSIONS:** Numerous adult education studies have shown that students learn more from trainings and material when they actively participate through a blended, guided process, than individual readings or exercises alone.

## 447

### Community Engagement Within the FEM-PrEP Clinical Trial at Setshaba Research Centre, Soshanguve, South Africa

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**BACKGROUND:** Setshaba Research Centre is conducting FEM-PrEP, a phase-3, placebo-controlled, safety and effectiveness study to assess the role of Truvada as oral pre-exposure prophylaxis (PrEP) to reduce HIV acquisition in women. This trial is led by Family Health International (FHI), in collaboration with local sites. Community Engagement activities began prior to the socio-behavioral and community preparedness (SBC) activities by developing partnerships with a broad range of stakeholders.

**METHODS:** The Community Educating Officer (CEO) initiated the engagement process by communicating with various community stakeholders including the Department of Health (DOH) about the planned clinical trial; community advisory board (CAB) representatives were recruited. Stakeholders were invited on site and provided with an overview of the upcoming clinical trial and the roles and responsibilities of a CAB. Some interested community members volunteered and others were identified and asked to be CAB members. Educational needs assessment surveys were conducted in the community to evaluate the knowledge of HIV prevention and clinical research. Then the community at large was educated about HIV and clinical research. The CEO conducted education sessions at meetings, workshops and participated in community forums. This was done in collaboration with formal and informal stakeholders such as ward councilors and street committee members; thus establishing rapport and trust between the community and the CEO.

**RESULTS:** A memorandum of understanding was established with the Department of Health (DOH) to build a strong referral networking system for trial participants. Fifteen representatives of the various community stakeholders volunteered to be CAB members and were trained in research ethics, basics of clinical trials and specifics of the FEM-PrEP clinical trial. The CAB members were engaged in this process prior to the initiation of the clinical trial.

The design of the education sessions were based on the community survey to provide appropriate educational content. The community education sessions were sequenced strategically to enable the recruiting officers to gain access to establishments such as taverns, where they are able to provide study information sessions. As a result of community engagement, the CEO receives frequent invitations from community organizations to attend health related community events and utilize the opportunity to provide information on the FEM-PrEP clinical trial.

**CONCLUSIONS:** Community engagement before, during and after the study is very important in gaining trust, acceptability and to develop and sustain good working relationships. Furthermore, this process facilitates communication between the study team and the community. Community engagement with the CAB assists the study team on issues such as language usage and cultural acceptability in the research documents, as well as advice on recruitment strategies.

**448**

**Virtual or In-Person?: Evaluating the Impact and Cost-Effectiveness of New Teaching Methods to Expand HIV Prevention Research Literacy**

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**BACKGROUND:** HIV prevention research literacy is a global endeavor. Despite the increasing demand for training of both advocates and trial staff unfamiliar with research, resources for research literacy are constrained. It is important therefore, to continually evaluate teaching methods to ensure they are effective, efficient, value-for-money and deliver demonstrable impact. GCM compared two approaches to increasing learners' interaction with the e-course material. The first, called the Microbicides Research Literacy Training (MRLT) was an in-person two day-long training. The second, called the Virtual Classroom was a bi-weekly series of webinar sessions. GCM conducted a formal evaluation of these different learning methods to guide future resource allocation.

**METHODS:** GCM created an in-depth evaluation as well as developed tools to compare method acceptability and information retention among (1) learners who took the e-course, (2) those participating in the MRLT, and (3) those who engaged with the Virtual Classroom. Pre- and post-tests were used to measure the retention of core information and understanding of key concepts. Acceptability data were collected through evaluation forms completed by students as well as by an evaluator who targeted a random sample of students via structured telephone interviews. All data were then analyzed to assess the advantages and disadvantages of each training methods, as well as the relative costs of each technique.

**RESULTS:** Participants across all study methods reported a positive learning experience and increased knowledge. Some of those studying alone appreciated being able to take the course at their own pace, without disrupting their work commitments. However many confirmed that they would have preferred to have taken the course in a group and preferably in a classroom environment. Participants in the Virtual Classroom reported a benefit from reviewing modules via webinars and having access to experts in the field. Students who attended the in-person trainings expressed increased confidence in public speaking and taking advocacy forward in their own communities. We present here vigorous evaluation methodology and tools, results of the evaluation, and an insight into the lessons learned to inform future pedagogic techniques.

**CONCLUSIONS:** It is important to continually develop and assess training and evaluation activities that address and target the complex learning needs of advocates engaged in HIV prevention.

**449**

**Leveraging Web-Based Technology to Build the Capacity and Knowledge of Advocates**

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<sup>1</sup>Global Campaign for Microbicides, PATH, Washington, DC, USA; <sup>2</sup>Global Campaign for Microbicides, London, UK

**BACKGROUND:** The Global Campaign for Microbicides (GCM) launched a virtual classroom in June 2009. By September 2009, fourteen European and African students successfully completed the course with demonstrable proficiency. The virtual classroom drew content from the *Microbicides Essentials* on-line course, a self-instructional tool created by GCM.

**METHODS:** Over the course of four months, students from as far apart as Zambia, Denmark, Rwanda and Portugal came together to participate in seven cyber-space study sessions. Equipped with headsets, computers, and phones, students engaged with expert guest speakers and GCM staff, viewed scientific animations and participated in interactive activities. Topics examined in depth included female condoms, vaginal and rectal microbicides and pre-exposure prophylaxis (PrEP).

**RESULTS:** By blending the self-instructional aspects of the *Microbicides Essentials* course with group sessions via a virtual classroom, students were able to gain greater understanding of the material, enabled them to apply their new-found knowledge with confidence in their advocacy role.

**CONCLUSION:** The use of asynchronous and synchronous distance learning activities provides an opportunity for advocates to strengthen their capacity and expand opportunities to engage with research and clinical trials productively. Web-based training tools can provide organizations with a cost-effective way to provide interactive training and connect participants from different time-zones and countries.

This presentation will provide an overview of the content, the technology utilized, as well as the successes and the challenges of training advocates on microbicides research literacy using asynchronous and synchronous distance learning.

**450**

**Result Dissemination: The Use of Annual Participant Events**

N. Mhlongo, M. Mzimela, H. Ndlovu, M. Gafos and the MDP team

Africa Centre for Health and Population Studies, University of KwaZulu-Natal, South Africa

**BACKGROUND:** The Africa Centre for Health and Population Studies in northern KwaZulu-Natal, South Africa was one of six sites participating in the Microbicides Development Programme MDP 301 clinical trial. Enrolment commenced April 2006, follow-up ended August 2009 and results were released December 2009. Follow-up was for 12 months so some women had exited the study 2.5 years before result dissemination. The site held 3 participant events (Aug '07; Nov '08; Dec '09) in order to maintain contact with participants to present the final results. We examine the characteristics of participants who attended the final participant event.

**METHODS:** Demographic, retention, gel allocation and clinical data were merged with event attendance registers for analysis in Stata 10. We defined time period of enrollment by dividing the 29 months of enrollment into six monthly categories. We calculated the proportion of enrolled participants who attended the final event. We used Pearson Chi<sup>2</sup> tests to evaluate whether attendance differed by characteristics or the occurrence of a clinical outcome during follow-up (HIV incidence, pregnancy, adverse event).

**RESULTS:** The first event attracted 75% of participants enrolled up to that time, the second event attracted 52% and the third 40% of the total 1,177 women ever enrolled. Of those who attended the final event 78% had attended a previous event. Univariously, there were no significant differences in the proportion who attended the final event based on gel allocation group ( $p=0.30$ ), HIV sero-conversion ( $p=0.33$ ), pregnancy ( $p=0.86$ ), experiencing a clinical adverse event ( $p=0.40$ ) or time period of enrollment ( $p=0.09$ ; test for trend= $-0.05$ ). Women who attended the event were significantly older (median 39 IQR:26-46) than women who did not (median 31 IQR:23-43). Attendance differed by clinic of enrolment (Mtubatuba 32%; KwaMsane 42%; Madwaleni 47%). 44% of those who completed follow-up attended; 17% of defaulters and 10% of women who withdrew also attended.

**CONCLUSIONS:** The annual participant events were successful in ensuring that almost half of the participants ever enrolled remained in contact with the site team to receive the results. A similar proportion of participants who experienced clinical events during the trial attended as those who did not, indicating positive ongoing engagement despite negative clinical outcomes. In rural settings with high mobility participant events can prove an effective long term engagement strategy.

# TUESDAY POSTERS

**451**

## What Does the Community Perceive as Engagement in Biomedical HIV Prevention Research?

G. Calazans, J.A. Beloqui, R. Mburu, M. Ukpong, T. Young

**BACKGROUND:** Report on the perception of various 'communities' on what it means to be engaged in research.

**METHODS:** 119 representatives of communities of laypersons, researchers and other stakeholders of biomedical HIV prevention research in Brazil, Nigeria, the US, and Kenya provided feedback on their perception on community engagement in research. The discussions were with a view of evaluating the Good Participatory Practice (GPP) Guidelines for biomedical HIV prevention trials and their appropriateness in facilitating community engagement in HIV biomedical research. Information was collected through face-to-face consultations and FGDs.

**RESULTS:** The community engagement process in biomedical research should support an enabling environment for community representatives to enable them review and give feedback to research protocols. This may include communities having the liberty to be able to consult other identified expertise to help provide feedback on protocols. Community participation is proportional to the time, space and autonomy given. Engagement should go beyond the existing CAB mechanism; there should be partnership with existing community structures. Engagement should be throughout the research lifespan. Engagement post trial should happen independent of the outcome of the research (good, bad, discontinued). The current level of engagement of communities in research is at best assessed as fair.

**CONCLUSIONS:** Engagement means participation in defining the research agenda, planning, and implementing the research as an ongoing process throughout the research lifecycle. This perception was the same independent of country, culture or language.

**452**

## Enhancing the Capacity of the Deaf to Manage and Cope with HIV/AIDS Pandemic

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**BACKGROUND:** In Kenya Research shows that majority of deaf people do not go beyond the primary level of education, often because sign language is so structurally and grammatically different from written English. Unlike the hearing population, deaf people have less ability to passively garner information like listening electronic media and thus miss a lot of H.I.V prevention messages that most Kenyans hear every day. This situation has led to deaf people's lack of knowledge about H.I.V/AIDS.

**METHODS:** The participants were selected from Thika National Deaf School. The cohorts comprised 150 deaf people who were divided into 2 equal groups to allow for effective training, the variables included; age (from 18 to 40 years), gender (equal males and females). Two professional counselors one of the counselors well conversant with the sign language met each group once a week for three months. The topics covered included; self-awareness, sexuality, H.I.V/AIDS information, life skills and basic counseling skills. These trained groups conducted outreach activities to disseminate updated information on HIV/AIDS to the deaf people in 3 districts; Nairobi, Kambui School of the Deaf and Machakos School of the Deaf.

**RESULTS:** In both groups and the outreach activities capacity for the deaf to manage the HIV/AIDS pandemic was enhanced. Approximately 150 deaf Members acquired updated information and contextualized HIV/AIDS. After the training it is expected that each participant came up with realistic and individualized strategies of reducing their risk of H.I.V infection. Outreach activities in Nairobi, Kambui and Machakos equipped 440 deaf people with skills, knowledge that will reduce their vulnerability to HIV and AIDS. The group is now a network for the deaf members with a strong base to handle the challenges pertaining to HIV/AIDS.

**CONCLUSIONS:** By the end of 2009, 590 deaf people had acquired updated H.I.V/AIDS information. The skills and knowledge equipped the deaf community to reduce their vulnerability to HIV and AIDS. From the trainings the deaf people embraced the information provided about upcoming Microbicides Research.

**453**

## Leading The Way: Faith Leaders Talk Microbicides

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<sup>1</sup>African HIV Policy Network, UK; <sup>2</sup>Terrence Higgins Trust, UK; <sup>3</sup>Naz Project London, UK

**BACKGROUND:** Faith leaders play an important role within African communities. They provide leadership, support and inspire change to local communities. The need for them to be engaged in HIV prevention is ever increasing due to the concentrated HIV epidemic in the UK. The UK African Microbicides Working Group has been working with faith leaders to increase their knowledge and understanding around the need for new prevention technologies and to fundraise for further research.

**METHODS:** In 2009, the working group partnered with the London Ecumenical AIDS Trust (LEAT) that provides faith-based response to the HIV epidemic in London and St Georges University delivered training and shared information about Microbicides. Through this partnership faith leaders received training on the Christian faith toolkit *Breaking the Loud Silence of HIV* which aims to increase levels of awareness on HIV and to change faith leaders perceptions of HIV and Africans in the UK. The training includes informing faith leaders about routes of HIV transmission, HIV related stigma and the Disability Discrimination Act (2005). The training was then linked to Microbicides research and development and the importance of Microbicides as an alternative prevention option, particularly in faith settings where condom use is infrequently discussed and promoted. The partnership is unique in that the idea to raise funds and awareness training on Microbicides has been led predominately by the men's fellowship group from the Springfield Methodist Church in London. The group consists of 10-15 men who are all enthusiastic and willing to learn about and engage in HIV-related work.

**RESULTS:** Through this work the working group found that faith leaders felt that churches must change their attitude towards HIV. Training sessions of this nature will increase faith leaders' awareness and understanding of HIV related stigma, discrimination and new prevention technologies. The partnership wished to encourage the faith leaders to be actively involved by participating in discussions with different organisations to enhance their knowledge about HIV and Microbicides research and development. There is lack of information within the Church about HIV, HIV testing, new prevention options, treatment and support needs. Raising awareness within Churches will equip faith leaders with the right information to communicate effectively with their congregation.

**CONCLUSIONS:** The partnership has demonstrated that engaging community leaders in advocacy and development of Microbicides raises awareness of new prevention technologies as well as general HIV awareness. The group is currently exploring the role of faith leaders in reaching their congregation with broader HIV prevention messages, including safer sex practices. We hope to encourage more faith leaders to be actively involved in advocacy and lobbying for Microbicides research and development globally.

**454****A Definition of Community Education for HIV Vaccine Trials**

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**OBJECTIVE:** Building a coherent and useful definition of community education about HIV vaccines and other HIV prevention trials that will result in ongoing, measurable, supported, and replicable community education programs that are complementary to, but separate from, study recruitment and retention efforts.

**METHODS:** To understand the baseline perceptions of community education, Samaritan's House conducted qualitative interviews with community educators, recruitment staff and Community Advisory Board members at HIV vaccine research sites. From these data, Samaritan's House created a working definition for community education and sought further input from targeted individuals through focus groups and conference calls.

**RESULTS:** The interviews underscored the need for a strategic rationale for community education, as most respondents could readily articulate specific activities of community education, such as presentations or community forums, but had a more difficult time describing the broad goals and reasons for community education. The authors define community education as a research site's commitment to conduct ongoing educational activities, not tied to any particular trial, which: 1) build the capacity of communities to understand biomedical HIV prevention research in order to make informed decisions about whether or not to support the research in their communities, 2) address stigma around HIV and build social support for individuals who do decide to participate in research, 3) foster a collaborative relationship with existing community structures in order to conduct more culturally appropriate research and to effectively communicate with the wider community, and 4) over time, build willingness to participate in research studies.

**CONCLUSIONS:** Creating a clear definition of community education will result in community education activities that are rational, measurable, replicable and adaptable. Furthermore, such programs can be measured by whether pre-established goals are met, rather than by enrollment numbers, allowing for better support for education, and increased understanding of the difference between community education and trial recruitment. As research for an HIV vaccine and other biomedical approaches to HIV prevention continues, quality community education programs are necessary to create successful working relationships with communities around the world and long-term support for research and its results.

**455****Knowledge of HIV Prevention and Willingness to Collaborate in Vaginal Microbicides Trials-Survey of Nigerian Gynecologists**J. Musa<sup>1</sup>, I. S. Adamu<sup>2</sup>, Z. Moh'd<sup>2</sup>, C. Egbodo<sup>3</sup>, C. Moh'd<sup>1</sup><sup>1</sup>Jos University Teaching Hospital, Jos, Nigeria;<sup>2</sup>Aminu Kano Teaching Hospital, Kano, Nigeria;<sup>3</sup>Medical Center, Nigeria Institute for Policy and Strategic Studies, Kuru, Jos, Nigeria

**BACKGROUND:** Heterosexual transmission accounts for 80% of HIV in Nigeria. Women are more vulnerable and account for 60% of the HIV burden in Nigeria. Gynecologists are advocates for women's sexual and reproductive health. Their knowledge and opinions on HIV prevention strategies as well as willingness to collaborate in trials on new prevention technologies is crucial in achieving success in the fight against HIV transmission. We hypothesized that the knowledge and opinions of Nigerian Gynecologists on the effectiveness of various HIV prevention strategies is significantly related to their willingness to collaborate in Vaginal Microbicide (VM) trials.

**METHODS:** A cross-sectional, self-administered questionnaire was distributed to Nigerian Gynecologists at the annual general meeting and scientific conference of the Society of Gynecology and Obstetrics of Nigeria, held between 17th and 21st November, 2009, Kano, Nigeria. A non-probability convenient sampling technique was used to select participants at the conference. An introductory letter stating the purpose of the survey was included in each of the questionnaire. Completed questionnaires were returned to the researchers at the end of the conference. The responses were scored on a Likert scale and statistical analyses were done on STATA version 11, college Station, TX, USA.

**RESULTS:** A total of 182 Gynecologist participated in the conference and 100 received the survey questionnaire. The survey response rate was 76.0%. The mean age of the respondents was  $42.54 \pm 6.75$  (range 30 to 58 years). Twenty five percent (25%) of the respondents had some knowledge of VM and only 7% could correctly name a VM for HIV prevention. The mean score of their opinions on the effectiveness of VM was significantly lower compare to other HIV prevention strategies (Abstinence, use of condoms, male circumcision, screening and treatment of STIs, and use of antiretroviral therapy; p values <0.001). There were no significant relationship between knowledge of VM and the mean scores on either willingness to collaborate, confidence on VM or readiness to recommend VM (P values >0.05).

**CONCLUSIONS:** The knowledge of Nigerian Gynecologist on VM for HIV prevention is relatively poor. They have a stronger opinion that other HIV prevention strategies are more effective than VM in preventing sexual transmission of HIV. They however have a strong willingness to collaborate on VM trials and are ready to recommend VM if effective in clinical trials.

# TUESDAY POSTERS

**456**

## Promoting Sexual Reproductive Health & Rights Of Women To Manage Their Sexual and Reproductive Life

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*Ambassadors of Change—NGO, Nakuru, Kenya*

**BACKGROUND:** Women and girls in Kenya presently comprise more than 60% of those living with HIV and the numbers continue to grow hence the need to acknowledge the devastating gender dynamics of HIV/AIDS demanding placement of women empowerment at the centre of programming, planning as a solution to the epidemic. Unintended pregnancy is a major public health challenge in Kenya, a key cause of induced abortion, most of which is unsafe, contributing to high maternal mortality. Electively aborted pregnancies are viewed as unintended. While Kenya is committed to ensuring access to family planning services for all, the reality is that many poor people, who form the majority of the country's population, are not using contraceptives due to stock-outs, attention shifting to HIV/AIDS, funds for buying the commodities dwindling, poor distribution to various health facilities and cultural factors. Under utilization of health facilities is a problem on reproductive health.

**METHODS:** The purpose of the study was to estimate levels of awareness among women of reproductive ages (14 years–49 years) on contraceptive methods, microbicides research, identifying critical barriers likely to challenge successful use of the products once research is over. Promoting sexual reproductive health, rights of women to manage their sexual and reproductive life. Make family planning real.

A cross-sectional design purposely utilized engaging 23 women, 17 married couples, focusing on understanding, acceptability of microbicides, use of existing HIV prevention tools, contraceptives including condoms consistent use. Both open-ended semi-structured interview guides were used for data collection through 8 group discussions, 10 in-depth interviews. A trained duo conducted focus discussions observing ethical approaches to gathering information from clients in a neutral, confidential location.

### RESULTS:

- Evidence on factors affecting use, non-use of contraceptives suggest that women's lack of decision-making power over sex among married couples, opportunity costs involved in seeking contraceptives, misconceptions, poor quality of public health centers in providing contraceptive services all play an important role in use and non-use of contraceptives among women.
- PMTCT should be an entry point for maternal treatment, early infant diagnosis and treatment.
- A multi-sect oral response to gender based violence e.g. addressing cultural norms and behavior regarding vaginal lubrication during sex i.e., accusation of infidelity for dry sex, which often vary between cultures.
- Community voices in research for policy.
- Pre-marital sexual behavior among youth and adolescents is rampant putting young women who engage in sexual relations in an extremely vulnerable situation and almost ¾ had experienced unintended pregnancy with no access to HIV prevention strategies including condoms.
- Social and cultural factors challenge efforts to improve health care delivery creating the role of communities to enhance efforts to increase utilization of health care services.
- Effective interventions exist to eliminate pre-natal HIV transmission, yet infants continue to become HIV infected e.g. PMTCT and Emergency Contraceptives.
- A third of women had a previous abortion with an average of 1.3 previous abortions. These were married women, (48% vs. 39%). Women with previous abortions reported having ever used contraceptives (59% vs. 43%) and reported higher use of short-term reversible methods (i.e. pills and condoms).
- Little knowledge about vaccines and microbicides.
- Skin-to-skin during sex was more popular among both female and male as it creates trust, intimacy, sexual pleasure and closeness hence the need for microbicides.

### CONCLUSIONS:

- Creating awareness around family planning through discussions, radio programs to lend medical advice on family planning methods and use.
- Highlight to couples to consciously decide on planning methods.
- Health facilities should provide free family planning services.
- The communities to play a role in promoting family planning and reproductive health by breaking through inhibiting cultural practices and beliefs.
- Men to support women in family planning and other reproductive health issues.
- Bridging the existing gaps between researchers and communities as key stakeholders.

Integrating reproductive health and HIV health care services

## 457

### Do Adherence Counselors Play Critical Roles as Community Agents to ARV Treatment Literacy and Adherence?

O. Osindele\* O. Onuoha O. Akanni  
*Journalists Against AIDS (JAAIDS) Nigeria*

**BACKGROUND:** The role and importance of the health workers as critical to the success of ARV treatment literacy and adherence is well acknowledged. However, emphasis on community empowerment for more participants in counseling is critical in the success of ARV treatment literacy and adherence in the community hence the need for adherence counselors, especially people living with HIV (PLWH). This abstract shares the experience of JAAIDS in addressing capacity building of adherence counselors as community gatekeepers for ARV treatment literacy and adherence.

**METHODS:** A two-day refresher training was organised for adherence counselors serving in ART sites within Oyo State, Nigeria. The two days were spent on knowledge and skills acquisition for the adherence counselors which was aimed at equipping them with new skills on HIV treatment. It was also spent equipping them on how to counsel PLWH accessing treatment as well as facilitate the decentralization process for ART services in the State. The adherence counselors had a mock counseling sessions where they were paired up to practise the new skills. Three of the adherence counselors at the refresher training were employed for one year and posted to specific ART sites in Oyo State while the rest returned to their ART clinics to continue their counseling.

**RESULTS:** This was part of the process aimed at the decentralization of ART services in Oyo state. The participants identified critical roles they could play as adherence counselors in ART treatment literacy and adherence. They also identified important steps JAAIDS could take to facilitate their ability to work effectively in their role. This included the need to train other people as counselors on ART treatment and adherence.

**CONCLUSIONS:** Adherence counselors in ART sites are critical community voices and they equally need to be empowered to be able to play roles in ARV treatment literacy and adherence.

## 458

### The Impact of HIV/AIDS Prevention Research Advocacy on Target Beneficiaries in Kisumu District, Nyanza Province, Kenya

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*HIV/AIDS Research & Advocacy Programme (HIRAP), Kisumu, Kenya*

**BACKGROUND:** Community education is needed in advance of clinical trials to prepare the community for the prevention research process and to lay the foundation for the eventual distribution and use of an effective prevention technology. Approach and timing needs to be considered as too much education may cause confusion and or raise unrealistic expectations in the community. Educational efforts should be carefully monitored to assess community response. The community education objective is to increase the level of the new prevention technologies knowledge among the NGO, CBO and FBO workers in Kenya that would eventually flow down to the general community and to create and sustain a network of community workers who are supportive of the AIDS prevention research. Kisumu District is a site for several researches including a discordant couple PrEP trial, an impending microbicide trial, and an incidence study all by known research institutions.

**METHODS:** The study was conducted in 30 randomly selected organizations in Kisumu District, data being collected using questionnaires. Purposive sampling was used to select one organization for the study data that had a total target population of 230 staffs and members. The study employed simple random sampling to select 30% of the population. The researcher was mainly concerned with knowledge of AIDS prevention research, views, opinions, perceptions, feelings and attitudes of the target communities of most community advocacy programs. The data collected was analyzed using descriptive statistics. The data was then summarized and presented in tables, charts and graphs. The analysis was done according to the major categories arising from the research questions.

**RESULTS:** The study concluded that 45% of respondents had heard of an AIDS prevention research trials before but only 10% had heard of a specific trial or impending trial in Kisumu District. The main source of information on prevention researches was the media at 32%. 57% of respondents felt that the message from the media was not sufficient while 43% felt the message was somewhat adequate. Those who felt they can participate in a research trial or encourage a relative to participate in a trial stood at 42% but only when provided with enough information concerning the trial, those that felt that they were not ready to participate due to fears or lack of information were 33% while 25% were undecided.

**CONCLUSIONS:** There is clear need for increased advocacy with clear targeted populations for an increase in informed engagement in any pending or ongoing research trial. There is also need to engage with different stakeholders in the community. The research organizations should work with the advocacy groups or networks to ensure correct information flows to the community. There is urgent need for donor funding for direct community advocacy initiatives to ensure that the community are effectively engaged in ongoing and impending trial and the benefits that would accrue from participating in a trial. IEC materials need to be developed and distributed in local dialects to ensure increased understanding of communities of prevention research. The media has proved to be a source of research information to many communities and they form a very important group that need to be engaged to ensure that information that passes through them are correct.

# TUESDAY POSTERS

**459**

## Perception and Attitudes of Stake Holders in the Dissemination of Result of Cellulose Sulfate III Study in Nigeria

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Health Matters Incorporated

**BACKGROUND:** HIV continues to infect and affect people, both adult and young in the world and Nigeria is not an exception. To date, there is no cure though there exist drugs that can curtail the virus. However these drugs have to be used for life. In Nigeria, an estimated 3.1 percent of adults between ages 15-49 are living with HIV and AIDS. Although the HIV prevalence is much lower in Nigeria than in other African countries such as South Africa and Zambia, the size of Nigeria's population (around 138 million) meant that by the end of 2007, there were an estimated 2,600,000 people infected with HIV.

The study was stopped in January 2007 because of safety concerns from a similar study in South Africa. There was a need to inform stake holders and participants alike on the project and its outcomes as well as reasons for its stoppage. To this end, dissemination seminars were organized to provide information to stakeholders on the outcomes of the study.

### Objectives:

- To present the results of CS phase III trial to the study participants and other stakeholders at the trial sites in a simple language
- To assess the acceptability of the CS phase III outcomes among stakeholders
- To assess stakeholders level of understanding of the research findings
- To facilitate discussion between the researchers and community members about result

**METHODS:** Dissemination took place at Ijora Badia, Boundary and Ikeja.

- A rapid assessment instrument was administered to 50 persons for each site.
- Administration of instrument was carried out by two outreach staff.
- The study participants were mobilized by phone call while a team of mobilizers went round the communities recruiting participants and key opinion leaders, NGOs, HIV activists, invitation letters were issued out, venue secure media invited.

### Structure of RAP Instrument:

- Participants were asked their level of satisfaction with the study procedure, outcomes and likely benefits
- Questionnaires were administered with the following categories: Highly satisfied, Satisfied, Some what satisfied, not satisfied

### RESULTS:

#### 1 Ijora Badia

30 Person reported 'Highly Satisfied'  
 11 Persons reported 'Satisfied'  
 8 persons reported 'Some what satisfied'  
 1 person reported 'Not Satisfied'

TOTAL 50

#### 2 Boundary

33 Persons reported 'Highly Satisfied'  
 9 persons reported 'Satisfied'  
 5 Person reported 'Some what satisfied'  
 3 persons reported 'Not satisfied'

TOTAL 50

#### 3 Ikeja

23 Persons reported 'Highly Satisfied'  
 17 persons reported 'Satisfied'  
 6 Person reported 'Some what satisfied'  
 4 persons reported 'Not satisfied'

TOTAL 50

### CONCLUSIONS:

- Studies of this nature need to have a process of feed back and interaction with both participants and other stakeholders.
- Stakeholders generally need to know the outcome of studies involving them or the studies in which they participated in.
- Participants felt that benefits of the study for the future will aid the search for an HIV vaccine/microbe.
- Participants and others felt honored to be included in the study.

## 460

### Community Dissemination of a Clinical Trial: Sharing Experience from a Site in Nigeria

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<sup>1</sup>University of Port Harcourt Teaching Hospital; <sup>2</sup>Diacore; <sup>3</sup>University of Lagos

**BACKGROUND:** Disseminating the outcome of a microbicide clinical trial is a key component of the trial. We share an approach used to disseminate a phase III trial assessing the effectiveness of cellulose sulphate vaginal gel for the prevention of HIV in Port Harcourt a trial site in Nigeria.

**METHODS:** Four separate sessions were held to ensure that appropriate communication methods were used. The first involved the study staff, the second the study participants. The third session brought together stakeholders from non governmental organizations through existing networks, community based organizations, representatives of the governmental State Action Committee on AIDS as well as media represented by newspaper journalist. The final session focused on researchers primarily from the university community. The sessions consisted of presentations by the Principal Investigator, followed by a discussion session and question and answer sessions. Volunteers from the study participant session were involved from the beginning of the process and co-moderated during session: A separate meeting was set up for the press to allow for further clarification and feedback on their understanding of the result.

**RESULTS:** All staff personnel participated actively. 157 participants of the 275 traced accepted to come for the sessions. 120 attended. Reasons for non attending were multiple. 47 representatives from the different networks attended while 52 persons from the research community were present in their session. Input from participants showed a high level of understanding and appreciation of the process. Feedback from all sessions was impressive. The media impression of the study was accurate. All stakeholders requested to be updated on activities from the microbicide arena.

**CONCLUSIONS:** The process was highly interactive. It was well received by all stake holders. The active involvement of participants in the process was highly rewarding. There is however the need to create a forum to develop and enhance their capacity as peer educators and advocates for microbicide.

## 461

### Comprehensive HIV/TB Care and Treatment Delivered by Community-Based Wellness Centres

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<sup>1</sup>Medical Research Council, HIV Prevention Research Unit, Durban, South Africa; <sup>2</sup>University of KwaZulu Natal, Department of Paediatrics and Child Health, Durban, South Africa

**BACKGROUND:** Recent failures of biomedical HIV interventions such as vaccines, microbicides, HSV2 suppressive therapy and vaginal diaphragms has resulted in major setbacks for HIV prevention strategies. As there is not one solution for prevention, the best hope currently is building synergies between prevention, care and treatment. Enrolled subjects benefit from participating in microbicide trials but a major challenge is the continuum of care for seroconverters, who are referred to local government health services. Conventional HIV care and treatment services in South Africa are overburdened, based on medical interventions provided by clinics and hospitals with long delays in diagnosis of TB and HIV staging. There is therefore a need for integration of research settings and referrals to local health care providers.

**METHODS:** An HIV care and treatment research programme integrating medical and psycho-social services while conforming to all current government recommendations of an ARV initiation site, was implemented to improve the wellness/health of clients at community level.

**RESULTS:** The integration of medical and psycho-social services (2002–2009) in a community based primary health care clinic in Durban enrolled 2761 HIV infected individuals, 690 adults (18.5% males) and 79 children on ARV therapy with low referral to hospital for patients (<5%). In a one-year closely monitored adherence surveillance study, the adherence was shown to be >94%. The characteristics of this successful programme were: it was a small programme with great commitment delivered by a multidisciplinary team whose roles were equally important and a refresher adherence course.

**CONCLUSIONS:** Community-based interventions through relatively small programmes are highly effective and feasible. The merging of research centres particularly in the VOICE study and local health care providers would be pertinent and beneficial in creating a symbiotic relationship between research objectives and relevance to the local health care context. The implementation effects would represent a massive new departure for the country in dealing with this epidemic.

## 462

### Research Literacy; the Place of Community Mobilization and Preparedness in Calabar Municipality Nigeria

M. Onah, L. Agaji, P. Onah  
*Safehaven international a.k.a Commercial Sex Workers Initiative*

**BACKGROUND:** Due to low level of research literacy in Nigeria, community mobilization and preparedness is very much needed for community understanding of research process, This makes the whole process transparent and more easily acceptable by the community.

When community preparedness is not undertaken properly and effectively, the research may be compromised. The need for community ownership/partnership to ensure success of trials and future use of research products can not be over emphasized. To ensure wholesome community mobilization and involvement trial efforts focused research literacy on increasing the enlightenment of the public to the implications and impact of trials. Community involvement in new HIV prevention technologies research and development ensures advocacy and education which is needed to ensure political commitment, resource mobilization, production distribution, acceptance and delivery of products.

**METHODS:** Community mobilization and preparedness efforts for clinical trials involve updating stakeholders about research process, site preparation, volunteer mobilization and community ownership

Wholesome community mobilization and involvement trial efforts focused research literacy on increasing the enlightenment of the public to the implications and impact of trials. The mechanisms applied include advocacy, open community mobilization/sensitization, seminars and workshops. Information education and communication materials of GCM were downloaded and given to participants

**RESULTS:** 250 participants were reached. 105 males and 145 females. 20 health workers, 15 traditional leaders, 10 religious leaders, 59 CSOs, 71 market women, 5 policy makers and 60 graduates benefited from the capacity building.

Majority of participants were hearing of NPT and especially microbicides for the first time and they were curious to learn. They were meant to go back with the knowledge gained back to their communities.

**CONCLUSIONS:** Community preparedness efforts that are established prior to the execution of a clinical trial or recruitment of volunteers, help to increase the community's understanding of research objectives, increase support for the research and research participants and in the long run, ensure the demand for the product in the community.

# TUESDAY POSTERS

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### The Community Involvement Community of Practice: A Model for Collaboration in the Field

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**BACKGROUND:** Authentic community engagement helps trials to proceed smoothly by cultivating the community's sense of ownership, building trust and deepening the researchers' knowledge of local realities. It can improve data quality by ensuring that trial protocols and procedures integrate effectively into local norms. This enhances their acceptability to trial participants and optimizes the likelihood of accurate self-reporting and protocol adherence by participants.

The learning curve toward meaningful community engagement, however, can be steep. Over the last decade, staff at many clinical trials have developed expertise on proven strategies, lessons learned, and better practices for doing this work. Sharing this expertise across trial networks can: (1) reduce the learning burden on network sponsors and individual research staff, (2) foster a culture of collaboration, and (3) help networks and individual trial sites to optimize their community engagement efforts and, thus, safeguard the good reputations of their trials and of the field.

**METHODS:** In 2007, the Global Campaign for Microbicides (GCM) held a meeting to discuss how the field of community engagement in HIV biomedical research could best move forward. Meeting participants asked GCM to establish a community of practice around community engagement in HIV prevention trials.

**RESULTS:** The resulting "Community Involvement Community of Practice" (CoP) brings together community liaison officers and other relevant staff through monthly teleconferences and annual in-person meetings. Currently, the CoP has over 65 members from 14 countries who work in vaccine, microbicide, and pre-exposure prophylaxis (PrEP) trials.

By enabling members to share lessons and techniques, discuss current challenges, and access the online CoP resource center maintained by GCM, the CoP facilitates collective problem solving within the HIV prevention research field.

**CONCLUSIONS:** Coordinated efforts to share lessons, materials and problem-solving strategies both within and across trial networks vital to the success of HIV prevention research. Until the establishment of the CoP, this kind of synergy happened within networks but little communication occurred across networks and independent research centers. The CoP offers an innovative model of collaboration that brings together staff working on various HIV prevention technologies in a way that serves as an example for the field at large.

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### Implementation of a Study Product Adherence Tool in a Pre-Exposure Prophylaxis Trial

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**BACKGROUND:** A high level of adherence (>95% as per treatment guidelines) to Pre-Exposure Prophylaxis is required to ensure activity of antiretrovirals and the prevention of resistance. The Microbicides Trial Network 001 study is a Phase 2 Adherence and Pharmacokinetics Study of Oral and Vaginal Preparations of Tenofovir. Botha's Hill Clinical Research Site in South Africa was one of the participating sites. Measurement of adherence to intervention poses several product related and social challenges. We developed an adherence checklist tool that recorded factors that are likely to impact on participants' adherence to product. This data was collected through face-to-face interviews at enrollment. At each follow-up visit thereafter, participants were asked on the factors that affected their product adherence using this baseline data tool. This tool collated all assessments of adherence factors at every visit and provides a single summary of information for review.

**METHOD:** At the end of the screening visit, factors affecting adherence was discussed with all eligible participants. The discussion consisted of a standard script that included the different reminder methods, potential storage locations and expected side effects of the product. Product sharing and partner awareness was also discussed. At the end of the enrolment visit participants were asked to select their preferred reminder method and storage location. Assessment of potential barriers to adherence like partner/family awareness, side effects and product use were also completed. This data formed the basis on the tool for future measurement to adherence. The adherence checklist tool enabled study staff to follow-up and reinforce all adherence factors that were discussed at enrolment at every subsequent visit. It was also a record to document participants' responses.

**RESULTS:** Using the tool at each follow-up visit ensured that every aspect of the participant's life that could affect adherence was assessed. The participants' response corroborated well with the pill count. Those who were not adherent were counseled on the aspect which impeded on their product adherence.

**CONCLUSION:** The adherence checklist tool provides valuable source documentation of factors that affect adherence. A tool that provides a single summary of ongoing participants' responses, trends, and challenges, will assist in devising a participant specific solution to adherence challenges.

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## Safety and Pharmacokinetics of a Pyrimidinedione Reverse Transcriptase Inhibitor in Pig-Tailed Macaques Using Elastomeric Intravaginal Rings

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**BACKGROUND:** Developing coitally independent controlled release of microbicides requires robust and relevant animal models to optimize dosage form properties, biocompatibility and drug release rates. The pyrimidinedione IQP-0532 is a potent small molecule inhibitor of HIV-1 RT with an IC<sub>50</sub> of 0.5 nM and therefore an ideal candidate for sustained vaginal delivery from intravaginal rings (IVRs). We formulated IQP-0532 in IVRs and performed 28 day pharmacokinetic studies in pig-tailed macaques.

**METHODS:** IQP-0532 and biomedical grade polyurethane was compounded at two different loadings (4.4 and 13.7% w/w) and hot melt extruded to form 25 mm diameter IVRs. The *in vitro* IQP-0532 release from IVRs was quantified using HPLC. Female pigtailed macaques were used to evaluate the IVRs with three groups of n=2: placebo, low dose (4.4%), and high dose (13.7%) IQP-0532 loading (w/w). Drug concentration in the polymer was determined pre and post study using a solvent extraction method quantified via HPLC. IVRs were retained from day 0 to day 28; vaginal fluid and plasma samples taken at -11, 0, 3, 7, 14, 21, 28 and 38 days were analyzed for drug content, microflora count, and cytokine analysis. Vaginal biopsies were taken at -11, 7, 21, and 38 days for drug levels.

**RESULTS:** *In vitro* drug release at 30 days under supersaturated conditions closely matched the *in vivo* release of ~10%. Vaginal fluid drug levels for both formulations, averaged from samples taken proximal and distal to the IVR, were >10<sup>5</sup> the IC<sub>50</sub>. In the low dose group micromolar tissue drug levels at days 7 and 21 were detected proximal to the IVR for both macaques, but no drug was found distal to the IVR placement. In the high dose group, drug was detected in the proximal and distal tissue samples only at day 7 in one animal. Drug was detected in the second high dose animal proximal at day 7 and both proximal and distal at day 21. Drug was not detected in plasma at any timepoint. There were no statistically significant increases in mucosal or plasma proinflammatory cytokines and normal vaginal microflora was maintained.

**CONCLUSIONS:** We evaluated the safety and biodistribution of IQP-0532 in pig-tailed macaques. We found that the amount of drug released *in vivo* closely correlated to supersaturated *in vitro* conditions thereby providing an *in vivo/in vitro* release correlation. Our drug-loaded IVR delivered IQP-0532 to vaginal fluid and tissue well above the compound's IC<sub>50</sub>, yet was safe by all measures.

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## Image-Based Noninvasive Evaluation of Sheep Vaginal and Rectal Tissue Response Following Topical Applications of Microbicides

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University of Texas Medical Branch—Galveston

**BACKGROUND:** Development of sensitive noninvasive methods to evaluate toxicity is important to ensure the safety of topical microbicides. A previous study showed feasibility of Optical Coherence Tomography (OCT) to determine benzalkonium chloride (BZK) toxicity in the sheep vagina. In the current study, we used OCT and colposcopy in the sheep model to evaluate the response of vaginal tissue following the topical application of Nonoxynol-9 (N-9) and the commonly used universal placebo Hydroxyethyl cellulose (HEC) in order to further validate the use of the sheep as a large animal model for the assessment of microbicide safety. In addition, we conducted preliminary studies to evaluate the sheep as a model for rectal microbicide safety studies.

**METHODS:** Yearling virginal female sheep were treated with a single dose of 5mL HEC or N-9 vaginally, or 8mL 0.2% BZK rectally. Colposcopy and OCT images were obtained at baseline (BL) and after 24 hours (24hr) of intra-vaginal treatment; OCT images were obtained at BL and after 20 minutes (20min) of rectal treatment. After post-treatment imaging, biopsies were obtained and H&E stained. Colposcopy findings were evaluated using modified WHO criteria. Vaginal and rectal OCT images were graded using a previously reported vaginal scoring system. Vaginal epithelial thickness was measured from OCT images and histology. Rectal histology slides were evaluated qualitatively.

**RESULTS:** After vaginal treatment, peeling was observed by colposcopy in 2 of 4 N-9 treated sheep. Vaginal OCT findings were similar at BL in all sheep and 24hr after treatment with HEC. After N-9 treatment, vaginal OCT images indicated thinned or absent epithelium. No evidence of injury was noted after treatment with HEC. Mean vaginal epithelial thickness by OCT and histology were similar at BL and 24hr after HEC treatment and reduced after N-9 application (p<0.0001 for OCT and histology). Rectal OCT image reflected histology findings, indicating loss of mucosal layer architecture after 20 min BZK.

**CONCLUSIONS:** Effects of vaginal and rectal microbicide application can be evaluated noninvasively in the sheep. After a single vaginal dose of N-9, both colposcopy and OCT showed epithelial injury, with the colposcopy finding of peeling in half of treated sheep and OCT findings in all treated sheep, which was confirmed by histology. Furthermore, OCT appeared to be more sensitive in the detection of epithelial changes than white light imaging techniques such as colposcopy.

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## Rational Design of a Vaginal Microbicide Gel: Relating Composition to Properties and Performance

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**BACKGROUND:** Prophylactic potential of vaginal microbicide gels depends on both the potency of the API and the ability of the vehicle to deploy in the vaginal cavity. Although spreading and retention have been identified as two critical factors governing the success of vaginal microbicide gels, there has been no satisfactory way to relate composition to properties and performance of the gels during the early development phase. Here we present methodology to couple design of experiments and computational fluid dynamics to rationally design semisolid vaginal products.

**METHODS:** We developed a preclinical *in vitro* algorithm that allows us to *de novo* design vaginal gels by empirically relating gel composition, across a wide range in composition space, to gel properties. Using gel property data we then predicted *in vivo* performance *in silico* for different applied gel volumes. Mixture design of experiments (MDOE) was used to develop models of gel rheological properties, including viscosity and yield stress, as a function of gel composition: hydroxyethyl cellulose (HEC, 0–3% w/w) and Carbopol 974P (0–2.5% w/w). These models were used to predict—the influence of gel composition on gel properties following administration and after mixing/dilution with vaginal fluid. Next we defined an empirical function that scored these gel compositions based on spreading and retention in the vagina. This biomechanical model computes vaginal area coated and gel leakage—for both undiluted gels and gels diluted with vaginal fluid simulant.

**RESULTS:** All 14 gels selected by the MDOE software demonstrated non-Newtonian behavior and were shear thinning, with viscosities at 1 s<sup>-1</sup> and 37°C that ranged from 270 Pa.s to 36 Pa.s. Rheological behavior, and consequently gel performance score, varied with composition. Our models predicted gels composed of only HEC to provide enhanced spreading and coating of the vaginal epithelium compared to the gels that included Carbopol. Consequently in the composition space consisting of HEC (0–3% w/w) and Carbopol (0–2.5% w/w), our data indicated that the 3.0% HEC gel was the nearly optimal composition for a 3.5 mL microbicide gel.

**CONCLUSIONS:** Use of gel rheological measurements and fluid flow models in conjunction with the objective biomechanical model approach is a novel yet simple tool for improving understanding of the effect of gel composition on overall gel performance, and for optimizing vaginal semisolid gels prior to *in vivo* investigation.

# TUESDAY POSTERS

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## Case Report of a Possible Elite Controller Identified in MTN 015 in Durban, South Africa

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**BACKGROUND:** Elite controllers (EC) are defined as HIV-1-infected persons who control viral replication at <50 copies/ml without antiretroviral therapy (ART). These individuals are very rare, estimated to occur in 1 out of 300 HIV-infected individuals. A case report of a possible elite controller discovered during participation in MTN 015, an Observational Cohort Study of Women following HIV-1 Seroconversion in Microbicide Trials is presented.

**METHODS:** In HPTN 035, two rapid HIV tests were performed quarterly to screen for HIV infection. Upon positive rapid test results, Western blots were performed on two independently collected samples to confirm infection. During MTN 015 CD4-positive T cell counts and copies/ml of plasma HIV-1 RNA (Roche Amplicor v1.5 ultrasensitive) were routinely measured at each visit. HIV-1 Western blot and plasma HIV-1 RNA (Abbott M2000) were also performed in the MTN network laboratory (NL).

**RESULTS:** The participant was serologically confirmed to be HIV-infected in May 2007 while enrolled in HPTN 035, a Phase II/Ib vaginal microbicide trial of BufferGel and 0.5% PRO 2000 gel. She completed follow-up in HPTN 035 in September 2007 and enrolled in MTN-015 in April 2009. Enrollment visit plasma tested by the NL using Western blot unambiguously reconfirmed HIV-1 seroconversion. The participant reported that she has never used ARTs at any time and she has remained ineligible for treatment at her local clinic due to high CD4+ T cell counts. Plasma drug levels have not been tested. Regular crosschecks in the Medical Research Council co-enrollment database confirmed that she has never participated in any HIV vaccine trials. Two years after infection, her CD4 count has been stable at >1300 cells/ $\mu$ L. Her HIV-1 viral load was below the level of detection (<50 copies/ $\mu$ L, Roche; <40 copies/ml, Abbott) at three different time points between April 2009 and September 2009 as tested by three separate laboratories.

**CONCLUSIONS:** Available information indicates that this participant, who became infected during participation in HPTN 035, may be an elite controller. Confirmation of EC status will be established by longitudinal viral load testing at MTN-015 follow-up visits. Reporting the occurrence of such rare individuals is important as this will increase the pool of ECs in the world to be studied for effective vaccine development and new therapies against HIV.

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## Relevance of Discordant HIV Rapid Tests in Clinical Trials in Durban, South Africa

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Medical Research Council [MRC] of SA, HIV Prevention Research Unit [HPRU]

**BACKGROUND:** The MRC HPRU conducted HPTN 035 and MDP 301 clinical trials which used Rapid HIV tests for routine HIV screening based upon protocol requirements. The Rapid HIV included FDA-approved UnigoldTM Recombigen® HIV [HPTN 035] and OraQuick® Advance HIV1/2 [MDP 301], as well as Abbott Determine™ HIV1/2 [HPTN 035 & MDP 301]. Confirmatory testing varied by protocol, we looked at relevance of discordant HIV results at screening for both studies under HPRU.

**METHODS:** The HPTN 035 HIV testing algorithm utilises UnigoldTM Recombigen® HIV and Abbott DetermineTM HIV1/2, with confirmatory via Genetics SystemsTM HIV-1 Biorad Western Blot testing whilst MDP 301 uses FDA-approved OraQuick® Advance HIV1/2, and Abbott DetermineTM HIV1/2 with Abbott 4th Generation EIA as confirmatory. Data were tabulated monthly to monitor discordant rapid HIV, confirmatory test results and HIV outcomes.

**RESULTS:** Of the 2184 screening HIV rapids for HPTN 035, 7 (0.32%) were discordant on rapid HIV : 4 Abbott Determine [0.18%] and 3 Unigold [0.14%] were false positive, all confirmed negative on Western Blot testing with no false negatives. 1 participant with normal history remained repeatedly discordant post enrollment till study end, tested negative on OraQuick and western blot. Sample sent to Unigold showed cross reaction with bovine serum [possible consumption of bovine blood]. Of the 4690 rapid tests for MDP 301: 27 [0.56%] discordant on HIV rapids: False positives included 12 for Abbott Determine and 6 for OraQuick [confirmed negative on Elisa] with 2 and 3 false negatives for Abbott Determine and OraQuick respectively [confirmed positive on Elisa]. No participants discordant at screening seroconverted but 3 remain repeatedly discordant till study end. Therefore the false positive/negative rates across both studies were 0.44%/0.04% for Abbott Determine, 0.14%/0% Unigold and 0.13%/0.15% for OraQuick based upon comparative confirmatory. The sensitivity and specificity of such kits range between 99.0–99.6% respectively.

**CONCLUSIONS:** The percentage of overall discordant HIV rapids is minimal when compared to the number screened and tested. The rates were <1% for all rapid HIV kits used. While these rates are acceptable it is vital such results be monitored to identify, troubleshoot any random trends, increased frequency of discordancy/Rapid, increased additional confirmatory testing and costs, including prevention of enrolling false HIV negative participants. The monthly monitoring system is an excellent tool to view discordant HIV rapids at screening and subsequent visits enable proper comparison of performance between first and second line rapid HIV tests.

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## Uptake of HIV Related Care Among Seroconvertors—Implications for VOICE or Future HIV Prevention Trials

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HIV Prevention Research Unit, Medical Research Council

**BACKGROUND:** Access to HIV-related care in resource poor settings can be attributed to unavailability of health services, stigma, and financial constraints. As part of setting the standard of care for participants, referral systems were set up in collaboration with local HIV care centres. Participants requiring HIV-related care were referred to these centres. We examine the uptake of referral by participants as part of the MDP 301 trial and the SPARTAC (Short Pulse Antiretroviral Therapy at HIV Seroconversion) trial.

**METHODS:** The MDP 301 trial was implemented at 3 centres in Durban and enrolled 2391 women, who received HIV testing and counselling at each quarterly visit. During the trial 165 women HIV seroconverted. Onsite care for women included HIV counselling, STI testing and treatment, CD4 testing and referral. The SPARTAC trial, which began in 2003, is currently operating at 6 sites in Durban with 87 seroconvertors recruited from clinical trials. Women were referred from the onset for care, monitoring and enrolment into the National ARV programme.

**RESULTS:** Post-trial follow up of MDP seroconvertors show that 47% (n=77) took up the referral to local clinics. For most women it took several counselling sessions before these women eventually attended local clinics. 16 women did not take up referrals due mainly to denial, stigma and other commitments. 11 women joined the SPARTAC trial and are receiving care through this project however transition to long term HIV therapy at local clinics still remains a challenge. The 61 remaining women were either LFTU or could not be contacted post trial for follow up. Of the 87 SPARTAC participants 15 are not enrolled at a National ARV clinic despite decreasing CD4 counts and intensive counselling. Of these 15 women, 5 have never enrolled at a clinic while 10 have enrolled but failed to attend follow up visits and were removed from the system. Common challenges to access care include, concerns on the side effects of ARVs, fear of disclosure of HIV status, stigma and discrimination, and feeling healthy. Some women opted for traditional medicines while others continue to deny their HIV status despite being positive for 3 to 4 years.

**CONCLUSIONS:** As we move to implementing PrEP trials such as VOICE it becomes increasingly important to ensure there is adequate referral for HIV-related care. Understanding social issues around uptake of referrals should be evaluated in parallel to the ongoing trials especially in resource poor settings.

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**Perception of At-Risk Group About Social Factors Affecting HIV Infection & Microbicides**

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*Triple A International, Nigeria*

**BACKGROUND:** HIV prevention activities using behavioral change communication and education has been identified as a successful strategy in several countries, the HIV prevalence rate in Nigeria (the third worst affected country globally) is highest (5.6%) among the 25–29yrs age group this is higher than the national average of 4.6%. A study was therefore conducted among members of the general population to identify their perceived reasons for this high prevalence rate and if they will be willing to become advocates for microbicide development as well as participate in microbicide research as a means of reversing the high prevalence rate.

**METHODS:** A cross-sectional descriptive study was carried out among fresh higher institution graduates attending NYSC camp, all attendees between the ages of 20–29yrs were involved in the study, a total of 2160 people were at the camp with 1967 between the desired age group. A structured self-administered questionnaire was used to collect information after the group had been addressed at the general assembly about the study. Data collected was analyzed using epi info statistical software.

**RESULTS:** Among the 1967 questionnaires distributed, 1941 were corrected filled and returned, 69% of the respondents were females, social and environmental factors perceived as contributing to the high HIV prevalence in the age group include, inability of the parents to provide adequately all the needs of the young people, high standard of living, lack of employment opportunity for fresh graduates, peer pressure, feeling of invisibility, inability to identify prevention activities as a continuous and consistent issue. 92% of the respondents were not aware that microbicides were being developed as other HIV prevention options. Out of the 8% that are aware they will like to be better informed before they will consider participating in microbicide research.

**CONCLUSION:** Information about microbicides and microbicide development is very poor in this age group, social and environmental factors impacting negatively on HIV prevention in this age group needs to be addressed. In talking about the ABC of HIV prevention there is need to always include information available about microbicides.

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**Priority for Local AIDS Control Efforts (PLACE) Progress in Nigeria**

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*Youth Action on Tobacco Control and Health (YATCH)*

**BACKGROUND:** Given the significant number of people living in developing countries seriously affected by the HIV epidemic, it is crucial that work is undertaken to ensure that they are able to protect themselves. This involves providing them with access to information and resources, as well as providing free HIV tests for the hard to reach in the community. The consequences of HIV/AIDS can be far-reaching for people. Not only does HIV disease have terrible consequences for the individual, causing serious illness and eventual death, it has the potential to trigger negative social reactions.

**METHODS:** Identification of (Priority Prevention Areas) PPAs-Mapping of PPA, we noticed that the hard to reach people can be found most of the time in night clubs. Over 30 Venue Outreach Staffs (VOS) were employed, this people live very close to the PPAs and they were trained by our organisation to reach out to the hard to reach in the community through the ongoing PLACE project. Talking Drum was used to entertain and draw people's attention to the message, cultural dance and drama was also organized to entertain the hard to reach in the PPAs.

**RESULTS:** We discovered that rich people and politicians attend most of the PPAs for pleasure, we also noticed that most of them are afraid to do HIV test, about 8% of the people that did HIV test were positive within the community. This encouraged many of them to do the test and to know more about HIV prevention, condoms was distributed free of charge to this people after asking several questions on how they can be protected from HIV.

**CONCLUSIONS:** Since the hard-to-reach—politicians, bank managers, students, etc.—are easier to reach in clubs and night parties, it is more appropriate to support the PLACE project in communities where people don't have adequate information about HIV/AIDS and introduce preventive methods such as microbicides and other methods.

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**Microbicide Funding Trends in a Global Recession: Research and Development (R&D) from 2000 through 2009**

K. Fisher\*, C. Feuer, S. Ghosh-Robinson, T. Harmon, P. Harrison, W. Lee, M. Warren  
*AVAC: Global Advocacy for HIV Prevention, USA*

**BACKGROUND:** Since 2004, the HIV Vaccines and Microbicides Resource Tracking Working Group have employed their jointly developed, comprehensive methodology to estimate financial investments and trends related to microbicide research, development and advocacy, and levels of political commitment.

**METHODS:** Investment data were collected on basic, pre-clinical, and formulation research; clinical trials and trial preparation and infrastructure, behavioral research, and advocacy and policy activities, to estimate 2008 global funding and 2009 funding by G-8 countries.

**RESULTS:** This presentation will provide preliminary estimates of total 2009 funding levels by G-8 public-sector funders, an overview of trends from 2000 through 2008, and information on how funds for microbicide R&D were spent in 2008. Key anticipated findings are that, despite the onset of a global recession in 2008, public and philanthropic sector funding in 2008 was similar to 2007 levels; however, 2009 levels may prove to be lower.

**CONCLUSIONS:** The onset of the global financial climate had limited impact on funding for microbicides in 2008 but, due to variability in donor funding cycles, the effect of the recession on funding commitments may not be fully appreciated until mid-2010. A strategic revitalization of public- and private-sector investments will be required to acquire new, diverse, and more potent microbicide candidates; develop those; and fund clinical trials to test their safety and efficacy.

## TUESDAY POSTERS

**476****Does PrEP Need a Pipeline?  
Strategies for Identifying the  
Next PrEP Candidates**K. Fisher\*, L. Mille, M. Warren  
*AVAC: Global Advocacy for HIV Prevention, USA*

**BACKGROUND:** Over the next few years, results from a number of trials using antiretroviral (ARV) drugs as pre-exposure prophylaxis (PrEP) will be released. These trials test TDF (tenofovir disoproxil fumarate) and TDF/FTC (tenofovir disoproxil fumarate and emtricitabine) in oral and topical form in different populations and through different routes of HIV transmission. Ideally, PrEP will prevent HIV infection by delivering a safe and potent ARV at the right time in the appropriate concentration systemically or to the mucosal area while avoiding drug resistance. Whether the current trials show no, low or high effectiveness in preventing HIV, there are still good reasons to test other ARVs as a PrEP agents.

**METHODS:** During 2009, AVAC interviewed researchers, product developers, treatment implementers, treatment advocates, and regulators to ask them to describe the attributes of an optimal PrEP drug and identify potential next-generation PrEP agents from the current licensed and experimental antiretroviral drugs.

**RESULTS:** The optimal PrEP drugs would have protection against HIV infection in the mucosa, a high barrier to resistance, acceptability to users, long-lasting activity, long-term safety data, and would not be part of any treatment regimen. Although there was no consensus on one agent, four antiretroviral drugs mentioned most often were: lamivudine; maraviroc; rilpivirine and raltegravir. Each drug had advantages and concerns, and all drugs had a number of areas where the lack of data made assessment difficult. A major concern is that TDF and TDF/FTC are used as first line ARV treatment, thus PrEP use with those ARVs may fuel drug resistance to these vital drugs. Creating a new prevention-specific ARV could take years. One alternative might be to take an existing ARV drug, such as lamivudine or maraviroc out of treatment regimens.

**CONCLUSIONS:** There is no clear consensus on the next PrEP agent to test after TDF and TDF/FTC. Many stakeholders would like to see an alternative PrEP agent that is potent, safe and not used in treatment programs. There is no mechanism for coordinating the PrEP field and deciding the next drug to be developed in the PrEP pipeline. While there are significant regulatory, financial and logistical obstacles to testing another PrEP candidate, it is critical to bring together HIV prevention research stakeholders along with treatment advocates and providers in the discussions and development of a robust PrEP pipeline.

# LATE BREAKERS

## POSTERS

Tuesday, May 25, 2010

## LB5

### Effects of BV-Associated Bacteria and Sexual Intercourse on Vaginal Colonization with the Probiotic *Lactobacillus crispatus* CTV-05

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**BACKGROUND:** Several fastidious bacteria have been associated with bacterial vaginosis (BV), but their role in BV recurrence and lactobacilli recolonization following antibiotic treatment and application of exogenous lactobacilli under development as probiotics is unknown. We studied the effect of seven BV-associated bacterial species and two *Lactobacillus* species on vaginal colonization with *L. crispatus* CTV-05 (LACTIN-V).

**METHODS:** Twenty four women with BV were treated with a 5-day course of metronidazole vaginal gel then randomized 3:1 to receive either LACTIN-V or placebo applied vaginally once daily for 5 initial consecutive days, followed by a weekly application for 2 weeks. Vaginal swabs for *L. crispatus* CTV-05 culture and 9-bacterium specific 16S rRNA gene quantitative PCR assays were collected at screening, enrollment (2 days after antibiotic treatment) and 28 days after randomization for the 18 women who received LACTIN-V.

**RESULTS:** Vaginal colonization with CTV-05 was achieved in 44% of the participants at the day 28 visit. Participants not colonized with CTV-05 generally had higher median concentrations of BV-associated bacteria compared to those who colonized. Between enrolment and day 28, the median concentration of *Gardnerella vaginalis* reduced from  $10^{4.5}$  to  $10^{4.3}$  16S rRNA gene copies per swab in women who colonized with CTV-05 but increased from  $10^{5.7}$  to  $10^{7.3}$  in those who failed to colonize ( $p=0.19$ ). Similarly, the median concentration of *Atopobium vaginae* reduced from  $10^{2.7}$  16S rRNA gene copies per swab to below limit of detection in women who colonized with CTV-05 but increased from  $10^{2.7}$  to  $10^{6.6}$  in those who failed to colonize ( $p=0.04$ ). The presence of endogenous *L. crispatus* at enrollment was found to be significantly associated with a reduced odds of colonization with *L. crispatus* CTV-05 on day 28 ( $p=0.003$ ). Vaginal intercourse during the study significantly impaired successful *L. crispatus* CTV-05 colonization ( $p=0.018$ ).

**CONCLUSIONS:** Vaginal concentration of *G. vaginalis* and *A. vaginae*, two BV-associated organisms that produce epithelial biofilms, appeared to inhibit colonization with *L. crispatus* CTV-05. In addition, vaginal intercourse during treatment and presence of endogenous *L. crispatus* at enrollment were associated with failure of CTV-05 to colonize the vagina following treatment for BV. Future research on prevention of bacterial vaginosis needs to include a detailed assessment of fastidious bacteria.

## LB6

### High HIV Incidence and Willingness To Use Rectal Microbicides Among Argentine MSM: Potential for Rectal Microbicide Studies

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**BACKGROUND:** Studies conducted in Buenos Aires, Argentina, repeatedly show high HIV incidence per 100 person years among MSM. Rates were 6.7 in the Vignoles et al. (2006) cross-sectional study that used STARHS, and 3.9 in the Segura et al. (2007) study of a prospective cohort followed up for 12 months (retention: 97.2% at 6 months and 91.5% at 12 months). With prevention efforts limited to condom promotion and no locally developed, proven effective, behavioral interventions, new strategies are urgently needed. We assessed HIV prevalence and incidence and studied acceptability of microbicides among MSM in Buenos Aires.

**METHODS:** 500 MSM were recruited through Respondent Driven Sampling. They provided blood samples and underwent CASI interviewing on microbicide acceptability. HIV-positive plasma samples were tested using a detuned version of an HIV-1 enzyme immunoassay (Vironostika HIV-1 Microelisa System; bioMerieux Inc, North Carolina, USA) to sort out potential recent infections (less than 6 months) from chronic infections using the STARHS strategy. Microbicide acceptability was measured with questions on willingness to use a gel microbicide for anal sex measured on 10-point Likert scales ranging from 1= completely unwilling to 10 = completely willing.

**RESULTS:** Sample HIV prevalence was 15.7 % (CI: 11.8-20.2), being higher among gay identified men (30.6 %) than non-gay identified MSM (12.9%,  $\chi^2 p < .001$ ). When the 85 HIV-positive plasma samples were tested using a detuned version of the HIV-1 enzyme immunoassay, 23 cases were identified as possible recent infections, this yielding an HIV incidence of 11.4 per 100 persons/year. Incidence was significantly higher among gay men (22.6) than non-gay identified MSM (8.3). Concerning willingness to use a gel microbicide during anal sex, although the mean score was 6.0 (neither willing nor unwilling), gay identified men scored significantly higher (7.1, willing range) than non-gay identified men (5.6).

**CONCLUSIONS:** Gay identified MSM in Buenos Aires have high HIV prevalence and incidence and are willing to use gel rectal microbicides. Furthermore, the research infrastructure (i.e., laboratory facilities, demonstrated participant recruitment and retention success, record of scholarly activity of the University of Buenos Aires/Nexo Asociación Civil, and effective collaboration with international partners) suggests great potential for successful collaborations in Phase 2 and 3 micro trials.

## LB7

### Acceptability Lessons Learned in a Phase 1 Microbicide Trial Involving Product Use During Vaginal Intercourse

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**BACKGROUND:** Despite limitations imposed by strict study designs, Phase 1 microbicide trials can shed important light on acceptability issues that need attention before product development can advance. As part of a safety and acceptability trial of SPL7013 Gel, known as VivaGel™, we studied the acceptability of three gel products (VivaGel, SP Placebo, and universal HEC Placebo) by young, ethnically diverse women who used them twice daily, including during vaginal intercourse.

**METHODS:** 61 participants ages 18–24 were randomized to use one of three gels twice a day for 14 days. Acceptability data were collected via Web-based CASI and in-depth video teleconferences. Structured assessments used 10-point Likert scales ranging from negative to positive with a middle, neutral point (e.g., 1= Extremely Unlikely; 5= Neither Likely nor Unlikely; 10= Extremely Likely), and were analyzed using ANOVA and chi-square tests.

**RESULTS:** Participants' average overall rating in response to how much they liked the gel was 6.21, corresponding to neither liked nor disliked. Concerning intentions to use in the future, average scores were 7.48, indicating a likeliness to use in the future. Although in both cases the VivaGel condition scored lower than the two placebo conditions, the differences did not reach statistical significance ( $p=.892$  and  $p=.710$  respectively). Despite these apparently favorable scores, many participants complained about leakage and related problems. In the qualitative interviews, over half of the participants described the gel as "messy," "gross," "disgusting," "slimy," or "cold," while others did not experience discomfort. Almost all participants had to wear pantiliners during the study and change them often. Concerning use of the gel during sexual intercourse, a third of the participants responded positively to the increased vaginal lubrication, but most complained that the gel was "squishy," "unnatural," and "gooey." Those who complained found the gel to be messy and to leak during sex, soiling the bed sheets, themselves, and their partners. Several women complained about not being able to receive oral sex while using the gel.

**CONCLUSIONS:** Participant concerns about gel leakage and messiness during intercourse pertained not only to VivaGel, but also to the universal HEC placebo. Concerns about gel leakage are not new but remain unaddressed. The potential interference with microbicide use in Phase 2 and 3 trials should not be underestimated.

# LATE BREAKERS

## LB8

### Introduction of a Quality System to On-Site Laboratories at IPM Research Centres

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A. Nel

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**BACKGROUND:** Accurate and reliable on-site laboratory testing is imperative for the successful conduct of HIV prevention trials. On-site HIV rapid testing is an essential tool in establishing participant eligibility and end-points for microbicide trials. HIV rapid tests are performed at screening, during the trial and at the end of the trial.

**METHODS:** To ensure the accuracy of HIV rapid test results, a quality system was introduced at 10 research centres (7 in South Africa and 3 in East Africa) focusing on high quality, reliable testing. All research centre staff responsible for HIV rapid testing (nurses and laboratory technologists/technicians) participated in ongoing training sessions on a variety of relevant topics such as introduction to quality systems in HIV rapid testing, internal quality control and external quality assurance concepts. The success of the quality system at each research centre was measured by the centre's performance on an HIV Serology external proficiency testing program. Eighteen blinded samples were sent in three cycles, February, June and October 2009 (6 samples per cycle), to each research centre. The trained staff performed HIV rapid testing on these samples and submitted the results to the service provider.

**RESULTS:** Seven of 10 research centres scored 100% on all samples tested in each cycle. Two research centres did not submit results for one cycle, but scored 100% on the 2 cycles that were submitted. One research centre scored 83% on one cycle (5/6 samples correct) and 100% in the other two cycles of samples.

**CONCLUSIONS:** Introduction of a quality system at 10 research centres in Africa resulted in almost 100% accuracy on HIV rapid test results as measured by an external proficiency testing program. Although not a perfect measure of quality and competence, this test provides a practical and objective assessment of quality control procedures at research centres. The quality system will be maintained through ongoing training and competency assessments, as well as quality control processes.

## LB9

### Subliming Solids Matrices as a Novel Delivery System for C5A

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**BACKGROUND:** C5A represents the prototype for a new generation of HIV microbicides since it i) exhibits a broad range of antiviral activity against primary HIV isolates; iii) prevents transmigration of HIV through genital cells; iii) prevents HIV transfer from dendritic to T cells; iv) is potent at a low pH; and v) offers protection in a humanized mice vaginal transmission model. However, C5A has obstacles to overcome. It must achieve long-term protection and coitus-independent administration if it is to become an accepted prophylactic in real world conditions.

**METHODS:** Subliming solids matrices are chemically inactive and continuously hydrophobic, allowing proteins stored within, and releasing from, such matrices to be more stable than when stored in the solid state in moist air. A subliming solids-based delivery system can provide a broad range of release rates and durations independent of the nature (size, hydrophobicity) of drug, and independent of the environment in which the drug is being released. Such matrices release incorporated proteins at the rate at which they sublime, which in turn is determined by mole fraction composition. We formulated C5A in cyclododecane (CD) solid matrices and tested them for cellular cytotoxicity and sustained C5A release with preserved anti-HIV activities.

**RESULTS:** Subliming CD solids do not exert any cellular toxicity to PBMC and cervical explants. C5A remains constantly released from CD solids over a period of 30 days. Importantly, C5A released from CD solids from day 9 to 30 completely neutralize HIV. CD solids loaded with C5A were kept at room temperature for 3 weeks before use. A same amount of non-formulated C5A placed under similar culture conditions lost its antiviral activity in less than a week, suggesting that C5A formulation into CD solids preserves its antiviral properties.

**CONCLUSIONS:** By showing that subliming CD solids release C5A with anti-HIV activities for a sustained period of time, these promising data serve as a proof-of-concept that the unique attributes of subliming solids-based drug release could offer an opportunity to overcome unresolved formulation problems intrinsic to a large panel of anti-HIV microbicides.

## LB10

### Natural Humic Acids as Active Components for New Microbicides

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**BACKGROUND:** At present there is a large data set on ability of humic substances to induce non-specific immune response in living organisms. In particular, antiviral activity of humic substances have been shown. However, the reported effects are mostly obtained for synthetic humic substances. Systematic studies on antiviral activity of natural humic materials are missing. The objective of this study was to assess anti-HIV activity of a broad set of natural humic materials. The set of humic materials tested included samples of coal and peat humic and fulvic acids as well as non-fractionated materials and more narrow fractions. All samples were isolated and purified in laboratory conditions using standard protocols of International Humic Substances Society (IHSS).

**METHODS:** Anti-viral activity of compounds was defined in modeling HIV-infection using laboratory adapted HIV-1 strains and T-lymphoblastoid cell lines. The level of virus reproduction in infected cells at presence of tested compounds was detected with p24 HIV-1 antigen ELISA. The cytotoxicity was defined as the viability of cells cultivated at presence of different doses of tested compounds with MTT-test.

**RESULTS:** All substances tested in this study showed weak cytotoxicity (10–15%) at concentrations 1.0–1.5 mg/mL. While their anti-HIV activities were high enough. The ED<sub>50</sub> values ranged from 3x10<sup>-3</sup> to 4x10<sup>-2</sup> mg/mL. The HIV-activity depended strongly on the source and fraction composition of HS.

**CONCLUSIONS:** Given low cytotoxicity and high efficacy of the humic materials, they can be considered as promising group of compounds suitable for further therapeutic developments. Hence, the performed experiments allowed us to propose new natural humic compounds as active and potent agents for microbicide formulations.

# LATE BREAKERS

POSTERS  
Tuesday, May 25, 2010

## LB11

### Male Circumcision as MICROBICIDES Technique—A Preventive HIV Transmission Strategy—Acceptability Study among Parents at Dr. Kutikuppala Surya Rao Hospital Visakhapatnam, India

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**BACKGROUND:** In India male circumcision is a traditional practice among Muslims alone but its acceptability in other religions in general is not known. There is currently no information on the acceptability of male circumcision in India. In the wake of significant role of male circumcision to decrease the risk of HIV transmission, an attempt is made to study the acceptability of male circumcision among Indian parents of male children.

**METHODS:** A cross-sectional study was conducted among a convenient sample of 1000 parents attending a general health clinic at Dr.Kutikuppala Surya Rao Hospital, Visakhapatnam, India during January 2008 to December 2009 after obtaining their consent and approval of relevant ethical committee. In view of superstitions the educational back ground of the parents also studied.

**RESULTS:** Out of the 1600 enrolled eligible Hindu couples 1000 couples agreed to participate (response rate = 62.5%). 350 (35%) of respondent couples had no schooling, (150 )15% couples had primary schooling, 250 (25%)couples had high school education 175 (17.5%) couples are graduates 75 (7.5%) couples are post graduates.. After the couples were informed about the risks and benefits of male circumcision, 810 (81%) couples with uncircumcised children told that they would circumcise their sons if the procedure is offered in a safe hospital setting, free of charge, or nominal charges and 130 (13%) said they would consider the procedure in due course and 60 (6%) said that they would not consider male circumcision saying that the sexual pleasure will be at hammering due to circumcision.

**CONCLUSIONS:** Since male circumcision has been found to decrease risk of HIV infection among men up to 65% in some studies, it is important to know about the attitude of parents and to determine its acceptability as a potential HIV prevention strategy in India. There is also need to bring awareness about the potential role of circumcision as an HIV prevention strategy among all sections and religions of people. This study found male circumcision to be highly acceptable among a wide collection of parents with male children in Visakhapatnam, India.

## LB12

### High-Resistance Barrier for Macromolecular CCR5 Inhibitors of HIV-1 Entry

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**BACKGROUND:** Small molecule, allosteric inhibitors of CCR5 can select for resistant HIV-1 variants that utilize the drug-bound form of CCR5 as the entry coreceptor, but no resistance to macromolecular CCR5 inhibitors (e.g., PSC-RANTES, 5P12-RANTES) had been reported until the recent publication of Dudley et al. One SHIV162P3 variant from one macaque exposed to PSC-RANTES in a preclinical microbicide trial harbored two amino acid substitutions that were reported to confer 5- to 7-fold resistance to PSC-RANTES. This result was surprising since multiple long-term selection experiments *in vitro* (including the one reported here) had failed to select for resistance to either PSC- or 5P12-RANTES.

**METHODS:** The two "resistance" mutations in the above-mentioned report, K315R in the V3 loop and N640D in HR2 of gp41, were introduced into SHIV162P3. Entry function and sensitivity to PSC-RANTES or the small molecule allosteric inhibitor TAK-779 were assessed by single cycle infection of U87.CD4.huCCR5 or rhCCR5 target cells. We also performed long-term selection experiments with escalating concentrations of the small molecule CCR5 inhibitor maraviroc (MVC) or macromolecular 5P12-RANTES in human PBMC cultures infected with HIV-1 CC1/85, an isolate previously demonstrated to develop resistance to MVC after 16 weeks of selection by increasing concentrations of inhibitor.

**RESULTS:** The combination of K315R and N640D mutations resulted in no significant change in IC<sub>50</sub> values for PSC-RANTES or TAK-779 from the SHIV162P3 wildtype (K315, N640) sequence. The long-term selection experiment resulted in >1000-fold resistance to MVC by 16 weeks of virus passage, but only transient 4–8 fold resistance to inhibition by 5P12-RANTES after 28 weeks, followed by loss of virus replication. The highly MVC-resistant viruses were fully sensitive to inhibition by 5P12-RANTES.

**CONCLUSIONS:** These results confirm the high barrier to resistance for macromolecular CCR5 inhibitors that act by occluding viral access to CCR5 and/or sequestering CCR5 at intracellular sites. We were not able to confirm the one report of resistance to PSC-RANTES.

The MVC-resistant isolates had identical mutations to those previously reported by the Pfizer group, suggesting a single pathway to resistance to MVC. There is no cross-resistance between small and macromolecular CCR5 inhibitors. Use of 5P12-RANTES in microbicide products would thus not be affected by prior or contemplated use of MVC as a systemic antiviral agent.

## LB13

### The Effects of Twice-daily Use of Either VivaGel, VivaGel Placebo, or HEC Placebo on the Vaginal Microflora

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**BACKGROUND:** VivaGel is a polyananionic dendrimer based gel that has anti-HIV activity *in vitro*. The objective of this study was to assess the impact of twice-daily VivaGel use on vaginal microflora.

**METHODS:** Healthy, non-pregnant, sexually active women aged 18–24 were enrolled in a Phase I, double blinded, randomized, controlled comparison with 14 days of twice daily exposure to VivaGel, VivaGel placebo, or HEC gel. Women were recruited in Tampa, FL, San Juan, Puerto Rico, and Pittsburgh, PA and screened for sexually transmitted infections prior to enrollment. Vaginal swabs were collected at enrollment, 1 week and 2 weeks after daily use, and at 1 week after completion of product use for quantitative culture in a central laboratory. Vaginal smears were stained and the flora assessed according to the Nugent criteria. Generalized estimating equations were used to evaluate the marginal effect of gel usage on prevalence and concentrations of vaginal microflora and on Nugent scores.

**RESULTS:** 58 of the 61 women enrolled had culture results for all 4 visits; VivaGel (n=21), VivaGel placebo (n=21), and HEC placebo (n=16). The prevalence of *Enterococcus* increased significantly after 1–2 weeks among women using VivaGel (OR 2.0, CI 1.1–3.5, P=0.01) compared to baseline and final visit off product, whereas women using either HEC or VivaGel placebo had no change. The prevalence of the following organisms decreased significantly (P<0.02) among women using VivaGel: *Lactobacillus* spp., *G.vaginalis*, and pigmented anaerobic gram negative rods (AGNR). With VivaGel placebo there was a significant (P<0.03) decrease in prevalence of H2O2 negative *Lactobacillus*, *G.vaginalis*, non-pigmented and pigmented AGNR. Women assigned to HEC gel had a significant decrease in group B Streptococcus (P=0.001). Women using VivaGel also had an increase of > 1 log in the concentration of *Enterococcus* (P=0.002), Group B *Streptococcus* (P=0.03), and coliforms (P=0.005), whereas women using HEC or VivaGel placebo had no significant increase in these organisms. None of the women had significant changes in Nugent scores of the vaginal flora (P>0.6).

**CONCLUSIONS:** Twice daily exposure to VivaGel resulted in shifts in the vaginal microflora including an increased prevalence and concentration of *Enterococcus*, and increased prevalence of GBS, and coliforms. While there was some inhibition of organisms associated with bacterial vaginosis among women using VivaGel, there was no overall impact on BV as assessed by Nugent score.

# LATE BREAKERS

## LB14

### Exploring the Vif-ABOEC3G Pathway as Novel Mechanism to Prevent HIV Sexual Transmission

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**WBACKGROUND:** APOBEC3G (A3G) and ABOEC3F (A3F) are expressed in the cytoplasm of various immune cells in the human female reproductive tract and in colorectal tissue, two portals of entry for HIV. A3G and A3F are members of the AID/APOBEC family of cytidine deaminases that introduce lethal hypermutations into retroviral cDNA. Vif counteracts A3G/F by targeting these cellular proteins for ubiquitin-mediated proteasomal degradation.

**Methods:** The Vif inhibitor, RN-18 was identified from a 30K compound library on the basis of its ability to stabilize A3G in the presence of Vif. Two RN-18 analogs, AE-17 and AE-47, were generated. RN-18 and its two analogs were tested for *in vitro* antiviral activity against a primary HIV-1 subtype B isolate (HT/92/599) and *in vitro* toxicity in human PBMC. Also, RN-18 was tested against subtype A, C, D, E, F, G, and O primary isolates and evaluated against two drug-resistant isolates. A TaqMan RT-PCR was developed simultaneously detecting human and monkey A3G RNA using *in vitro* transcribed human A3G cDNA as standard.

**RESULTS:** Against the HT/92/599 isolate (B clade), RN-18 had an IC<sub>50</sub> ranging from 9.8–20.8 μM in PBMC cultures from 4 different donors, AE-47 from 6.0–19.7 μM, and AE-17 had an IC<sub>50</sub>>50μM in 2 of 4 donors (IC<sub>50</sub>=29.5–31.4 μM). None of the compounds were toxic to human PBMCs (TC50>50μM). RN-18 was highly active against at least 1 out of 3 strains from clade A, C, D, E, F, and G (IC<sub>50</sub> ranging from 0.5–7.6 μM, an IC<sub>50</sub> of <10μM was considered active) but not against two subtype O viruses BCF01 and BCF03. There was minor activity against the O type strain BCF02 (IC<sub>50</sub>=27.7 μM). RN-18 was active against the protease-resistant strain Merck 144-44, but not against the multi-drug resistant strain MDR769. Moreover, it demonstrated activity against several HIV-2 strains and SLVmac251. A3G cDNA was expressed to variable degrees in monkey vagina, cervix, and uterus. Investigation of expression of A3G in the colon and rectum is in progress.

**CONCLUSIONS:** Vif inhibitors will prevent A3G from proteasomal degradation and create a Vif-minus phenotype at mucosal sites. The broad antiviral activity against viral subtypes that are responsible for the majority of mucosal infections worldwide, as well as the expression of A3G in tissues involved in mucosal transmission, support the notion that Vif antagonists, such as RN18, are a potential new class of agents that can be used to prevent mucosal HIV transmission.

## LB15

### The Impact of a Prescription-Only Microbicide on Women's Access in Urban South Africa

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**BACKGROUND:** With first generation microbicides, there was great hope for an over-the-counter (OTC) product that could be widely distributed and easily accessed outside of health services. Current microbicides in the pipeline are largely ART based and will need to be distributed through health care facilities or pharmacies on prescription. This study looks at women's preferences for different distribution channels to consider the impact of restricted prescription only products in Johannesburg, South Africa.

**METHODS:** A discrete choice experiment was administered to 1017 adult sexually active women in three Johannesburg townships to estimate their preferences for different distribution strategies and predict uptake through each channel (Clinic, Pharmacy, Supermarket, Spaza (township cornershop)). Also elicited were women's preferences for how they would be advertised (HIV prevention, pregnancy prevention, enhanced pleasure, women's empowerment).

**RESULTS:** This study showed that the distribution channels were very important to women. Given the choice of these four channels, 33% would collect from a pharmacy, 32% from a clinic, 20% from a supermarket and 16% from a spaza. Even with restricted, prescription-only access, 65% of women would still be able to access product through their preferred outlet. This was fairly robust across different groups of women (cohabiting, socio-economic status, and employment status). When removing OTC distribution outlets, 49% preferred clinic and 51% chemist distribution, with clinic appealing slightly more to lower SES and unemployed women. We are unable to predict the proportion of women who would no longer access microbicides without the OTC outlets, nor account for reduced uptake of 2nd generation due to required and repeated HIV testing. The type of promotional messaging used was also important, with 'enhanced pleasure' generally least and 'women's empowerment' most liked; preference heterogeneity was identified, suggesting a potential for market segmentation to increase overall uptake.

**CONCLUSIONS:** Though OTC distribution of microbicides was preferred by a third of the participants, the remaining two-thirds would still have access through their preferred channel if it were introduced as a prescription-only product. It will be very important to include both clinic and chemist distribution as together they are likely to provide good access for both higher and lower income women in urban South Africa.

## LB16

### Potential Impact of Circumcision on Herpes Simplex Virus Type 2 Prevalence Among Spouses in Five Northeastern States of India

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**BACKGROUND:** Herpes simplex virus type 2 (HSV-2), the most common cause of genital ulcer disease worldwide has shown increasing evidence to have synergistic activity on human immunodeficiency virus (HIV) acquisition by two fold or more. So, there was a need to know the prevalence of HSV-2 to develop intervention strategy especially in the high HIV prevalent states of northeast India. A study was therefore conducted among antenatal women to assess the prevalence of HSV-2 infection as well the role of spouse's circumcision on HSV-2 prevalence.

**METHODS:** A total of 1640 antenatal women from five different northeastern states of India, namely Assam, Arunachal Pradesh, Manipur, Meghalaya and Mizoram with diverse ethnic background were enrolled after informed consent. They were screened for IgG antibody status against HSV-2 using HerpeSelect 2 ELISA IgG kits form Focus Diagnostics, USA. A structured questionnaire was used to evaluate different risk variables.

**RESULTS:** The median age of the subjects was 24 years (SD±4.8) with inter quartile age of 22–28 years. The overall prevalence of HSV-2 (IgG) positive was 8.6%, while prevalence was highest in Arunachal Pradesh (15%) and was lowest in Manipur (2.74%). There was a significant association of HSV-2 infection with history of vaginal discharge with pelvic pain ( $p=0.0001$ ) and genital ulcer ( $p=0.02$ ). Regular condom user's had a low HSV-2 prevalence of 1 % compared to 10.3 % in infrequent or non-condom users (OR=11.1, 95% C.I.= 3.5 – 35.2,  $p<0.0001$ ). HSV-2 prevalence was 1.7% in women with circumcised spouses compared to 9.2% among uncircumcised spouse (OR= 5.7, 95% C.I.=1.4 – 23.4,  $p=0.01$ ).

**CONCLUSIONS:** The study documented a variable difference of prevalence of HSV-2 among the different states of northeast India. The independent association of spouse's circumcision with HSV-2 sero-status in pregnant women documents the role for circumcision in decreasing the transmission of HSV-2 in the community. This is the first study from India that substantiates the usefulness of circumcision as a modifiable preventive measure in Indian settings that can be utilized to prevent the spread of HSV-2 which may also have a role in lowering transmission of other STIs and HIV prevalence in the community.

## LB17

### Partner HIV Testing as a Strategy for Recruiting HIV Serodiscordant Couples into an HIV Prevention Clinical Trial: Experiences from the Partners Center, Tororo, Uganda

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**BACKGROUND:** Partners Center Tororo is affiliated to The AIDS Support Organization (TASO); it is one of 9 sites in Uganda and Kenya implementing the Partners PrEP Study, a phase III randomized trial of antiretroviral pre-exposure prophylaxis (PrEP) for HIV prevention. Our site recruitment goal is 500 HIV-1 discordant couples over 2 years. The site works closely with the local TASO center and several VCT sites, and has implemented partner HIV testing, a new approach to identifying serodiscordant couples.

**METHODS:** We identified HIV+ individuals in the TASO Tororo client database who were not on ART and had partners with unknown HIV status; we also gave talks at TASO clinics. TASO counselors, field officers, nurses and community volunteers contacted those identified at their homes. They were told about discordance and the importance and availability of testing their partners for prevention and care. With their permission, we offered their partners an HIV test. All staff involved in tracing and testing had training in home-based HIV testing. Confidentiality was upheld. Couples found to be serodiscordant were told about the Partners PrEP Study; those interested were referred to the study site for screening.

**RESULTS:** 99% of the partners approached accepted an HIV test. Of these, 64.7% were female, 81.5% were negative. Between Nov 2008 and Nov 2009, a total of 645 HIV discordant couples were screened and 385 enrolled into the Partners PrEP trial at our site. Of those HIV discordant couples screened for eligibility, 345 (53.4%) were identified through the partner testing strategy; 121 (18.7%) from couples tested at VCT centers; 99 (15.5%) from a pool of discordant couples already known before the trial began. The remaining strategies contributed 12.4%. Costs up to the time of screening included allowances for the staff and community volunteers, fuel for the site vehicles, transport refund to couples on screening day. Total costs were \$37 for the partner testing strategy, \$37 for testing at VCT centers and \$100 for couples from the pool of previously known discordant couples.

**CONCLUSIONS:** Multiple concurrent strategies are needed in order to recruit high numbers of eligible serodiscordant couples into clinical trials. Confidential, voluntary HIV testing of partners of HIV+ people whose serostatus is not known is an efficient way to identify HIV serodiscordant couples for HIV prevention trials, and for future implementation of effective HIV interventions.

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