Eastern Virginia Medical School

End-of-Project Technical Report for CONRAD Proprietary Products Research and Development Program (CONRAD PPRD)

September 5, 2008 - September 4, 2014

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CONRAD Proprietary Products Research and Development (PPRD)

End-of-Project Technical Report

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CONRAD Proprietary Products Research and Development (PPRD)

USAID - GPO-A-00-08-00005-00

Final Report Summary

I. Executive Summary

The CONRAD Proprietary Products Research & Development (PPRD) program was a Cooperative Agreement with USAID from September 5, 2008-September 4, 2014.

Overview of Program Objectives, Expected Outcomes and Deliverables

Program goal: The initial objective of the PPRD program was to develop and clinically test at least one single-entity and one combination microbicide based on CONRAD's proprietary compounds, as well as develop two dual-protection technologies, that could be used on-demand or coitally-dissociated. The PPRD program also proposed to develop and implement novel preclinical models to evaluate potential cervicovaginal toxicity induced by CONRAD-PPRD compounds.

In order to achieve these overall goals, the PPRD program sought to address several key objectives in the development of HIV prevention and multi-purpose technologies (MPT) microbicide products, including:

1) To develop *effective microbicides* by using ARV (e.g., TFV) and other CONRAD proprietary compounds alone or in combination
2) To evaluate and select the best microbicide candidates using standard and newly-developed *assay models*
3) To develop immediate and controlled-release microbicide *delivery systems*
4) To design and test *dual-protection technologies* displaying microbicidal and contraceptive properties
5) To test the selected products *clinically*

The major expected outcomes and deliverables of the PPRD program were:

1) the preclinical development and early clinical testing of single- and multi-entity microbicides based on CONRAD proprietary compounds,
2) expanded preclinical characterization of CONRAD compounds-based formulations and delivery systems,
3) development and early clinical testing for the safety and efficacy of multi-purpose prevention technologies (MPT), and
4) establishment of new markers and biomarkers of microbicide safety and efficacy.

At the conclusion of PPRD in September 2014, CONRAD had successfully achieved all planned deliverables related to these outcomes. Notably, the PPRD program progressed tenofovir (TFV) 1% gel towards licensure, developed two new single-entity and combination microbicide formulations and delivery systems (TFV and TFV/FTC tablets; TFV-only intravaginal ring (IVR)), developed two novel MPTs (TFV/LNG and TFV/ACV rings), and updated preclinical testing endpoints, models and algorithms in order to more effectively screen pipeline microbicide candidates.

Summary of Program Activities

At the start of the PPRD program, the most advanced compounds in CONRAD's microbicide portfolio were UC781 and TFV 1% gel. While the initial goal was to develop combinations of the two microbicides into vaginal gels and intravaginal rings (IVRs), potential safety/irritation concerns observed in preclinical studies of UC781/TFV combination gel, instability concerns for UC781 in IVR formulations, and bioanalytical challenges associated with UC781, coupled with the availability of promising alternative antiretrovirals (ARVs) such as TFV, emtricitabine (FTC), MIV-170, dapivirine and TDF, resulted in the decision to terminate development of UC781-based products.

TFV 1% gel was advanced to clinical Phase III studies following the proof of concept demonstration of a statistically significant 39% reduction in the HIV incidence overall and 51% protection against HSV-2 when used in a pericoital dosing regimen in the seminal CAPRISA 004 Phase IIb trial. CONRAD also provided gel to the MTN-led Phase IIb VOICE study, which ultimately failed to demonstrate by intent-to-treat significant protection with daily TFV gel use. In parallel with VOICE and as a confirmatory study for CAPRISA 004, CONRAD initiated the Phase III FACTS 001 trial of pericoital TFV 1% gel use in 9 South African sites. In addition, several pharmacokinetic (PK)/pharmacodynamic (PD) studies of TFV gel, including CONRAD 095, 113 and 114, were conducted which provided critical information on drug concentrations in the female genital tract following topical dosing. Collectively, under work funded by PPRD, TFV 1% gel was progressed towards NDA submission for HIV-1 indication, with potential for licensure for HSV-2 indication.

Also under the PPRD program, CONRAD was able to develop new microbicide formulations and delivery systems. In addition to providing TFV and placebo gels to numerous clinical trials, CONRAD oversaw reformulation of TFV gel into a reduced glycerin formulation for improved osmolarity (for rectal and potentially vaginal use), and helped develop cost-effective paper applicators as an alternative to prefilled plastic applicators. To expand our platform for on-demand, pericoital dosage forms, CONRAD developed TFV and TFV/FTC fast dissolve vaginal tablets. Preclinical PK and safety studies were conducted in vitro and in rabbits and nonhuman primates, which demonstrated that these new tablet formulations provide similar PK profiles to TFV 1% gel, that coformulation with FTC did not alter the PK profiles, and that the formulations may be safe for clinical testing. These positive preclinical findings led to two new IND submissions with the FDA and the clinical testing of these tablets in a safety and PK Phase I trial (CONRAD 117). The results of this study, which revealed good safety and promising TFV and FTC PK profiles despite slower-than-expected in vivo tablet disintegration, has led CONRAD to continue optimization of the TFV tablet formulation and development of the platform dosage form.

CONRAD also developed a longer-acting delivery system, the TFV IVR, with a target duration of 3 months. After investigating several IVR technologies, and subsequently advancing development of several IVR platforms for microbicide delivery (including the pod-based IVRs and polyurethane IVRs), CONRAD moved forward with development of a novel polyurethane reservoir design capable of delivering approximately 10 mg of TFV per day in a tightly controlled and sustained manner, thereby achieving the target 3 month duration. Preclinical studies of this TFV IVR demonstrated vaginal TFV levels in sheep and non-human primate models are sustained at levels comparable to peak levels seen with TFV 1% gel. Under the PPRD program, the TFV IVR was evaluated through a suite of IND-enabling nonclinical safety studies, the technology was transferred to contract manufacturers enabling clinical supplies production, and a new IND was filed with the FDA in parallel with a separate IND for its sister MPT product, TFV/levonorgestrel (LNG) IVR, which is based on the same customizable technology. The TFV-only and TFV/LNG IVRs are currently being evaluated in a Phase I safety and PK study (CONRAD 128), which was initiated in October 2014, under a new USAID/GH/PRH Cooperative Agreement AID-OAA-A-10-00068 (APS Aim 4).

The PPRD program directly supported CONRAD's development of several novel MPTs for the prevention of HIV, HSV and/or unintended pregnancy. For longer-acting, coitally-dissociated formulations, CONRAD developed two initial TFV/ACV ring technologies (pod-based and PU matrix designs) at the preclinical level, and advanced the aforementioned TFV/LNG IVR through IND-enabling activities, thereby enabling readiness for clinical testing. For on-demand contraceptive and HIV prevention, CONRAD collaborated with PATH to use the Caya diaphragm, which has market approval in the US and Europe, as an inexpensive delivery system for TFV 1% gel. At the conclusion of PPRD, this late-stage product was poised for Phase I clinical testing, with plans for TFV gel reformulation to enhance contraceptive efficacy under a new USAID/GH/PRH Cooperative Agreement AID-OAA-A-11-00064 (APS Aim 2). Furthermore, the technologies developed under PPRD have also enabled the development of other novel MPTs in CONRAD's pipeline.

In order to better guide the rational selection of microbicide candidates, several product development-associated activities under the PPRD program identified and validated biomarkers and new endpoints of safety. By combining in vitro efficacy data, in vitro, ex vivo and animal data on cervicovaginal mucosal safety, and in vitro and in vivo PK/PD data, CONRAD created an expanded preclinical testing algorithm for microbicides and MPT candidates. Using microarray gene analysis, we characterized a panel of gene markers in vaginal cell models, explants and vaginal tissue biopsies that discriminate pro-inflammatory from non-inflammatory compounds. We also expanded the rabbit vaginal irritation (RVI) model to include newly-identified RNA-based biomarkers and immunohistochemistry-based markers for mucosal inflammation and irritation. Under PPRD, CONRAD refined a novel PD model of ex vivo HIV-1 infection in ectocervical and vaginal tissues, which has been incorporated as an important safety and efficacy endpoint in numerous clinical studies conducted by CONRAD and other organizations. CONRAD also pioneered development of composite biomarkers of product adherence, protocol compliance and semen exposure that can be used to objectively confirm product usage by participants in microbicide trials.

Interagency Agreements and Field Support

NIH/NICHD

NICHD interagency funds were focused on three areas. In the first of these, CONRAD's Intramural Preclinical Research Program (IPR) explored various new and improved technologies for contraception as well as the prevention of sexually transmitted infections including HIV/AIDS. Initial studies were aimed at studying a combination of anti-microbicide and contraceptive agents together, such as the formulations of Tenofovir (TFV) & UC78 or TFV & ContraGel. CONRAD was also interested in identifying the dual activity compounds that exhibited both microbicide as well as spermicide activities. Among 400 compounds that were tested, several of fatty-acid nucleoside derivatives of the Reverse Transcriptase Inhibitor (RTI), and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), and a CCR5 antagonist were identified as the lead candidates. These compounds are further being evaluated for their dual activities.

The goal of the second activity was to study and compare two new female condoms (PATH condom and FC2 condom) in a clinical study that enrolled 490 couples. The results showed that the PATH condom had higher acceptability ratings than FC2, was associated with less female discomfort than FC2, and was non-inferior to FC2. CONRAD had also completed a separate sub-study funded by NICHD comparing the same two female condoms in 150 additional couples. Through comparison of the results of the two studies, CONRAD is exploring to reclassify the female condoms as Class II-FDA devices.

In its third activity, CONRAD focused on developing devices such as Intravaginal Rings (IVRs) that released combinations of anti-microbial and spermicidal compounds. An IVR that released Levonorgestrel (LNG; a contraceptive) and TFV (microbicide) as well as a second IVR that released TFV and ACV were developed. These rings will need to be evaluated further as the candidates for providing dual protection. In addition to

IVRs, CONRAD explored the properties of 1% TFV as a potential contraceptive agent. While men taking oral tenofovir (Viread®) showed a reduction in progressively motile sperm population in their semen leading to decreased pregnancy rates, the data was NOT statistically significant. While CONRAD's initial efforts to reformulate the TFV gel to increase its sperm immobilizing properties were not successful, CONRAD is continuing its efforts to reformulate TFV as part of multipurpose prevention technologies.

NIH/NIAID/DAIDS

Funds from an interagency agreement with NIAID/DAIDS were provided for identification and validation of biomarkers of cervicovaginal mucosal alteration. The main goal of this project was to identify gene-expression profiles caused by the microbicides, vaginal products having mucosal altering properties, known pro-inflammatory compounds and non-inflammatory compounds, using microarray technology in cervicovaginal cells and biopsies. CONRAD was successful in identifying a panel of discriminatory gene markers in a vaginal cell model. At the end of the project period, studies were underway to define the roles for some of the differentially expressed gene products in vaginal inflammation, immune activation, and in compromised barrier function.

CDC

Interagency funds from CDC were provided to co-fund the development, monitoring and evaluation of ongoing and planned microbicide activities supported by USAID and co-fund two main activities. The first of these was a clinical trial to assess the effects of the diaphragm and candidate microbicide (BufferGel) use on the acquisition of Neisseria gonorrhoeae and Chlamydia trachomatis among women in five cities in Madagascar. Unfortunately, the planned trial was not initiated due US Government decisions to suspend all funded activities in Madagascar in 2009 and the need to identify an alternative microbicide than was originally planned. The funds were then used to for a second clinical study addressed prospective evaluation of the impact of bacterial vaginosis and its treatment on endpoints of cervicovaginal safety and mucosal susceptibility to HIV infection. This study demonstrated that common safety endpoints used in phase I clinical trials, such as genital tissue immune cell populations and anti-microbial activity of the CVL is significantly altered by the presence of abnormal vaginal flora.

USAID-South Africa Field Support

Field Support funds from AID-South Africa were provided in co-support of CAPRISA 008, an open-label randomized controlled trial in South Africa to assess the implementation effectiveness and safety of 1% tenofovir gel provision through family planning services in KwaZulu-Natal, South Africa." Participants will begin exiting the clinical phases in Q1 2015; results and data analysis will be available by Q3 2015.

Summary of Program Achievements

CONRAD was able to leverage the outcomes of PPRD-related activities to improve microbicide and MPT research and development methods. Lessons learned in early preclinical selection include the importance of creating a target product profile (TPP) for product evaluation, careful API and delivery system selection, and soliciting early feedback on user preferences and adherence from target populations. CONRAD also established an algorithm and selection criteria with go/no-go decisions to be implemented prior to initiating product development, and utilized an integrated preclinical/clinical evaluation system including sensitive measures of safety, efficacy, PK and PD early in clinical development. By monitoring timelines and reprioritizing product development efforts at regular intervals, CONRAD was able to successfully meet all outcomes of the PPRD program.

Through dissemination of results and technologies from the PPRD program, CONRAD has impacted the greater microbicide field. Work initiated by and funded through PPRD has culminated in 150 peer-reviewed publications from CONRAD (see Appendix A), as well as over 100 meeting presentations. CONRAD also prepared quarterly letters to the HIV prevention community, biannual financial reports to USAID, and regularly updated our organizational website in order to highlight our most recent findings to the field. CONRAD implemented workshops with published papers summarizing updates on biomarkers, product development and alternative clinical trial design, sponsored major scientific conferences (e.g., Microbicides, R4P), supported advocacy organizations (e.g., AMD, AVAC) and participated in working groups and consultations with USAID, NIH, CDC, WHO and Gates Foundation. Cumulatively, CONRAD's successful PPRD-initiated activities not only advanced CONRAD's product development program, but also positively benefitted the microbicide field as a whole, through provision of clinical supplies, development of new drug candidates and delivery systems, and improved preclinical testing technologies.

In summary, CONRAD achieved all planned deliverables of the PPRD program, and contributed significantly to advancing the microbicide field. Through large-scale, late-stage clinical trials, and smaller supporting clinical studies initiated through PPRD funding, CONRAD progressed the regulatory pathway of TFV 1% gel towards NDA submission. We expanded our microbicide formulation and delivery system pipeline to include new on-demand and longer-acting methods (fast dissolve vaginal tablets and vaginal rings, respectively), and advanced a novel MPT, the TFV/LNG reservoir IVR, to early clinical testing. CONRAD also completed multiple activities that supported product development, including drug discovery of new microbicide compounds, identification and validation of new biomarkers and endpoints of safety and adherence, and the development of an in vivo/ex vivo pharmacodynamics model as a surrogate of microbicide efficacy.

Upon completion of this cooperative agreement, CONRAD achieved all major expected project outcomes and deliverables, attaining the goal of improving global health by developing products designed to prevent HIV acquisition by women, especially in resource-constrained regions of the world such as sub-Saharan Africa.

II. Locations (Countries) of PPRD Activities

Denmark

Dominican Republic

Germany

India

Madagascar

South Africa

Switzerland

United Kingdom

US

Zambia

III. Financial Summary

As presented in the previously submitted SF-425 for PPRD, the total expenditures amounted to $99,999,870, which included $95,359,142 from USAID/Micro, and Interagency Agreement funds from NICHD ($1,776,272), NIAID ($2,000,000) and CDC ($864,456). The majority of these funds (65.7%) were awarded to collaborating extramural investigators and contractors via subagreements and service contracts to achieve the goals and objectives of CONRAD's workscope, as shown in Figure 1.

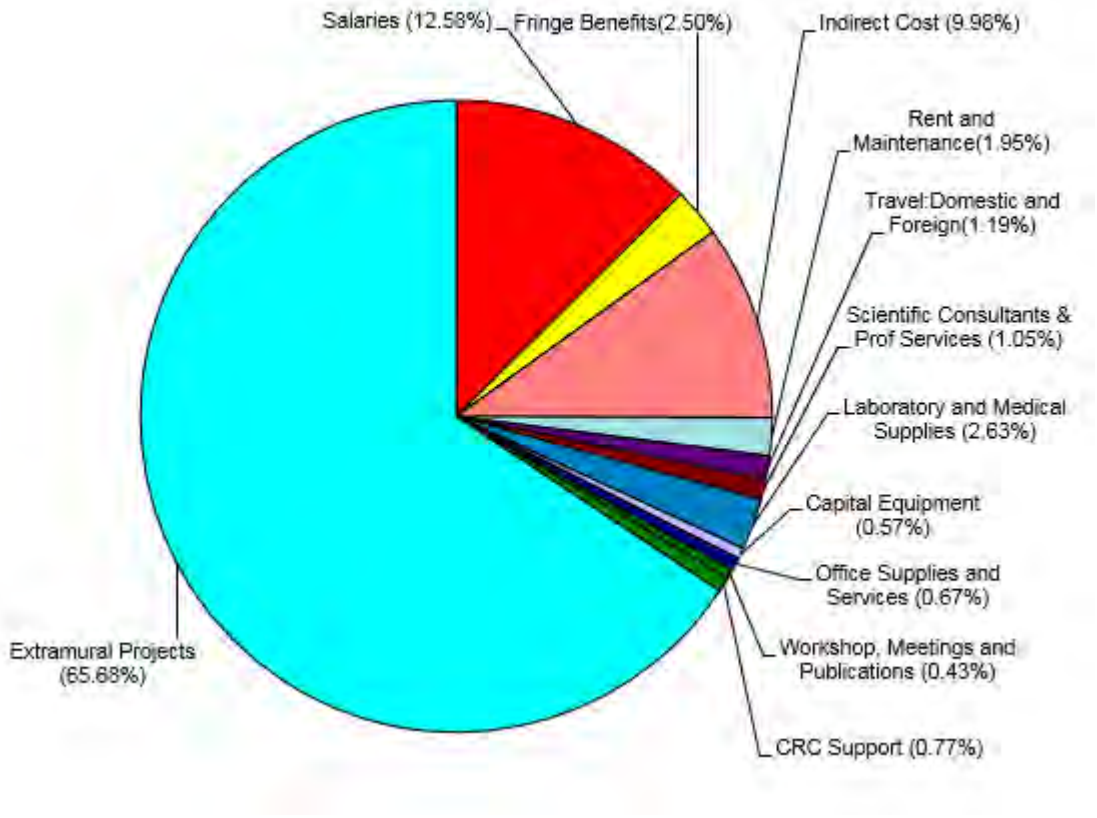

Figure 1. CONRAD- PPRD Total Expenditures for the Period: 9/05/08 - 09/04/14

Essentially all of the work carried out under subawards, totaling $65,680,628, was focused on microbicide development (Figure 2), either alone or as part of multipurpose prevention technologies (MPTs) (Activities 1-5, as well as the Interagency Agreement funds received the Centers for Disease Control and Prevention (CDC), NIAID and NICHD).

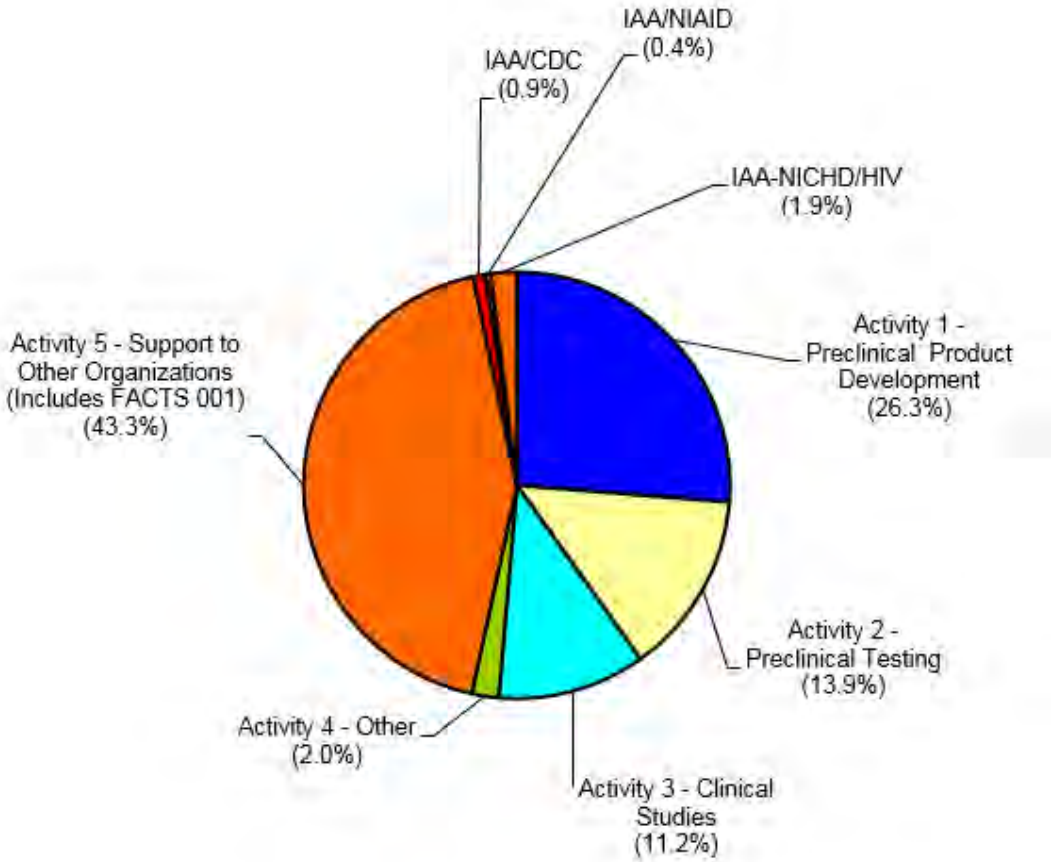

Figure 2. CONRAD- PPRD Extramural Subawards for the Period: 9/05/08 - 09/04/14

Cost-Share

The CONRAD–PPRD Cooperative Agreement funding was $100,000,000 and the required 10% Cost-Sharing Percentage (Non-Federal) of $10,000,000 was cost shared for the period 11/01/08 – 03/31/13 by the Gates Microbicides Development Project (GMDP) awarded by the Bill and Melinda Gates Foundation to CONRAD EVMS on October 23, 2007 for 5 years at $28,460,969 (10/23/07 – 12/1/12). On October 15, 2012 the GMDP award ceiling was raised to $35,292,841 and extended through June 30, 2015. As of June 30, 2011, $10,411,582 total cost share had been provided by the GMDP award.

Appendix A. PPRD Final Report Summary Workplans and Accomplishments

1. Preclinical Characterization, Formulation, and Delivery…… 10

1.1 Active Ingredients
1.2 Primary Dosage Forms and Formulations
1.3 Alternative (Innovative) Dosage Forms and Formulations
1.4 Dual-Protection Technologies
1.5 Manufacture of Clinical Supplies

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2.1 Standard Preclinical Evaluation of CONRAD PPRD Microbicide Candidates
2.2 Models and Biomarkers of Microbicide Safety to Evaluate CONRAD PPRD Compounds
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2.4 Characterization of Cervicovaginal Mucosal Markers and Early HIV Infection in the Tenofovir Gel Trial CAPRISA 004

3. Clinical Studies……. 35

3.1 Studies of Gels – Pharmacokinetics, Pharmacodynamics, and Markers of Mucosal Safety
3.2 Exploratory Studies: Site Identification/HIV Incidence in India
3.3 Exploratory Studies: Influence of Steroid Contraception on Pharmacokinetics/Pharmacodynamics and Vaginal Safety in TFV Gel Users (*was* "Influence of Steroids on Vaginal Mucosal Inflammation")
3.4 Imaging Study of Combination Microbicide Formulations
3.5 Exploratory Studies: 1) Using Indicators of Vaginal Inflammation to Assess New Microbicide Candidates, and 2) Using Explants as Early Indicators of Microbicide Efficacy
3.6 Phase III Effectiveness Trial of the PATH SILCS Diaphragm plus BufferGel
3.7 Prospective Evaluation of the Impact of Bacterial Vaginosis and its Treatment on Endpoints of Cervicovaginal Safety and Mucosal Susceptibility to HIV Infection
3.8 Safety and Pharmacokinetic Studies of Dual-Protection Tenofovir/Levonorgestrel Intravaginal Ring
3.9 Collaboration and Support for Microbicide Trials Network Studies
3.10 Clinical Support Services and Consultants
3.11 A Phase I Clinical Trial Assessing the Safety, Pharmacokinetics, Pharmacodynamics, and Disintegration Time of Vaginal Tablets Containing Tenofovir and/or Emtricitabine
3.12 Tenofovir Intravaginal Ring Studies
3.13 Interference with Semen Biomarkers due to Vaginal Product Use
3.14 Biomarkers of Semen Exposure
3.15 Behavioral Assessments of Combination Formulations
3.16 Exploratory Studies – Vaginal Taggants for Compliance Monitoring
3.17 Tenofovir Phase III Trials for Safety and Effectiveness: Support for Mazabuka Site in Zambia
3.18 TFV/MIV-170 Intravaginal Ring (IVR) Studies (originally "Safety and PK of UC781/TFV Combination Ring")
3.19 Safety and PK/PD of Combination Gel

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4.1 Biomarkers of Inflammation, Infection, and Adherence (formerly titled "Conference on Semen Biomarkers for Female Condom Development")
4.2 Integration of Biomarkers in Comparative Studies of Barrier Devices: Application to New Female Condoms
4.3 Public Relations Services
4.4 Regulatory Support for 1% Tenofovir Gel Investigational New Drug (IND) Application and New Drug Application (NDA) Submissions
4.5 General Support Services
4.6 Manufacturing and Packaging of Tenofovir 1% Gel and Clinical Oversight of a Phase I Safety Study of the PATH Applicator

5. Support to Other Organizations……. 97

5.1 Regulatory Support:
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5.1b Regulatory Support and Trial Oversight and Management for TFV gel Adolescent Studies
5.1c Regulatory Support and Trial Oversight and Management for CAPRISA 008

5.2 Support for AVAC
5.3 Support for the Alliance for Microbicide Development and CAMI

5.4 CDC Collaborative Activities:
5.4a Support of CONRAD Staff
5.4b Diaphragm plus microbicide: Expansion of ongoing clinical study to prevent STDs
5.4c Overcoming barriers to adherence (OBA): Qualitative research to identify strategies for high-risk women to achieve use of the diaphragm with candidate microbicide during sexual intercourse
5.4d Cross-sectional study to measure gonorrhea and chlamydial infection among women at high risk in Madagascar

6. Interagency Agreements and Field Support ……. 119

6.1 IAA - NICHD/HIV
6.2 IAA - NIAID
6.3 IAA - CDC
6.4 Field Support – South Africa

CONRAD
Microbicide Account - Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#1 – Preclinical Characterization, Formulation, and Delivery
Activity Number and Title:	#1.1 – Active Ingredients
CA Activity Numbers:	Numerous
Primary Country of Activity:	USA
Technical Coordinator:	David Friend
Implementers:	CONRAD, University of Utah, Particle Sciences , Absorption Systems, MatTek Corporation, PPD Laboratories, MPI Research, Regis Labs, Dow Pharmaceutical Sciences, Cirrus Pharmaceuticals, MedCrystalForms, Micron Technologies, University of Rhode Island, Xenobiotic Labs, Huntingdon Life Sciences
Period:	8/1/08 – 9/4/14

Objective:

The initial objective was to collect sufficient preclinical characterization and pre-formulation data to permit the appropriate formulation of UC781, tenofovir (TFV), and fatty acid nucleoside analogs. Specifically, the physicochemical characterization and in vitro tissue absorption properties would be assessed. Another key objective of this activity is to determine the ability to co-formulate two actives in the same formulation. After development of UC781 was abandoned, the focus shifted to formulation of tenofovir (TFV), and combinations of other antiretrovirals (emtricitabine [FTC] and MIV-170) with TFV. Compatibility of these actives with each other would be thoroughly investigated. IND/NDA-enabling studies on drug substances are also included in this Activity.

Activity Description:

UC781 is a rapid-acting, tight-binding NNRTI with very potent activity against HIV-1. It has been extensively characterized by multiple laboratories, possessing the largest amount of published data for any NNRTI currently in development as a microbicide. TFV is a nucleotide analog of deoxyadenosine monophosphate with activity against HIV-1, -2 and hepatitis B virus. CONRAD has developed fluorinated and non-fluorinated fatty acid derivatives of nucleosides such as azido and fluorothymidine and deoxycitidine (similar to FTC). Two selected analogs, KP-1 and KP-17, show the highest selectivity indices, with activity against wild-type and multi-drug resistant virus. In vitro, they display no cytotoxicity or proinflammatory potential. CONRAD has filed a patent application to protect their use as microbicides.

In addition to the compounds listed above, CONRAD would search for new compounds, especially small molecule entry inhibitors. CCR5 antagonists are the newest agents in clinical HIV-infection treatment. CONRAD supported the development of two RANTES analogs which have demonstrated outstanding activity (100% efficacy) in monkey studies. New gp-120 blockers and integrase inhibitors were also to be explored.

This activity (as well as others) was revised substantially around 2010, based on unfavorable results related to UC781. Therefore, development of some products (e.g., combination of UC781 and TFV) was discontinued

while other drugs were identified as potential combinations. Based on the success of the CAPRISA 004 study, CONRAD focused its development efforts around TFV, a nucleotide analog of deoxyadenosine monophosphate with activity against HIV-1, HIV-2 and hepatitis B virus. In addition to UC781, CONRAD was developing fluorinated and non-fluorinated fatty acid derivatives of nucleosides (KP compounds) such as azido, fluorothymidine and deoxycytidine (similar to FTC). Work on the KP compounds was also terminated.

CONRAD obtained rights to develop MIV-170, a potent NNRTI from Medivir. CONRAD performed a wide range of nonclinical studies on MIV-170, which was planned to be used in combination with TFV. Due to changes in workplans, development of MIV-170 was discontinued. Another compound obtained from Gilead is FTC. This compound is currently marketed in combination with tenofovir disoproxil fumarate for treatment of HIV infection (Truvada®). A combination of TFV and FTC in a fast-dissolve tablet formulation was investigated (see Alternative Dosage Forms under Activity 1) along with a TFV-only fast-dissolve vaginal tablet.

By 2012, the primary effort in this activity has been focused on collecting data on TFV drug substance to support the filing of an NDA in the US and its equivalent in South Africa.

The final area in this activity is related to creation of dual-activity compounds (i.e., microbicide and contraceptive properties). Some molecules and mechanisms used by sperm to fertilize the egg are similar, if not identical, to those used by HIV to infect host cells. These commonalities provide the biological framework to develop bifunctional inhibitors with both antimicrobial and contraceptive properties.

Progress:

This activity included the synthesis of significant quantities of API (active pharmaceutical ingredient). During preparation of a second batch (5 kg) of UC781 drug substance, a new impurity was identified; with a modest change in the process, it was removed from the final product. Additional supplies of KP-1 and KP-17 were synthesized and some preformulation activities completed. In vitro PK (using EpiVaginal™ tissues) studies of TFV and UC781 from single entity and combination gels were performed.

Most of the development work on UC781 was completed with the exception of preparation of more soluble forms of the API. Three different UC781 powders underwent a formal stability study and in vitro testing at EVMS. These new compositions possessed a 3-order of magnitude increase in the water solubility of UC781. Much of the work focused on KP-17. Additional supplies of KP-17 were prepared at the University of Rhode Island and the synthetic process was transferred to a commercial manufacturer (Regis). Regis successfully prepared a batch of 100 g KP-17 and could prepare larger amounts under GLP/GMP for toxicology and clinical testing. Particle Sciences completed preformulation work with KP-17 and formulation development work was initiated. The formulation prepared was a gel containing UC781. MatTek examined the effect of vaginal gel formulations on EpiVaginal tissue permeation. Xenobiotic Labs developed a sensitive bioassay for UC781 in human plasma.

CONRAD brought all UC781 related activities to a close over the course of the year starting in mid- 2010. Likewise, work with KP-17 was discontinued due to funding constraints and re-prioritization of the CONRAD portfolio. MatTek completed a safety evaluation of MIV-170 and it was found to be well tolerated. MIV-170 drug substance was also found to permeate through EpiVag tissues as expected. Considerable work was performed on the preformulation assessment of MIV-170. MIV-170 was also micronized to permit preparation of gels and IVRs with consistent dose uniformity and enhanced solubility compared with the unmicronized form. Due to funding constraints, development activities focused on the TFV/MIV-170 combination was became a lower priority.

Several additional products were assessed for in vitro safety and permeability using the MatTek EpiVaginal system. CONRAD focused considerable effort on validation of several drug substance methods including assay and impurities, optical purity, residual solvents, and similar activities. In addition, Cirrus (a contract lab) completed a forced degradation study of TFV drug substance. This information was submitted via amendment to the CONRAD IND with the US FDA covering TFV 1% gel. Three lots of TFV drug substance were set up on stability as required to file the South African NDA (the Medicines Control Council requires 1 year room temperature stability data at the time of filing). These data will also be required for filing of the U.S. NDA.

PPD completed method transfer/validation of all methods required to assess TFV drug substance. Data over 24 months was collected. The 6 month data, including accelerated test conditions (40°C/75% relative humidity) indicating the 3 lots were stable. However, at 12 months at room temperature, one of the tests (clarity of solution) failed the acceptance criteria; however, after an extensive investigation, it was decided that these data would still be included in submissions to the NDA filings. The 2 impurities synthesized were used to complete the validation of the assay/impurities method for TFV drug substance and TFV 1% gel drug product.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

The contribution of this activity to Microbicide R & D was the providing sufficient preclinical information on TFV drug substance to support an NDA filing for TFV 1% gel. Similar data collected for the other APIs, particularly KP-17 and MIV-170, were still valuable and would still be suitable in the pathway towards clinical assessment, if funding was available.

Involvement of Other Donors and Interested Parties:

CONRAD co-funded some of the specific and related activities using its funding from the Gates Foundation to develop new, multi-target combination microbicides (~$1.1M was used for activities related to this overall activity). CONRAD-III USAID/Micro funds also co-supported some of the initial activities (~$300K).

CONRAD
Microbicide Account - Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#1 – Preclinical Characterization, Formulation, and Delivery
Activity Number and Title:	#1.2 – Primary Dosage Forms and Formulations
CA Activity Numbers:	Numerous
Primary Country of Activity:	USA
Technical Coordinator:	David Friend
Implementers:	CONRAD, Dow Pharmaceutical Sciences, Particle Sciences, Queen's University of Belfast, University of Utah, Oak Crest Research Institute, Northwestern University, MPI, DPT Laboratories, NAMSA, Foster Corp, DSM-PTG, Absorption Systems, MatTek, Duke University, Huntingdon Life Sciences, others
Period:	8/1/08 – 9/4/14

Objective:

The objective of this activity is to prepare the primary microbicide dosage forms: gels and intravaginal rings (IVRs). These dosage forms will be developed to support preclinical and clinical evaluations.

Activity Description:

Gels provide the drug(s) in a readily available dosage form for in vitro, animal and clinical testing. Gels are also the most adequate form to study drug combinations, both at preclinical and early clinical stages. Owing to their physicochemical properties and human safety record, cellulose derivatives will be our first choice as polymeric carriers.

A clear rationale also exists for providing long-term, controlled release of vaginal microbicides in order to afford continuous protection against heterosexually transmitted HIV infection and to improve user compliance. In order to address these issues, the intent was to develop a microbicide-releasing device (IVR) that is placed around the cervix and delivers microbicides for an extended period of time.

Silicone elastomer rings are currently being pursued as a controlled-release strategy to deliver microbicides. Queen's University of Belfast (QUB, Malcolm) worked with CONRAD and CDC to develop and test a UC781-containing IVR. Preliminary testing of this device showed consistent and sustained in vitro release of drug as well as absence of vaginal side effects and good vaginal distribution in a monkey study. CONRAD intended to pursue the development of prototype rings to deliver active compounds of different solubility such as TFV and UC781. IPM (International partnership for Microbicides) intended to collaborate with CONRAD on the development of a TFV-releasing ring. Another approach to the use of silicone is polyurethanes. They are available in a range of properties ranging from relatively hydrophobic to relatively hydrophilic. Due to the wide range of physicochemical properties of UC781, TFV, the KP compounds, such flexibility aids in development of a combination IVR product designed to release two microbicides simultaneously.

The novel hydrophobe-modified derivative, Q-2, is a cationic polymer with gel- and film-forming properties. Cationic and hydrophobe-modified polysaccharides are preferred excipients for personal care products. Furthermore, they are hydrophilic, film-forming, compatible with many therapeutic agents, non-penetrating and non-irritating.

Progress:

The clinical gel formulation of UC781 (micronized) was being characterized. CONRAD established contracts with QUB and Utah to develop IVRs for UC781, TFV, and their combination.

Both projects were to enter clinical trials (UC781-single entity gel into a Phase 2 safety study and UC781/TFV into a Phase 1 safety/PK study). However, a decision was made in July 2010 to discontinue development of all UC781-based products. As a result, all work on UC781 single entity gel and combination (gel and IVR) of UC781 and TFV were ended.

At the same time work on UC781 was discontinued, work on a novel non-nucleoside reverse transcriptase inhibitor (NNRTI), MIV-170, was started. A gel formulation in combination with TFV was developed at DPT and tested for PK and in a rabbit vaginal irritation (RVI) study. However, work on MIV-170 was discontinued at the end of Q1 2011 at the request of USAID.

Oak Crest completed development of an IVR for the sustained release of TFV. In vitro and in vivo data in rabbits and pigtail macaques demonstrate the ability of this technology to deliver TFV into vaginal tissues for at least one month (with a target of three months). However, the levels proved to be inadequate to prevent infection in pigtail macaques.

By 2013, work on transitioning TFV 1% gel into a commercial product was initiated following the results obtained in CAPRISA 004. This work included validation of drug product methods, qualification of impurities, forced degradation studies, process validation, and manufacture of registration batches. CONRAD initiated development of a new package for TFV 1% gel (in 100g tubes). Stability of TFV 1% gel in tubes was confirmed.

Gel from the tubes would be administered via a user-filled disposable paper applicator. This packaging configuration will markedly lower the cost of TFV 1% gel compared with the prefilled plastic vaginal applicator.

A “reduced glycerin” formulation of TFV 1% gel was prepared at DPT and was successfully evaluated by MTN in a Phase I clinical studies involving rectal administration (MTN-007 and MTN-017). This study demonstrated significantly reduced lower gastrointestinal distress compared with the current high glycerin (20%) vaginal formulation. Additional minor adjustments were made to the “reduced glycerin” formulation (now called the TFV RG 1% gel). In addition to supporting the use of a safe and effective TFV RG gel for the MSM population (men who have sex with men), the product will be better tolerated by women who choose to use the gel rectally.

Work on evaluation of extractables/leachables of both the pre-filled plastic applicators and the paper applicators were initiated.

Development work on a 90 d TFV IVR was completed. A novel ring design (reservoir with a rate limiting membrane composed of a unique polyurethane indicates that TFV can be released at a rate of 10-15 mg per day over 90 days. The IVR has been designed and tested in animals (rabbits and sheep). The IVR delivers the desired amount of TFV (10 mg/day) over a 90-day period. The technology was transferred to Foster (all extrusion activities) and DPT (final product manufacture). Most new methods were validated by September

2014. The investigational new drug (IND) application was successfully filed with the FDA in April 2014. Manufacture of clinical supplies was completed by Q3 2014.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

Work under this activity has led to the development of a viable commercial microbicide gel (TFV 1% gel) and a potentially improved form of TFV gel that could be administered both rectally and vaginally. Finally, the development of a novel 90-day IVR capable of releasing TFV at an appropriate rate could offer women a viable alternative pericoital gel product.

Involvement of Other Donors and Interested Parties:

CONRAD co-funded some of the specific and related activities using its funding from the Gates Foundation to develop new, multi-target combination microbicides, of which ~$2M was used for activities related to this activity. In addition, some of the early work was funded using CONRAD-III USAID/Microbicide funds (~$1.5M).

CONRAD
Microbicide Account - Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#1 – Preclinical Characterization, Formulation, and Delivery
Activity Number and Title:	#1.3 – Alternative (Innovative) Dosage Forms and Formulations
CA Activity Numbers:	Numerous
Primary Country of Activity:	USA
Technical Coordinator:	David Friend
Implementers:	CONRAD, University of Pittsburgh, MonoSol Rx, University of Auckland , Aptalis Pharma (formerly Eurand), MPI Labs, Absorption Systems, MatTek, FHI 360
Period:	8/1/08 – 9/4/14

Objective:

The objective of this activity was to prepare alternative microbicide dosage forms: fast dissolve films, fast dissolve tablets/capsules, sponges, and cervical caps. These dosage forms would be developed to support preclinical evaluations leading to testing in Phase 1 clinical trials.

Activity Description:

Compared to creams, gels, and other semi-solids, solid films may be easier to apply since they do not require an applicator. In addition, films are potentially less messy than semi-solids, potentially leading to increased user acceptability. Currently, this type of dosage form for vaginal delivery of pharmacologically active agents is limited. For this reason, research is required to optimize utilization of this dosage form for vaginal microbicide use. A film dosage form for UC781 would result in efficient delivery of UC781 to the tissue. Stabilization of UC781 in a solid polymer matrix and rapid release of the drug is possible in the film. With respect to the combination product of tenofovir (TFV) and UC781, the film dosage form has the ability to simultaneously release these two molecules, which possess very different chemical properties.

CONRAD also initiated a project investigating fast-dissolve tablets and gelatin capsules. Both dosage forms are easy to administer and potentially can improve the stability of APIs such as UC781 and TFV.

A vaginal contraceptive sponge, the Today® Sponge, was introduced in 1983 and became the largest-selling over-the-counter (OTC) female contraceptive in the United States. Sponges have major advantages over gel formulations, since they act both as an applicator of the microbicide, coating the vaginal wall during insertion, but also providing a reservoir from which further microbicide is released during each coital act. CONRAD proposed to develop a sponge that does not only incorporates the active agent in the matrix like Today® Sponge, but that retains it in the pores of the sponge still allowing it to be readily released during insertion and during and after intercourse. In addition, CONRAD planned to investigate a vaginal foam product capable of delivering 30 doses from an inexpensive metered-dose canister.

CONRAD also proposed to develop and evaluate a microbicide-releasing cervicovaginal barrier. The device would be composed of a cervix-covering flexible thin dome which ends in a circumferential thicker rim that

extends anteriorly and posteriorly into the respective fornices with smooth conical, crescent-shaped protrusions. The device would be made of elastomers capable of releasing hydrophilic and lipophilic microbicides in a controlled fashion. In addition to the sustained release of microbicides by the device, the rapid bursts of release during insertion and intercourse would provide increased concentrations of actives when they are most needed.

CONRAD also planned to collaborate with PATH on the development of a product combing the SILCS diaphragm and BufferGel. PATH initiated an exploratory investigation with Karl Malcolm of the Queen's University Belfast on the feasibility of incorporating drug into the SILCS diaphragm as a vehicle for sustained release delivery.

Progress:

Vaginal fast dissolve films were developed at the University of Pittsburgh. The ultimate goal of this work was to prepare a film combining UC781 and TFV. Several prototype films were prepared and put on stability and tested for release into vaginal tissues. UC781 films were able to deliver the drug into vaginal tissues in a manner similar to that observed in UC781 gels. A GMP manufacturer of fast dissolve films (ARx) was identified. Work on this dosage form with this combination was discontinued based on the decision to terminate all efforts involving UC781. Work on a TFV-only film was continued until funding of a U19 grant from NIH was awarded to the University of Pittsburgh.

Fast dissolve tablets containing the single entities (UC781 or TFV) and their combination have been prepared at the University of Auckland. Three month stability data under accelerated conditions indicated that the combination tablet formulation was stable. However, development of fast-dissolve tablets containing the single entities (UC781 or TFV) and their combination at the University of Auckland was terminated by 2011.

A contract with Eurand (now Aptalis Pharma) was initiated in 2010 as an alternative to the Auckland tablet approach. The Eurand technology is a proven fast dissolve tablet system that should provide more rapid dissolution when applied to the vagina than the tablets under development at Auckland. Based on a rabbit PK study and a study in pigtail macaques at the University of Washington (D. Patton), the Aptalis-based tablets containing TFV have proven to be a viable alternative to gels, confirming that TFV was rapidly bioavailable. This work was expanded to prepare tablets capable of delivering TFV, FTC, and their combination.

The tablet format permits the formulation of compounds including actives such as emtricitabine (FTC), which is unstable in aqueous gels. Therefore, development of both a single entity tablet (40 mg TFV) and a combination of TFV (40 mg) and FTC (40 mg) was initiated. Ten (10) mg versions of these dosage forms were tested in rabbits for local irritation and pharmacokinetics. In addition, the CDC tested the tablets in pigtail macaques. The tablets proved to be well-tolerated, with acceptable pharmacokinetics (determined through comparison with TFV 1% gel pharmacokinetics).

Progress on these vaginal tablets has remained on schedule. Tablet supplies were manufactured to initiate a Phase I study (CONRAD-117) in Q3 2012. Disintegration data from the study was assessed and formulation optimization was initiated to modify disintegration properties. Despite slower than desired tablet disintegration, the pharmacokinetics of TFV were similar to those observed following vaginal administration of TFV 1% gel.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This activity markedly advanced the prospect of a less expensive and a more discrete dosage form of TFV (and potential a wide range of additional microbicide candidate drugs) compared with vaginal gels. This product

may be more acceptable to women and could potentially improve user adherence.

Involvement of Other Donors and Interested Parties:

CONRAD co-funded some of the specific and related activities using its funding from the Gates Foundation to develop new, multi-target combination microbicides, of which ~$500K was used for activities related to this activity. In addition, some of the preliminary work was funded using CONRAD-III USAID/Microbicide funds (~$250K).

CONRAD
Microbicide Account - Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#1 – Preclinical Characterization, Formulation, and Delivery
Activity Number and Title:	#1.4 – Dual-Protection Technologies
CA Activity Numbers:	Numerous
Primary Country of Activity:	USA
Technical Coordinator:	David Friend
Implementers:	CONRAD, University of Utah, MPI, NAMSA, Controlled Therapeutics, Particle Sciences, University of Utah, Oak Crest Research Institute, DSM-PTG
Period:	8/1/08 – 9/4/14

Objective:

The objective of this activity was to prepare the dual protection technologies including anti-HIV/contraception and anti-HIV/anti-STIs properties.

Activity Description:

The high rate of HIV and other infections acquired through sexual activity and the concurrent need to avoid or delay pregnancy warrant development of novel compounds displaying contraceptive and microbicidal properties. Some of the molecules and mechanisms used by sperm to fertilize the egg are similar, if not identical, to those used by HIV and other STD-pathogens to infect host cells.

Research on this topic has been ongoing since 2003 and has yielded significant discoveries that led to the filing of a patent of noncytotoxic bifunctional anti-HIV-1 microbicides with sperm-immobilizing activity by CONRAD. Furthermore, conjugation of anionic polymers with fatty acid derivatives of AZT has shown contraceptive properties both in vitro and in vivo, in addition to HIV entry and reverse transcriptase (RT) inhibitory activity. Dual-activity compounds will be formulated and delivered as described below.

Contraceptive microbicide. CONRAD would develop an intravaginal ring (IVR) that releases levonorgestrel (LNG) and UC781. LNG is a 19-nortestosterone derivative of proven safety and efficacy. It is available in the market in different dosage forms including a LNG-releasing intrauterine system, which releases LNG directly into the uterine cavity at a constant rate of 20 µg per day. Preliminary data indicate that release rates of LNG from an IVR are adequate to achieve contraceptive efficacy. The controlled release of LNG and UC781 from a vaginal ring device will protect women against sexual acquisition of HIV and unplanned pregnancies.

In addition to the contraceptive microbicide LNG/UC781-releasing ring, dual-activity compounds described above would be formulated in polycarboxylic acid (e.g., Carbopol) and cellulose derivatives (e.g., Q-2 and HEC) to form a gel. It was expected that a formulation containing specific carbomers at appropriate concentrations would display contraceptive properties similar to those of BufferGel.® The use of sperm-inhibiting vehicle ingredients in conjunction with microbicidal or dual-activity agents constitutes another strategy to develop dual-protection contraceptive microbicide products.

Dual-activity microbicide (anti-HIV and -HSV activity). Genital herpes is the leading cause of genital ulcer disease worldwide, and it is one of the most common sexually transmitted diseases. Genital herpes is caused by herpes simplex virus (HSV) type 2 in 60–80% of cases, while HSV-1 is responsible for as many as 35%. Acyclovir suppressive therapy, which leads to a reduction in relative risk for recurrence, has been used since the early 1980s. Several trials of suppressive therapy with acyclovir derivatives daily found significant reductions in genital and plasma HIV loads. Adherence to daily dosing, however, is key to the success of the therapy. Using a polyurethane-based ring and the same technology developed to incorporate UC781 and TFV, we plan to vary drug loadings until release rates match those desired to achieve effective doses in vivo. The development of a simple gel would be used to test the active agents for safety and efficacy (especially against HSV-2) in small animal models. The development of a product that offers protection against HIV and other STIs, such as the highly prevalent genital herpes, would significantly benefit the population of women at risk of acquiring HIV/STIs but desiring conception.

Progress:

Contraceptive-Microbicide. Three IVR projects were initiated for co-delivery of UC781 and LNG. Progress on these projects (University of Utah using polyurethanes, Particle Sciences using ethylene-vinyl acetate copolymer (EVAc), and Oak Crest using their pod-based technology) has led to the demonstration of in vitro release of both compounds. The polyurethane- and EVAc-based polymer IVRs were found to release UC781 effectively in vivo in rabbits. A bioanalytical method to assess tissue and plasma concentrations of UC781 and LNG in plasma in rabbits was developed by MPI. By 2011, however, work at Particle Sciences and Oak Crest was discontinued because their technical approaches were unsuccessful in meeting target release rates.

Work continued on the polyurethane-based approach under development by the University of Utah and CONRAD. As stated above, UC781 has been replaced with TFV and, separately, considerable progress on the TFV IVR was achieved in support of the combination IVR for TFV and LNG. Polyurethane IVRs were developed which could control the release of relatively large amounts of TFV (10 mg/day). The LNG-releasing component of the combination IVR was developed and optimized.

Prototype IVRs were tested in rabbits (segments) and sheep for PK and safety. Adequate amounts of TFV were released from these IVRs over 90 days. Cervical concentrations of LNG were maintained at a constant level for 90 days in a dose-dependent manner.

In addition to assessing drug release and stability of the contraceptive-microbicide IVR, methods for its manufacture were optimized through the use of a unique welding system. The IVRs were able to withstand elongation in excess of what a human could perform.

By 2013, a commercial line of polyurethanes (Tecoflex® and Tecophilic®) became available for use in IVRs. Therefore, CONRAD discontinued development of the custom synthesized polyurethanes to save both time and money.

All IND-enabling studies were completed. A pre-IND meeting was held in July 2013 and an IND filed in April of 2014. The IND was accepted by the FDA and the clinical evaluation of the TFV/LNG IVR began in Q4 2014.

Dual-Activity Microbicide (anti-HIV and anti-HSV activity). Two programs were initiated for the co-delivery of TFV and ACV. One program (Oak Crest) has developed a pod-based system capable of co-releasing both compounds at a constant rate for one month. In a second program, Controlled Therapeutics designed specific polyurethanes with optimized properties for co-release of these two relatively water soluble compounds from a matrix-based IVR. Both projects were terminated at the request of USAID.

Development of an IVR releasing TFV and acyclovir was initiated, but put on hold due to USAID funding constraints and lower prioritization.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This activity led to the first combination IVR capable of releasing both a microbicide and a contraceptive agent to enter clinical trials. This was a major contribution to the field since it is believed that women will be more motivated to use a contraceptive product compared with a microbicide-only product. Thus, an area that has plagued clinical evaluation of microbicides (adherence) may be obviated.

Involvement of Other Donors and Interested Parties:

CONRAD co-funded some of the specific and related activities using its funding from the Gates Foundation to develop new, multi-target combination microbicides, of which ~$1M was used for activities related to this overall activity. NICHD/HIV Interagency Agreement funds (~$654K) have co-supported this activity and some of the preliminary work was funded using CONRAD-III USAID/Microbicide funds (~$500K). CONRAD was also awarded an USAID APS Agreement for clinical studies of the IVR (up to $5M).

CONRAD
Microbicide Account - Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#1 – Preclinical Characterization, Formulation, and Delivery
Activity Number and Title:	#1.5 – Manufacture of Clinical Supplies
CA Activity Numbers:	Numerous
Primary Country of Activity:	USA
Technical Coordinator:	David Friend
Implementers:	CONRAD, DPT Laboratories, Logenix, Foster, Aptalis Pharma (formerly Eurand), Dow Pharmaceuticals, Patheon Pharmaceuticals, Azopharma, Particle Sciences, Logenix, Bilcare
Period:	8/1/08 – 9/4/14

Objective:

The objective of this activity was to prepare clinical supplies for studies conducted by CONRAD and to provide clinical supplies to other organizations such as CAPRISA, MTN, and FACTS for Phase I, II and III trials. This activity also included good manufacturing practice (GMP) manufacture of other primary dosage forms including tenofovir (TFV) intravaginal rings, alternative dosage forms, and combination dosage forms.

Activity Description:

CONRAD has been responsible for the manufacture of GMP quality clinical supplies for several microbicide trials, including efficacy studies such as CAPRISA 004 and 008, VOICE, and FACTS-001. All current and planned clinical trial materials involving tenofovir (TFV) 1% gel were prepared at DPT and Patheon under the direction of CONRAD. CONRAD intends to continue to be responsible for the manufacture of TFV 1% gel for future studies. CONRAD was also responsible for the GMP manufacture of UC781 gel (0.1% and 0.25%). This work was conducted at Dow Pharmaceutical Sciences. This activity also includes required product development-related regulatory activities to support manufacture of TFV gel for registration. CONRAD also has manufactured other dosage forms for it. These include vaginal tablets and intravaginal rings (IVRs).

Progress:

The UC781 clinical formulation (0.1% and 0.25%) was prepared using micronized UC781 for stability, toxicology and future clinical evaluation. Clinical supplies for the Phase I PK/safety evaluation of UC781 gel were manufactured at Dow Pharmaceuticals. Azopharma manufactured two placebo gels under GMP for clinical evaluations (acceptability and spreading). Development and production of the combination gel containing UC781 and TFV was transferred from the University of Utah to DPT, which was to manufacture the Phase I clinical supplies. Due to unexpected potential safety issues, work on all UC781-containing products was discontinued in 2010.

The manufacture of polyurethane-based IVRs required several organizations. DSM Biomedical was identified as an acceptable manufacturer of the polyurethane thermoplastic (DSM). However, once polyurethanes became commercially available from Lubrizol, work at DSM was stopped.

Foster Corp was selected as the manufacturer for tube extrusion and coaxial extrusion contraceptive ring segments of the 90-d TFV IVR. DPT was selected to perform the final manufacturing steps required to prepare TFV IVR clinical supplies including the LNG ring segments. DPT was selected for manufacture of combination LNG/TFV IVRs for Phase I clinical testing. IVRs were manufactured under GMP at DPT in the summer of 2014 and the rings were released for clinical work in Q3 2014 (CONRAD 128).

DPT manufactured 2 additional lots of TFV 1% gel packaged in 100 g tubes. Including a previous lot, 3 different lots were put up on stability to support this packaging configuration, as required for regulatory purposes.

During 2011-2014**,** CONRAD manufactured several large batches of TFV 1% gel for the VOICE and FACTS 001 studies (co-funded with the Gates Foundation). In addition, CONRAD manufactured clinical supplies of TFV reduced glycerin (RG) 1% gel (for the MTN clinical studies, with funding primarily from NIH).

Finally, disintegrating vaginal tablets were manufactured for clinical evaluation at Aptalis Pharma.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This activity was critical to advance of the field of microbicide development by providing quality products for clinical evaluation of TFV 1% gel (FACTS-001), TFV RG 1% Gel (MTN-017), TFV and TFV/LNG IVRs (CONRAD-128) and TFV and TFV/FTC vaginal tablets (CONRAD-117).

Involvement of Other Donors and Interested Parties:

CONRAD co-funded some of the specific and related activities using its funding from the Gates Foundation to develop new, multi-target combination microbicides, of which ~$1.3M has been used for activities related to this overall activity. In addition, some of the manufacturing costs for MTN studies were co-funded by the University of Pittsburgh/Magee-Women's Hospital (~$2.1M).

CONRAD
Microbicide Account - Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#2 – Preclinical Evaluation of Toxicity, Pharmacokinetics, and Efficacy
Activity Number and Title:	#2.1 – Standard Preclinical Evaluation of CONRAD PPRD Microbicide Candidates
CA Activity Numbers:	Numerous
Primary Country of Activity:	USA
Technical Coordinator:	Gustavo Doncel, Meredith Clark, Tim McCormick
Implementers:	CONRAD/EVMS, BWH, Drexel, SRI, Rega Institute, ITM, University of Washington, University of Texas, AECOM, University of Pittsburgh, Dartmouth Medical Center, MPI, MatTek, Huntingdon, NAMSA, InPheno, Cirrus, Particle Sciences, Morseth Consulting, St. George's Medical Hospital, others, Dartmouth Medical Center, MPI, MatTek, Huntingdon, ABL, others
Period:	8/1/08 – 9/4/14

Objective:

Existing animal models are insufficient to adequately characterize the preclinical efficacy of a microbicide candidate. The objective of this activity was to conduct preclinical studies needed to evaluate compound toxicity, PK and efficacy using in vitro, ex vivo and animal systems. CONRAD's PPRD microbicides and screen other promising agents would be evaluated that, through licensing, may be incorporated in CONRAD's portfolio. IND-enabling tests would be performed on all clinical candidates. At the completion of the studies, the safety, efficacy and preclinical pharmacokinetics of new formulations and delivery systems, providing the basis for a rational selection of clinical candidates will have been characterized.

Activity Description:

Accurate preclinical efficacy is key to the successful development of effective microbicides. Currently, there is no widely accepted animal model for studying HIV transmission. Non-human primate models are considered to be the best model for the assessment of microbicides, but recent reports highlight the feasibility of using humanized mouse models for such evaluation. Primate models were previously developed or further developed in collaboration with Drs. Cheng-Meyer (ADARC) and Tsai (Univ. of Washington), and small animal models with Drs. Garcia (UTSWMC), Akkina (Univ. of Colorado) and other investigators. Using these models, pharmacokinetic and pharmacodynamic parameters were to be correlated with microbicide efficacy in vivo and ex vivo.

CONRAD would also support further development of an RT-SHIV (with and without HIV *env*) monkey model to assess UC781 and its combinations, both in semi-solid and solid dosage forms in collaboration with Dr. Tsai.

Given the discouraging results of recent microbicide clinical trials, some of which showed increased risk of

infection in subgroups of the test arm, the development of a primate model that evaluates potential enhancement of HIV transmission is imperative. CONRAD previously collaborated with Dr. Miller on such a model. Once established, it would be used for the evaluation of final formulations of UC781, TFV and their combinations.

CONRAD also planned to adapt the existing humanized mouse models to vaginal microbicide efficacy testing, establishing their most appropriate protocol conditions, validating the model with clinically tested microbicides, and, finally, using the model to assess safety and efficacy of UC781, TFV and other CONRAD proprietary compounds. The new generation of humanized BLT and Rag2 mouse models showed great promise to assess microbicide efficacy at early stages of development. Humanized mice engrafted with CD34 hematopoietic progenitor cells harbor HIV-1-susceptible human cells in the rectal and vaginal mucosa and are susceptible to HIV-1 infection when exposed to cell-free HIV-1 either via vagina or rectum, without any prior hormonal conditioning or mucosal abrasion.

Biological activity evaluation of drug substances and preliminary formulations (thin gels, at the beginning) for preclinical safety and efficacy would follow CONRAD's standard algorithm with some recent modifications. In vitro anti-HIV activity evaluation of multi-ingredient compounds would follow a standard combination matrix to determine the cooperative effects of individual components. After synergistic or additive activity has been confirmed, further anti-HIV and anti-STD pathogen evaluation would follow. Single entity and combination microbicide candidates would be tested for resistance, by assessing their capacity to induce and their efficacy to inhibit resistant virus. To characterize full-strength formulations regarding safety, efficacy and PK, animal testing would be first conducted in small animal models (see Animal Models workplan). Final formulations would then be tested in the low-dose, repeated challenge SHIV monkey model (Tsai). An RT-SHIV would be used as needed to evaluate the efficacy of a non-nucleoside reverse transcriptase inhibitor (NNRTI)-containing product (see Animal Models workplan). Preliminary and final formulations also would be evaluated in the refined and improved rabbit vaginal irritation model. Mucosal injury and inflammation markers will be assessed and later compared with mucosal safety endpoints gathered in Phase I clinical trials. Intravaginal rings would be tested in sheep and monkeys. Dual-protection methods would be assessed in vitro for their sperm function and HSV inhibiting properties and in vivo (rabbit, mouse and macaque models) for contraceptive and anti-STD efficacy. Pre-IND toxicity studies would be completed prior to the initiation of Phase I clinical trials. Standard FDA-required toxicology (general acute, subacute and chronic toxicity, reproductive segment I and II, and mutagenicity) would be performed as needed.

Due to funding constraints during the later stages of the activity, CONRAD focused the algorithm only on lead microbicide candidates. Again, due to funding constraints, non-human primate (NHP) testing was reduced to a minimum. Dual-protection methods are assessed in vitro for their sperm function and HSV-inhibiting properties and in vivo, when the appropriate model exists. Pre-IND toxicity studies were completed prior to the initiation of Phase I clinical trials. Standard FDA-required toxicology (general acute, subacute and chronic toxicity; reproductive segment I and II; and mutagenicity) was performed as needed.

Progress:

The safety and pharmacokinetic properties of single-entity and UC781/TFV combination gel formulations was characterized. Gels of different compositions and rheology were evaluated in cell-based, explant-based, and animal models. Furthermore, micronized and non-micronized UC781 (API and gels) were investigated for safety, PK and efficacy. As a result of this comprehensive preclinical evaluation, a gel containing 0.25% micronized UC781 was selected for clinical and long-term toxicology testing and for further development in combination with 1% TFV.

Four new compounds have shown outstanding antiviral properties, especially in combinations. Two are nucleoside derivatives owned by CONRAD, which are products of collaboration with the University of Rhode

Island, and the other two are an integrase inhibitor and an NNRTI. These compounds represented backups and alternatives for the lead candidates. Through collaboration with ImQuest, in which NIAID and IPM were also parties, CONRAD characterized and selected a pyrimidinedione-based combination for further development.

We also made progress in the development of dual-protection technologies. Intravaginal rings containing UC781 and LNG and containing TFV and ACV successfully released the active drugs. Good tissue absorption of TFV released by a ring was demonstrated in monkey models. Preclinical safety and PK studies continued. Bioanalytical methods were developed.

As a result of comprehensive preclinical evaluation, CONRAD decided to discontinue the development of UC781-based combinations in 2010. The focus then shifted to TFV. A TFV gel was reformulated with reduced glycerin and assessed for safety and PK. TFV tablets were assessed for PK in rabbits and for safety in macaques. TFV rings were evaluated for safety and PK in tissue constructs and rabbits. A decision was made to pursue a reservoir-type ring. TFV in combination with MIV-170, FTC or an integrase inhibitor was evaluated for antiviral and cytotoxicity/proinflammatory properties. TFV/FTC and TFV/MIV-170 entered product development stages in tablet and gel dosage forms, respectively.

By 2012, TFV gel was reformulated with reduced glycerin and assessed for safety and PK in rabbits and tissue constructs. TFV and TFV/FTC tablets were assessed for safety and PK in rabbits, tissue constructs and macaques. TFV and TFV/emtricitabine (FTC) tablets were assessed for safety in the guinea pig sensitization model. TFV rings were reformulated in a reservoir device design and were evaluated for safety and PK in rabbits and sheep; safety and PK assessment in macaques was also conducted in collaboration with the CDC. TFV/LNG rings, reformulated in a segmented dual-reservoir device design, were evaluated for PK and safety in rabbits and PK in sheep. Biocompatibility testing of TFV and TFV/LNG IVRs was also completed. Results from all of these studies were used to support the submissions of four new INDs (two for tablets and two for rings) and an amendment to the existing IND for TFV gel (reduced-glycerin formulation). Exploratory endpoints of safety in cell-based, tissue-based and animal models, however, have revealed signs of possible TFV-mediated effects on epithelial integrity and lymphocyte infiltration which need to be further investigated.

Also completed was the evaluation started during the preceding period of new TFV/MIV-170 combination gels in PK and safety studies in rabbits and tissue constructs as well as a sensitization study in guinea pigs. Genotoxicity studies and oral toxicity studies in rats and dogs were also performed on the MIV-170 drug substance to support the nonclinical development of the new microbicide candidate. Though these studies showed favorable results, development of this combination microbicide product was halted due to funding constraints.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This activity advanced several leading microbicide candidates preclinically and enabled their clinical development. Some of the outcomes of this activity also contributed to decisions made to halt development of some products (e.g., UC781/TFV gel). These contributions have helped shape the microbicide field's approach to evaluating safety and efficacy of vaginal microbicides preclinically, and have helped shape the field of microbicide products now leading the development pipeline.

Involvement of Other Donors and Interested Parties:

About $1M of Gates MDP grant funds was used to co-fund some of the above described activities. In addition, some of the early work was funded using CONRAD-III USAID/Microbicide funds (~$200K) and Gates CS Phase III Reprogramming (~$200K). Testing of the IVRs in macaques was primarily supported by CDC intramural funds.

CONRAD
Microbicide Account - Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#2 – Preclinical Evaluation of Toxicity, Pharmacokinetics, and Efficacy
Activity Number and Title:	#2.1 – Models and Biomarkers of Microbicide Safety to Evaluate CONRAD PPRD Compounds
CA Activity Numbers:	PPA-09-034
Primary Country of Activity:	USA
Technical Coordinator:	Gustavo Doncel
Implementers:	CONRAD/EVMS, Brigham & Women's Hospital, Albert Einstein College of Medicine
Period:	8/1/08 – 9/4/14

Objective:

Based on lessons learned from previous microbicide trials, no new candidate or formulation should be tested in large clinical trials without advanced safety, pharmacokinetic, and pharmacodynamic (efficacy) evaluations. Furthermore, with the predicted disappearance of placebo-controlled studies, new models and biomarkers are essential to properly evaluate these candidates.

This activity involved the development, validation and implementation of preclinical models needed to evaluate potential cervicovaginal toxicity of CONRAD's microbicides in development. Existing assays are clearly insufficient to adequately characterize the preclinical profile of a microbicide candidate. Therefore, new assays currently in development would be validated and used to further evaluate CONRAD's microbicides.

At the completion of the studies corresponding to this activity, it was intended that CONRAD will have characterized the safety of UC781- and TFV-based new formulations and delivery systems, providing the basis for a rational selection of clinical candidates. In the process, new models would be established to evaluate local microbicide safety, including possible interference with innate and adaptive immune responses, determined mechanisms of microbicide-induced inflammation and factors regulating this reaction (e.g., hormones, seminal plasma and microflora), and validated biomarkers of microbicide safety for preclinical selection and clinical assessment of new microbicide candidates.

Activity Description:

Based on the observed discrepancies between preclinical and clinical data for microbicides whose Phase III clinical trials have been completed or stopped (i.e., nonoxynol-9 [N-9], C31G [Savvy], cellulose sulfate [CS], BufferGel [BG], PRO2000), it is obvious that new, more predictive models and biomarkers are urgently needed to more properly evaluate the safety and efficacy of microbicide candidates. Safety issues, undetected by standard tests, may have been at the core of the enhancement of HIV transmission seen with those microbicides. Therefore, it is imperative to develop new models and markers that allow for a better selection of safe formulations of UC781, TFV and other CONRAD proprietary compounds, before they get into clinical trials. The work in this activity would be focused on three main specific aims: identification and validation of predictive models and biomarkers of microbicide safety, influence of vaginal environmental factors on

microbicide safety endpoints, and impact of microbicide candidates on genital innate and adaptive immunity. By 2013, this activity was re-focused to address two main specific aims: 1) validation and implementation of predictive models and biomarkers of microbicide safety, and 2) development of a pharmacodynamic ex vivo model of microbicide efficacy.

Progress:

The impact of microbicides and placebos on epithelial cells from the human reproductive tract was studied. A group of selected proinflammatory compounds (PIC: N-9, C31G and BZK) and non-inflammatory compounds (NIC: HEC and CMC) and one agent considered "unknown" regarding this property (CS) were evaluated for cytotoxicity on epithelial cells from different parts of the reproductive tract. The ability of PIC and NIC to induce inflammation-related cytokines in these cells was further studied.

In order to broaden the pool of potential biomarkers for genital mucosal injury or dysfunction, human vaginal cells (VK-2) were incubated with subcytotoxic doses of PIC and NIC for analysis of gene expression by DNA microarray (genomics). Certain gene products appear to be consistently better than others in differentiating PICs from NICs. For instance, the prostaglandin endoperoxide synthase 2 (PTGS2), a potent enzyme linked to inflammatory processes, was significantly upregulated in PIC (≥10-fold in comparison with baseline values), but unchanged or down-regulated with NIC and CS. Up-regulation of selected genes and protein expression were confirmed by RT-PCR, immunofluorescence, Western blot and tissue immunohistochemistry.

The immune system of the genital tract displays unique characteristics, and it is essential for the maintenance of a healthy vaginal ecology. Topical microbicides should not interfere with these immune responses. During this period, we studied the impact of selected PIC, NIC and polyanions. Unlike NIC, the polyanionic compounds, dextran sulfate and polystyrene sulfonate, significantly inhibited mRNA and protein levels of chemokines (IL-8) and anti-viral cytokines (e.g., IFN-β), following epithelial stimulation with microbial Toll-like receptor (TLR) ligands.

Following a suggestion from CONRAD's advisory committee (SAC), the number of non-cytotoxic proinflammatory compounds (PIC) used as positive controls were increased for identification of discriminatory biomarkers. CONRAD's lead microbicide candidates, UC781, tenofovir and KP-17, were also tested. Using a genomic approach, a group of 12 vaginal epithelial gene products were identified as consistently better than others in differentiating PIC from non-inflammatory compounds (NICs). In addition to IL-8 and CCL20, the prostaglandin endoperoxide synthase 2 (PTGS2), a potent enzyme linked to inflammatory processes, and IL-13RA2, a subunit of the receptor for IL-13, a cytokine involved in allergic inflammation, were significantly upregulated in PIC, but unchanged or down-regulated with NICs and CS. Other genes followed a similar pattern. A custom-made "heat map" with all 12 genes allowed a clear identification of PIC.

Using a human vaginal cell system and proinflammatory, noninflammatory and microbicide compounds, a set of discriminating biomarkers was identified which, when up-regulated, indicate mucosal alteration leading to increased risk of HIV infection. In particular, the impact of selected PICs and microbicide compounds on human cervical/vaginal cells colonized with *Prevotella bivia* (Pb) and *Lactobacillus acidophilus* was investigated, as microorganisms representing bacterial vaginosis-associated and normal microflora. Genital tract cells recognized pathogenic bacteria and mounted an immunoinflammatory response characterized by activated nuclear factor kappa β (NFκβ) and secretion of IL-8. Three critical signal transduction pathways involved in epithelium-triggered mucosal inflammation were also activated by all PIC tested. These were NFκβ, mitogen-activated protein kinase (MAPK) and EGF receptor kinase (ERK). A set a biomarkers of mucosal safety has also been identified for human explanted cervical tissue and rabbit mucosal tissue from the RVI model.

Since vaginal formulations are better tested in tissues than in cell monolayers, a group of biomarkers was

identified in tissue constructs and explants. Explants yielded a consistent set of new biomarkers. In order to evaluate these biomarkers in rabbit cervicovaginal tissues, a quantitative nuclease protection assay was developed which enables us to assess gene expression in formalin-fixed, paraffin-embedded rabbit vaginal mucosa, and a quantitative PCR-based assay. A set of new biomarkers is capable of differentiating inflamed from non-inflamed mucosa in an objective manner. These markers were validated using RVI tissues from TFV gel and UC781/TFV gel RVI studies. In the final stage of biomarker identification and validation, we began enrolling participants for a small, exploratory, cross-over study from which samples were to be collected from women using Conceptrol (nonoxynol-9), Aldara (imiquimod cream), and Universal placebo and analyzed for biomarker expression.

In summary, we have pioneered the use of vaginal biopsies as a pharmacodynamic model or surrogate of microbicide efficacy using ex vivo HIV challenge of in vivo microbicide-treated mucosal tissue. Although far from being standardized, this model represents the future of microbicide clinical testing. We have continued trying to improved methodological conditions.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This activity has advanced the design and use of several preclinical and clinical (ex vivo) models for testing the safety, PK and efficacy of topical microbicides. Some of the outcomes of this activity also contributed to decisions made to halt development of some products (e.g., UC781/TFV gel). These contributions have helped shape the microbicide field's approach to evaluating safety and efficacy of vaginal microbicides in preclinical and clinical trials, and have helped shape the field of microbicide products now leading the development pipeline.

Involvement of Other Donors and Interested Parties:

Gates Foundation funding (Gates MDP) co-supported biomarker testing (~$500K) and NIAID co-funded this overall activity through an Interagency Agreement (IAA), beginning in FY11 (total award of $2M).

CONRAD
Microbicide Account - Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#2 – Preclinical Evaluation of Toxicity, Pharmacokinetics, and Efficacy
Activity Number and Title:	#2.3 – Animal Models of Microbicide Efficacy to Assess CONRAD PPRD Compounds
CA Activity Numbers:	MSA-06-442
Primary Country of Activity:	USA
Technical Coordinator:	Gustavo Doncel
Implementers:	CONRAD/EVMS, University of Washington, St. George's Medical Hospital, CDC, Univ. of Texas, Univ. of Washington, ADARC, Univ. of Colorado, University of Texas
Period:	8/1/08 – 9/4/14

Objective:

Existing animal models are insufficient to adequately characterize the preclinical efficacy of a microbicide candidate. Therefore, the objective of this activity is to develop and implement small-animal and macaque models needed to evaluate the microbicide efficacy of CONRAD-PPRD compounds. The expected outcome of this activity is the animal model efficacy characterization of UC781/TFV lead formulations and delivery systems and the establishment of new animal models of microbicide efficacy.

Activity Description:

Accurate preclinical efficacy is key to the successful development of effective microbicides. Currently, there is no widely accepted animal model for studying HIV transmission. Non-human primate models are considered to be the best model for the assessment of microbicides, but recent reports highlight the feasibility of using humanized mouse models for such evaluation. Primate models were to be developed in collaboration with Drs. Cheng-Meyer (ADARC) and Tsai (Univ. of Washington), and small animal models with Drs. Garcia (UTSWMC), Akkina (Univ. of Colorado), Potash (Columbia University) and other investigators. Using these models, pharmacokinetic and pharmacodynamic parameters would be correlated with microbicide efficacy in vivo and ex vivo.

Primate model development was to include an RT-SHIV (with and without mutations) monkey model to assess UC781 and its combinations, both in semi-solid and solid dosage forms in collaboration with Dr. Tsai and on development of a primate model that evaluates potential enhancement of HIV transmission in collaboration with Dr. Miller (UC-Davis). The RT-SHIV monkey model would also be used to assess non-nucleoside reverse transcriptase inhibitors (NNRTIs) and their combinations with TFV.

Given the discouraging results of recent microbicide clinical trials, some of which showed increased risk of infection in subgroups of the test arm, the development of a primate model that evaluates potential enhancement of HIV transmission is imperative. In collaboration with Dr. Miller, CONRAD is currently working on such a model. Once established, we plan to use it for the evaluation of final formulations of UC781, TFV and their combinations.

CONRAD also planned to adapt the existing humanized mouse models to vaginal microbicide efficacy testing, establishing their most appropriate protocol conditions, validating the model with clinically tested microbicides, and, finally, using the model to assess safety and efficacy of, initially, UC781, then with TFV and other CONRAD proprietary compounds. The new generation of humanized BLT and Rag2 mouse models showed great promise to assess microbicide efficacy at early stages of development. Humanized mice engrafted with CD34 hematopoietic progenitor cells harbor HIV-1-susceptible human cells in the rectal and vaginal mucosa and are susceptible to HIV-1 infection when exposed to cell-free HIV-1 either via vagina or rectum, without any prior hormonal conditioning or mucosal abrasion.

Due to funding constraints by 2012, animal model development was halted and testing efforts concentrated on testing lead candidates and formulations in existing animal models.

Progress:

In previous collaboration with Dr. Cheng-Mayer, a low-dose, repeated X4/R5 SHIV challenge model was established and compared the predictive capacity of this model with that of the single high-dose Depo-treated model using 6% cellulose sulfate (CS) gel. The recently completed single-dose study yielded 1 of 12 versus 7 of 12 animals free of systemic infection (protected) in the placebo and CS groups, respectively. PRO2000 gel (0.5%) protected 4 of 6 animals. CS prevented systemic viremia in all animals in the low-dose model and in 60% of the animals in the high-dose model. Given the differences in the models, the results were consistent and demonstrated preclinical efficacy of both CS and PRO2000 gels. Results from the low-dose SHIV model were compared with the high-dose model to determine which one predicts clinical effectiveness better.

In previous, pilot dose-finding studies of the RT-SHIV model (Tsai), intravaginal administration of 4800 $TCID_{50}$ of RT-SHIV after a single intravaginal challenge without Depo-Provera treatment productively infected 5/5 animals and a single dose of 1200 or 300 $TCID_{50}$ infected 4 of 5 animals. Studies were then undertaken to fine-tune the intravaginal infectious dose ($IvagID_{50}$) of RT-SHIV in 4 groups of monkeys (2400, 1200, 300 and 75 TCID50) before performing pharmacokinetic and efficacy studies with UC781 and TFV gels. Microbicide efficacy testing of micronized and non-micronized UC781 and TFV gels showed partial protection (not statistically different from placebo) in spite of tissue drug concentrations in excess of those required to inhibit the virus in vitro.

A repeat low-dose viral inoculum challenge to rule out a possible effect related to the high virus inoculum was conducted in which seventeen female macaques were randomly distributed in three groups (tenofovir gel, placebo gel, and virus control), and challenged intravaginally with 50 $TCID_{50}$ RT-SHIV after 30 min of gel/saline administration. Four of six macaques (67%) pretreated with tenofovir 1% gel were protected from repeated low dose challenges with RT-SHIV, while 100% of the animals in both the placebo and virus control groups were infected. This contrasts with the results obtained after a single high-dose challenge (2,400 $TCID_{50}$) in which no protection was observed after a single or double dose of TFV gel, when compared to placebo gel.

In collaboration with Dr. Miller, three groups of animals (rhesus macaques) were used to test a model of viral infection enhancement based on 14 daily applications of the compound (in this case, CS and placebo) prior to a challenge with a suboptimal infectious dose ($\sim ID_{20}$) of SIV. No evidence of CS enhancement of mucosal SIV infection was seen under these testing conditions.

We also collaborated with Dr. Garcia in the design of a protocol for testing enhancement of HIV infection using a humanized BLT mouse model.

Two new mouse models were developed to evaluate safety and efficacy of microbicide lead formulations. In Dr. Akkina's model, intravaginal infectivity of an R5-HIV-1 stock was titrated in humanized RAG2 mice,

primarily using funding previously provided by the Gates Foundation. Preliminary data show that while 100% of the animals were infected in the placebo group, only 66% (partial protection) were infected in the TFV-treated group. UC781 gel protected 100% of the animals and a combination of TFV/UC781 protected 84% of them.

In Dr. Potash's mouse model, vaginal transmission of a chimeric EcoHIV has been established after mating of immunocompetent mice. In a preliminary study, TFV protected female mice from becoming infected. No further work has been funded for the Potash model using Gates or USAID/PPRD support.

Transgenic rabbits for human CD4 and CCR5 were successfully generated at St. George's Hospital in London (Shattock). CONRAD/EVMS received tissues to assess human CD4- and CCR5-expressing cells. In an exciting discovery, both types of human cells colonizing rabbit vaginal tissues. The next step was to cross breed animals to generate double-transgenic rabbits, as these lab animals would be useful for assessing safety and efficacy in a model without the limitations of macaques or humanized mice. However, due to funding constrains, this model was not further supported.

Using partial support from CDC intramural funds at CDC, macaque safety and PK studies were performed for TFV and TFV/emtricitabine (FTC) tablets and TFV intravaginal rings (IVRs). No safety issues were found in either study, and the PK of TFV was found to be promising in comparison to the PK of TFV gel in this model. Based on CDC's previous studies with TFV gel and other TFV-based products in this animal model, the vaginal concentrations of TFV from both of these new TFV dosage forms are expected to be effective at preventing SHIV infection in their repeated low-dose model.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This activity advanced several leading microbicide candidates preclinically and enabled their clinical development. This activity also advanced the design of several preclinical models used for testing the safety, PK and efficacy of topical microbicides. These contributions have helped shape the microbicide field's approach to evaluating safety and efficacy of vaginal microbicides preclinically, and have helped shape the field of microbicide products now leading the development pipeline.

Involvement of Other Donors and Interested Parties:

Testing of the TFV and TFV/LNG IVRs in a macaque model was primarily supported by CDC intramural funds. In addition, Gates Foundation funding (Gates MDP) supported related animal development (~$750K) and. CONRAD-III USAID/Micro funds also co-supported some of the non-human primate activities (~$900K).

CONRAD
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USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#2 – Preclinical Evaluation of Toxicity, Pharmacokinetics and Efficacy
Activity Number and Title:	#2.4 – Characterization of Cervicovaginal Mucosal Markers and Early HIV Infection in the Tenofovir Gel Trial CAPRISA 004
CA Activity Numbers:	PPC-09-046
Primary Country of Activity:	USA
Technical Coordinator:	Gustavo Doncel
Implementers:	CAPRISA (University of KwaZulu-Natal, South Africa), CONRAD/EVMS
Period:	7/1/09 – 06/30/12

Objective:

CAPRISA 004, a Phase IIb trial, showed the safety and effectiveness of the vaginal microbicide tenofovir (TFV) 1% gel to prevent HIV infection in women. An ancillary project, dubbed TRAPS (Tenofovir gel Research for AIDS Prevention Science) by the PI of CAPRISA 004, Dr. Salim Karim, was designed to address questions regarding the impact of tenofovir 1% gel on the cervicovaginal mucosal immune system and its correlation with the characteristics of the infecting virus. CONRAD would collaborate with Dr. Karim in the conduct and coordination of the proposed studies. Given CONRAD's interest in developing TFV as a topical microbicide, alone and in combination, the information obtained in this project is uniquely relevant and will be directly applied to product development decisions.

Activity Description:

In collaboration with Dr. Salim Karim, blood and cervicovaginal lavages from the participants of CAPRISA 004 would be studied. Using multiplex technology, immune and innate mediators in samples collected at baseline (before product use) and before and after seroconversion would be measured. A group of matching participants who have not seroconverted and a group of seroconverters from the same population who are not participating in the study would be used as comparisons. Comparisons between TFV and placebo would be performed after the data are unblinded. Preliminary human as well as animal data suggest a possible association between increased levels of IL-6 and decreased levels of IL-8 and increased risk/susceptibility to infection. We would attempt to establish patterns of mucosal and blood immune mediators conferring susceptibility to HIV infection in the presence and absence of TFV.

A second aim of this study would be focused on the characterization of the genotype and phenotype of the infecting virus in CAPRISA seroconverters and a matching group of seropositive women from the same community, and their relation with immune mediator profiles in blood and CVLs. Preliminary work has shown an association between inflammation, assessed by increased proinflammatory markers, and increased probability of infection with more than one HIV variant.

The possibility of evaluating samples from the CAPRISA TFV gel Phase IIb study represented a unique opportunity to validate biomarkers of mucosal alteration and correlation them with HIV infection.

Progress:

The TRAPS cohort was followed up through the acute and chronic infection stages (Phases II to IV) and to the stage at which they are put on HIV treatment. Of the 98 participants enrolled, 94 remained in active follow up: 67 had not yet received treatment, while 27 receiving HAART therapy. Regarding viral diversity, recent results suggest that the viruses from early infection differ between the TFV and placebo arms. However, further analysis was required to identify the mechanisms responsible for these changes. Elevated CVL concentrations of IL-1, IL-6, IL-8, IP-10, MCP-1, MIP-1, GM-CSF and IL-10 were associated with increased risk of HIV-1 infection, irrespective of gel use. However, there were no significant differences found in any of the 12 inflammatory cytokines measured in CVLs in women using tenofovir gel compared to placebo gel. In contrast, significantly higher concentrations of inflammatory cytokines were found in pre-infection CVLs from woman who eventually acquired HIV versus women who remained uninfected. Data were further analyzed and stratified according to women with elevated inflammatory cytokines in CVLs (defined as at least 3 cytokines which were above the median of the group) compared to those with normal cytokine levels. Women with elevated inflammation in the genital tract had a higher relative risk of becoming infected with HIV compared to women with no inflammation, after adjusting for TFV use. These findings suggest that elevated levels of genital inflammation that are sustained over time may facilitate HIV-1 infection, even in women using 1% TFV gel.

The investigators continued analyzing the cohort of infected women to better determine the impact of TFV on disease progression (i.e., symptomatology, CD4 counts, viral load, etc.) and investigating the association of increased proinflammatory cytokines and concurrent genital infections. Additional analysis of pharmacokinetic (PK) data in relation to HIV acquisition was planned to try to establish a predictive surrogate for increased susceptibility to HIV infection

Due to funding constraints, CONRAD could not fund this activity beyond this period.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

The TRAPS (Tenofovir gel Research for AIDS Prevention Science) program was a series of basic science and clinical studies designed to contribute to understanding the outcomes of the CAPRISA 004 tenofovir gel trial, the only microbicide trial to demonstrate effectiveness against heterosexual HIV transmission. More broadly, TRAPS would contribute to the advancement of the HIV prevention field by enhancing the understanding of biological mechanisms at play in modulation of immunological processes that are largely responsible for HIV susceptibility/protection and clinical disease progression post ARV-based pre-exposure prophylaxis.

Involvement of Other Donors and Interested Parties:

The total activity costs were being co-funded by CAPRISA (~$1M).

CONRAD

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USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.1 – Studies of Gels – Pharmacokinetics, Pharmacodynamics, and Markers of Mucosal Safety
CA Activity Numbers:	Numerous
Primary Country of Activity:	USA
Technical Coordinators:	Jill Schwartz, Kim Linton
Implementers:	Gustavo Doncel, David Archer (EVMS); Profamilia (Dominican Republic); University of Pittsburgh; University of Pennsylvania, Advances in Health; other laboratories (Dezzutti, Herold, Kashuba, and Asin)
Period:	9/1/08 – 9/4/14

Objective:

The objective of this activity was to carry out clinical studies aimed at characterizing pharmacokinetics (PK), pharmacodynamics (PD) and markers of inflammation of UC781 and tenofovir (TFV) gels. The TFV studies would utilize the BAT 24 regimen versus daily and pericoital dosing. Long-terms goals will include bridging studies of the new and old UC781 formulations including tissue levels of drug, a 6-month expanded safety study, and safety and PK studies of the combination of UC781 and TFV and potentially other combinations with proprietary products such as fatty acid nucleoside analogs.

Activity Description:

CONRAD obtained rights to UC781 in 2006 for use in developing countries. Since that time, CONRAD completed a male tolerance study and planned to undertake additional clinical studies to develop UC781 as a microbicide and for use in a combination product with tenofovir (TFV) gel. CONRAD planned to initiate a single dose, 14-day PK/PD and expanded safety study with UC781 to assess local and systemic UC781 levels, the effect of UC781 on HIV-1 infection in an explant challenge, and local and systemic safety. In addition, CONRAD would continue to provide UC781 clinical supplies to several clinical research groups at CDC, UCLA and University of Pittsburgh, all of whom are collaborating with CONRAD.

Efforts were also planned to develop a combination IVR capable of controlled release of UC781 and TFV over 90 days.

The proof-of-concept for a microbicide, tenofovir (TFV) 1% gel, to prevent HIV infection was demonstrated by the CAPRISA 004 results and led to shifting priorities for CONRAD and the microbicide field. Registering TFV gel and improving TFV performance to increase protection became a higher priority. Several studies planned or underway would help provide a pharmacokinetic basis to explain the clinical efficacy of the pericoital dosing regimen used in CAPRISA 004 in which TFV gel used before and after sex resulted in reduced HIV infection by 39% compared to the daily dosing regimen of TFV gel used in MTN-003/VOICE that did not reduce HIV infection rates (HIV infection was 6.0% in the women assigned to daily tenofovir gel compared to 6.1 % in women in the daily placebo gel group).

It is unclear whether the VOICE study's unexpected outcome was due to the daily dosing regimen as opposed to the pericoital regimen, inadequate or non-use of the study gel, insufficient drug concentrations in women at the time of HIV exposure, or some other unknown reason. Analysis of plasma samples from a case-cohort subset in the VOICE study showed that TFV was detected in the plasma of only 22% of women using TFV gel, indicating a low level of adherence to the gel dosing regimen.

Progress:

UC781 was reformulated and other preclinical work completed. The clinical team developed the study design for the PK/PD protocol with input from preclinical and product development. A clinical pharmacology laboratory at the UNC-Chapel Hill developed an assay for UC781 drug levels in tissue. KAR Bioanalytical Inc. developed an assay for drug levels in genital tissue and fluids, and investigators at the University of Dartmouth, in conjunction with EVMS, developed an ex vivo explant HIV infectivity assay.

New UC781 API (active pharmaceutical ingredient) was manufactured and bridging toxicology studies along with preclinical work completed. A pilot study was performed at Eastern Virginia Medical School using the original API in order to prepare for the planned PK/PD/safety study when the new product was available. A total of 15 volunteers underwent three genital tract biopsies at baseline. The volunteers returned for Visit 2 to undergo intravaginal application of 0.1% UC781 (3.5 mL), followed 4 hours later by three genital tract biopsies. All baseline biopsies 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). Concentrations of UC781 showed tissue levels orders of magnitude higher than its in vitro effective concentration, following a single dose of UC781 gel and were higher with exposure to 0.25% compared to 0.1% UC781.

Toxicokinetic studies indicated that combination gel formulations (UC781 and TFV) were more irritating than either single entity gel in animal models. Coupled with the availability of new compounds and other issues concerning UC781, development of the combination UC781/TFV gel was terminated in 2010. As such, plans to initiate a single dose and 14-day PK/PD and safety study with UC781 and to provide UC781 clinical supplies to several clinical research groups were terminated.

Development of a combination IVR capable of controlled release of UC781 and TFV over 90 days encountered difficulties related to UC781 surface crystallization, drug stability, low tissue delivery, and metabolite formation; thus, further development was also discontinued in 2010.

Prior to conducting the planned PK/PD safety study of TFV, a pilot study was undertaken to establish collection, transport and assay methodology. Between December 2010 and March 2011, ten women underwent three genital biopsies at baseline, and again 4 hours post-treatment with 4 ml intravaginal TFV gel, during the same phase of sequential menstrual cycles. These fresh genital tissue samples were used to develop the infectivity protocol for the HIV explant challenge assay. An investigator at the University of North Carolina has developed assays for the measurement of TFV drug concentrations in genital tissue and fluids.

The PK/PD safety study (Pharmacokinetic and Pharmacodynamic Study of Tenofovir 1% Gel Using the BAT24 Regimen Versus Daily and Pericoital Dosing, Protocol #A10-113) was conducted to assess the impact of different vaginal dosing regimens (BAT24 versus daily and pericoital dosing regimens) on the PK and PD of TFV 1% gel and the impact of these dosing regimens on immune cell activation. TFV concentrations were assessed in blood plasma, aspirate, tissue, and cell pellet from cervicovaginal lavage (CVL); TFV-diphosphate (TFV-DP) in peripheral blood mononuclear cells (PBMCs), endocervical cells, tissue, and cell pellets from CVL; the effect of TFV on HIV-1 infection in an explant challenge model; anti-HIV and anti-HSV activity in

CVL; and immune cell activation in tissue after a single study-related sex act and after 2 weeks of twice weekly study-related sex.

TFV gel dosing regimens, assigned by randomization, were:

BAT24:	1 hour before and 1 hour after sex
Precoital:	1 hour before sex
Postcoital:	1 hour after sex
Daily:	Each day or evening at about the same time with the last study-related sex 12 hours after the final dose

Overall, 194 women were screened/enrolled in order to randomize 94 participants, meeting the target of 90 women completing the study.

After this reporting period, the funding for this activity continues under Cooperative Agreement MAPS1-14-002. These data were presented at R4P in Cape Town, SA on October 29th, 2014.

There does not appear to be a meaningful difference in drug exposure between single or multiple actor or dosing regimens. There is a slightly greater tissue exposure to TFV and TFVDP in the BAT24 arm. TFV-DP remains high at 72h after gel administration. All regimens provide concentrations that correlate with protection. The median TFV concentration in aspirates was $>10^3$ng/ml and the median TFV-DP concentration in tissue was $>10^3$ fmol/mg. HIV inhibition was noted in CVL after exposure to all dosing regimens up to 72 hours. There was a significant negative correlation between TFV-DP concentration in tissue and maximum/cumulative HIV-1 p24 with BAT24 multiple dosing.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

These clinical studies advanced the field in characterizing pharmacokinetics (PK), pharmacodynamics (PD) and safety of microbicide candidates with first in kind evaluations. The ex vivo HIV-1 infection assay in cervicovaginal explant tissues has been successfully implemented as a surrogate marker of mucosal susceptibility to infection in recent and on-going CONRAD studies.

The results of CONRAD Protocol 113 demonstrated that BAT24 and pericoital regimens provide similar PK concentrations consistent with protection. All regimens provided evidence of HIV inhibition in fluid, with BAT24 providing a statistically significant PK/PD (ex vivo explant challenge) concentration.

Development of UC781 was terminated in 2010. As such, plans to initiate a single dose and 14-day PK/PD and safety study with UC781 and to provide UC781 clinical supplies to several clinical research groups were terminated.

Involvement of Other Donors and Interested Parties:

FHI 360 provided data management and statistical support for the TFV PK/PD study through their PTA agreement with USAID. Completion of this overall work will be funded through APS Objective 1 (AID-OAA-A-14-00011), "Completion of Clinical Studies Required for TFV Gel Licensure."

CONRAD

Microbicide Account - Final Summary Report

USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.2 – Exploratory Studies: Site Identification/HIV Incidence in India
CA Activity Number:	PPA-08-001
Primary Country of Activity:	India
Technical Coordinators:	Marianne Callahan, Kim Linton
Implementers:	CONRAD; Indian Council of Medical Research (ICMR); National AIDS Research Institute (NARI)
Period:	9/1/08 –9/4/14

Objectives:

The primary objective of this study was to identify and prepare sites for conducting clinical trials of HIV-prevention technologies in India, including vaginal microbicides. Secondary objectives included:

- Estimating the current incidence of HIV infection among high-risk women, primarily sex workers, in selected sites in high HIV prevalence states in India by use of a sequential algorithm with BED capture enzyme immunoassay (BED-CEIA) as the screening tool and the Avidity Index as the confirmatory test;
- Estimating the prevalence of sexually transmitted infections (STIs);
- Assessing the needs of the population for female-controlled options for the prevention of HIV and other STIs;
- Assessing the willingness of study participants to participate in future HIV prevention trials; and
- Building capacity in sites in order for them to participate in expanded safety studies of products prior to effectiveness trials.

Activity Description:

The primary activity of the planned cross-sectional study at multiple sites in India was to estimate HIV incidence among high-risk women, primarily sex workers, using BED-CEIA and Avidity Index methodologies on HIV-positive samples and multistage pooling of HIV-1 negative sera using polymerase chain reaction (PCR). About 3-4 months before study initiation, health visitors and study counselors, supervised by the study coordinators at the various clinics, were involved in community awareness sessions during which women from the community were encouraged to come to the clinics for screening. If they met the criteria as specified by the protocol, were willing and able to provide written informed consent, they would be enrolled in the study. Demographic data was recorded and blood samples were collected for HIV and RPR (TPHA for confirmation) testing. Treatment for STIs and RTIs were provided based on self-reported symptoms. Blood samples testing positive for HIV were sent to National AIDS Research Institute (NARI) Serology Laboratory for BED-CEIA and Avidity Index incidence estimation along with all negative samples which were eventually pooled and tested for HIV by PCR. Data management and analysis was also carried out by NARI.

Progress:

Eligible women were enrolled in six districts with historical evidence of concentrated HIV epidemic from three high HIV prevalence states of India, namely Maharashtra, Andhra Pradesh and Karnataka. The six sites were: the Karnataka Health Promotion Trust (KHPT) sites in Karnataka and Bangalore, National Institute for Research in Reproductive Health (NIRRH) in Mumbai, National AIDS Research Institute (NARI) in Pune, and two National Institute of Nutrition (NIN) sites - Khammam and East Godavari - in Andhra Pradesh. Demographic data were collected and blood samples collected for HIV and rapid plasma reagin (RPR) (*Treponema pallidum* hemagglutination assay [TPHA] for confirmation) testing. Treatment for STIs and reproductive tract infections (RTIs) were provided based on self-reported symptoms.

The current algorithm and proposed study sample size was raised in 2010. Subsequently, the suggestion of adding CD4 counts and viral loads to the current algorithm was raised. Although this addition to the algorithm was feasible, it was decided to convene a small, targeted consultation of experts to review the protocol and make recommendations on this issue. Following recommendations from the expert consultation, testing of the blood samples for BED-CEIA and the CDC Avidity assay was adjusted to improve the accuracy of tests for recent infections (TRIs) based on biomarkers in cross-sectional samples. Multiple tests were used in the testing algorithm to obtain more accurate information. The NARI laboratories helped identify the assay window for the new avidity assay to be used in the study and the confirmation of the local window for the BED assay using archival samples. Individual false recency rates and false positive rates (FRR/FPR) for individual and combined assays using the archival samples were determined, then a revised power calculation was carried out to determine if the sample size needed to be adjusted above the initial study sample size of 1,000 women/site or 6,000 total. The final sample size was 1,500 women/site or 9,000 total. Finally, low CD4 counts were added to the algorithm to help in the elimination of immune-compromised HIV-infected subjects and to provide better interpretation of results of the BED assay and the Avidity Index.

The full study report was distributed to USAID, CDC and FHI 360 in Q1 2014. CDC and FHI 360 were very helpful in providing guidance on the method algorithm and reviewing and commenting on the draft report prior to finalization. A summary of the results and limitations of the study included:

- The HIV prevalence in this enrolled population of 9138 FSWs was 9.26%
- A syphilis prevalence of 1% was seen with no correlation with cases of recent HIV infection. One third of RPR reactive samples were TPHA positive.
- HIV Incidence was calculated using serological methods & pooled PCR.
- Serological methods used were: BED-Capture Enzyme Immuno-assay (CEIA) and two avidity assays: modified GS HIV ½ (Biorad) [avidity assay -1] and Lag HIV-1 (Sedia)[avidity assay-2]. In addition, molecular estimation was carried out on sero-negative samples using pooled PCR. Furthermore, HIV Incidence was calculated using Recent Infection Testing Algorithm (RITA-1) which was developed by including BED-CEIA, an avidity assay, and CD4 cell count in series.
- The HIV incidence in this population by serological assay ranged from 0.144 by avidity assay-1 (AI 20%) to 2.4 by BED assay.
- HIV incidence calculated by Pooled PCR was 9.27% which was more than 10 times the Incidence calculated by serological methods.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

India has the second largest burden of HIV in the world with an estimated 2.1 million infections. Of these, 39.3% are estimated to be women. This study presented a detailed picture of over 9,000 women from six districts with historical evidence of concentrated HIV epidemic from three high HIV prevalence states of India. While HIV prevalence was shown to be high at 9.26%, the incidence by serological assays ranged from 0.144

to 2.4% and it is the incidence rate that is important when one considers sites for future HIV prevention trials. Although this study showed that the incidence estimates varied with the test applied, two avidity based assays gave comparable results. As there is no "gold standard" test available, running multiple tests seems to be the best algorithm. This is the first study that has provided a detailed look at the incidence of HIV in India. Lag avidity test, considered to be the best amomg the available assays, is faced with the limitation that there is the lack of data on HIV-1 subtype C (Indian) specific mean recency period and the False Recent Rate (FRR) for the Indian population, and hence for employing this assay some assumptions based on global evidence was required. However, additional data from this study will be helpful with these issues.

As none of the serological tests used for the study showed an incidence of more than 3%, it may be concluded that Phase III HIV prevention trials, as currently designed, may not be feasible in this population as they may not yield adequate end-points in the study arms.

Involvement of Other Donors and Interested Parties:

ICMR cost-shared the study by providing the sites' personnel costs, estimated to be U.S. $40,000, assuming responsibility for the ICMR headquarters budget for the study (approximately US $22,000), covering the increase in the NARI laboratory budget due to the sample size increase from 6,000 to 9,000 (estimated to be US $131,000) and other expenses for a total ICMR contribution estimated to be US $653,000.

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Microbicide Account - Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.3 – Exploratory Studies: Influence of Steroid Contraception on Pharmacokinetics/Pharmacodynamics and Vaginal Safety in TFV Gel Users (*was* "Influence of Steroids on Vaginal Mucosal Inflammation")
CA Activity Numbers:	Numerous
Primary Country of Activity:	USA
Technical Coordinators:	Christine Mauck, Kim Linton, Gustavo Doncel
Implementers:	EVMS; Vivian Brache (Profamilia); Beatrice Chen (University of Pittsburgh); Charles Morrison (FHI 360); Raina Fichorova (Brigham and Women's Hospital); Magee-Womens Hospital; other testing labs (Herold, Kashuba, Dezzutti, and Asin)
Period:	9/01/08 – 9/4/14

Objective:

Studies in this category are designed to produce information that will facilitate the assessment of microbicide candidate safety and efficacy. They are exploratory in nature, but their ultimate goal is to assist in the development of CONRAD proprietary compounds.

Alterations in vaginal immunity may affect risk of HIV acquisition by changing innate immunity, causing inflammation, disrupting epithelial integrity, and altering vaginal microbiota. How contraception affects each of these is poorly understood. However, effective contraception is required or strongly encouraged for Phase IIb/III microbicide trials in which women are at risk of HIV acquisition. Furthermore, contraception is currently provided by USAID to women at high risk for acquiring HIV.

Publication in October 2011 of data from the Partners in Prevention trial suggested that the use of DMPA and possibly oral contraceptives may increase both female susceptibility and female-to-male transmission of HIV. A WHO panel (including Drs. Doncel and Mauck from CONRAD) reviewed the epidemiologic, animal, and human data, and concluded that WHO should continue to recommend no restrictions (MEC Category 1) on the use of any hormonal contraceptive method for women living with HIV or at high risk of HIV. However, the group recommended that a new clarification (under Category 1) be added stating that women using progestogen-only injectable contraception should be strongly advised to also always use condoms, male or female, and other HIV preventive measures. It further stated that expansion of the contraceptive method mix and further research on the relationship between hormonal contraception and HIV infection is essential. CONRAD's work in this area, ongoing for several years, is made even more important by these events.

This activity was aimed at assessing the effect of contraception on markers of vaginal immunity. The first part of this activity was a collaboration with FHI 360 on a nested case-control analysis of immune and inflammatory biomarkers within cervical swabs stored from a large prospective study of the effect of contraception on HIV incidence (the Hormonal Contraception [HC]-HIV study). The nested case-control study allows linkage of

biomarkers to incident HIV infection and subsequent cervical HIV shedding with correlation to contraceptive method use.

As the second part of this activity, CONRAD originally planned a prospective study (Protocol A09-112) to examine how four contraceptives alter the vaginal environment in healthy women. This study was to examine biomarkers of inflammation and innate immunity identified in the recently completed study of new markers of inflammation among users of the universal placebo, cellulose sulfate (CS) and nonoxynol-9 (N-9), CONRAD Protocol A04-097. The study was not conducted as planned, but was incorporated into a prospective open-label study (CONRAD A10-114) entitled, "Assessing the Effect of Contraception and the Menstrual Cycle on Pharmacokinetics, Pharmacodynamics, and Vaginal Safety in Tenofovir Vaginal Gel Users" to examine the effects of oral and injectable contraceptives and the menstrual cycle on the PK and PD of tenofovir 1% gel and the effect of the contraceptive methods on markers of mucosal safety.

Activity Description:

This specific activity will look at the effect of endogenous and exogenous hormones on markers of inflammation.

CONRAD has been collaborating with Dr. Charles Morrison at FHI 360 to examine stored swabs from his multicenter prospective cohort HC-HIV study of 6,109 HIV-uninfected women in Africa and Thailand. This study evaluated the effect of combined oral contraceptives (OCs) and Depo-Provera® (DMPA) on HIV acquisition. Cytokine levels from cervical swabs were correlated with HIV acquisition and contraceptive method. Data were divided into 2 types of statistical analyses: a cross-sectional one (to include swabs from the visit before the women who became HIV-positive seroconverted and from a similar visit for women who did not seroconvert) and a longitudinal one (to include swabs from the visit at which seroconversion was documented plus the 2 visits preceding and 2 following it, with analogous visits from non-seroconverters). The cross-sectional analysis showed that seroconversion and shedding were associated with elevations in RANTES (regulated on activation, normal T cell expressed and secreted) and decreases in secretory leukocyte peptidase inhibitor (SLPI), as was Depo-Provera use. Oral contraceptives were associated with a more proinflammatory picture. In addition, STIs were associated with decreases in SLPI and increases in biodefensin2 (BD2). These analyses provide a crucial link between vaginal cytokine levels and HIV acquisition.

CONRAD Protocol A10-114 entitled, "Assessing the Effect of Contraception and the Menstrual Cycle on Pharmacokinetics, Pharmacodynamics, and Vaginal Safety in Tenofovir Vaginal Gel Users," was conducted at 3 sites (EVMS, University of Pittsburgh, and Profamilia in the Dominican Republic). Approximately 72 women (24 at each of 3 sites) were to be enrolled, half on OCs and half on Depo-Provera. The effects of oral and injectable contraceptives and the menstrual cycle on the PK and PD of tenofovir 1% gel were assessed as well as the effect of the contraceptive methods on markers of mucosal safety.

Progress:

Results of the cross-sectional analysis in the Morrison collaboration have been published in *JAIDS*. A poster on the link between biomarkers and shedding was presented at the R4P meeting in Cape Town, South Africa, October 2014, and a paper has been submitted to *AIDS Research and Human Retroviruses*. A paper on the link with RTIs has been submitted to *JAIDS*. New funding has been obtained from NIH to continue work, looking also at systematic markers.

The study of the universal placebo, CS, and N-9 (CONRAD Protocol A04-097, supported primarily using CONRAD-III/Microbicide funds was completed and results published in *AIDS Research and Human Retroviruses* and *mBio*. The markers and specific time points at which they were collected were used to help draft the protocol for CONRAD Protocol A10-114. That study was completed at three sites (EVMS,

University of Pittsburgh, and Profamilia in the Dominican Republic). Approximately 72 women (24 at each of three sites) were to be enrolled, half on OCs and half on DMPA. Overall, 222 women were screened/enrolled in order to have 32 oral contraceptive users complete the study and 29 DMPA users complete the study. All laboratory analyses have been completed and final data are expected from FHI in Q2 2015.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This activity contributes to our understanding of changes in genital immunity that affect susceptibility to and transmission of HIV and other RTIs, and the effect of contraceptives on genital immunity.

Involvement of Other Donors and Interested Parties:

FHI 360 donated the swabs stored from the HC-HIV study. In addition, FHI 360 provided data management and statistical support for CONRAD A10-114 through their PTA Cooperative Agreement with USAID. Completion of this work is being funded through CONRAD's new APS Objective 1 agreement (AID-OAA-A-14-00011), entitled "Completion of Clinical Studies Required for TFV Gel Licensure".

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USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.4 – Imaging Study of Combination Microbicide Formulations
CA Activity Number:	PPA-10-094
Primary Country of Activity:	USA
Technical Coordinator:	Jill Schwartz
Implementer:	Craig Hendrix (Johns Hopkins University)
Period:	07/01/09 – 12/31/12

Objective:

One of the critical properties of new vaginal microbicide formulations with antiretroviral drug combinations is their ability to release and distribute the drug product. Two different types of gel formulations are being developed for combination products: spreading gels and bolus gels. The traditional approach to microbicide gel formulation is to use a spreading gel that will coat the cervicovaginal surfaces to provide maximal protection. In contrast, thicker bolus type formulations do not coat the entire cervicovaginal surfaces but instead act as a reservoir for the drug. They are likely to be associated with less leakage and, therefore, greater acceptability and drug retention. The objective of this activity was to investigate whether assessing quantitative distribution of surrogate microbicide released from vaginal formulations can help inform the drug formulation process.

Activity Description:

Imaging of microbicide gel formulations has been done for many years using gadolinium and MRI. Craig Hendrix of Johns Hopkins University has developed a means of using radiolabeled antiretroviral size surrogate molecules to track distribution of the microbicide drug (as opposed to the gel formulation) in the female genital tract using sensitive imaging and modeling techniques. Two different types of gel formulations that have been selected from a screening algorithm will be studied in a phase I study at Johns Hopkins. The gel vehicles will be mixed with radiolabeled (gamma emitting) antiretroviral size surrogate molecules (Technetium-DTPA) and gadolinium in order to visualize both the surrogate marker and the gel. Gadolinium improves the imaging of the gel and allows distinct comparison of the gel from the surrogate marker.

Six volunteers in good health, at low risk for STIs, and protected from pregnancy were planned to be enrolled in a cross-over study design. It was originally planned that each volunteer would be randomized to order of gel use for three gels: a spreading gel (3.5 mL) and a bolus gel at two volumes (1.5 mL and 3.5 mL). However, the spreading gel was judged to be the appropriate formulation to move forward and the study was redesigned to compare the distribution, concentration, and persistence of this spreading gel formulation in the female genital tract through 24 hours using both SPECT-CT and MRI and to compare the spreading gel effects on vaginal mucosal permeability with N-9 as the positive control.

This was a comparative, open-label two-period crossover study to evaluate the distribution and retention of a 3.5 ml dose of a spreading gel and to compare vaginal mucosal permeability of the spreading gel with a 3.5 mL

dose of N-9 gel as a positive control. Gadolinium and ^{99m}Tc-DTPA were mixed into the spreading gel to facilitate imaging. Since no imaging was conducted with the N-9 intervention, the N-9 formulation was mixed with ^{99m}Tc-DTPA only. Following the screening visit, six research participants underwent two study intervention visits -- an imaging visit for the spreading gel followed by the N-9 positive control permeability assessment visit. The day of each dosing visit, a serum pregnancy test was obtained and confirmed negative prior to proceeding with gel administration. Following gel dosing, MRI was performed at 30 minutes, 4 hours, and 24 hours after gel application. SPECT/CT imaging was obtained at 1.5, 5, and 25 hours following dosing. Blood was collected at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 12, 16, and 24 hours after gel insertion and plasma was separated for measurement of ^{99m}Tc. Urinary excretion of radioactivity was measured by timed urine collections at 0-1, 1-3, 3-6, 6-12, and 12-24 hours post-dosing. A sanitary pad was placed following gel dosing and changed just prior to each SPECT/CT procedure. The pads and any tissue paper used following urination were saved and counted for radioactivity to account for leakage of isotope from the introitus.

Progress:

The study was fully enrolled and the clinical phases of the study complete by the end of 2011. Results were presented as an abstract at Microbicides 2012 in Sydney, Australia.
SPECT/CT showed uneven distribution of the spreading gel throughout the vagina with highest concentration of ^{99m}Tc-DTPA in the region of the fornices and the external surface of the cervix; signal intensity diminished over 25 hours. MRI showed the greatest accumulation of spreading gel in the vaginal anterior fornix, most notable 1-4 hours post-dosing. Median (interquartile range) isotope signal lost from the vagina through 6 hours was about 30%, Mucosal permeability to ^{99m}Tc-DTPA following spreading gel administration was negligible in contrast to the N-9 dosing which was associated with detectable radiosignal in plasma throughout the observation period. The spreading gel formulation distributed variably throughout the vagina, persisted for 24 hours, though over a quarter of the administered dose was lost within 6 hours. The greatest concentration of spreading gel signal was in the fornices and the ectocervix, consistent with the distribution of HIV surrogates (cell-associated and cell-free) after simulated intercourse in other studies. Based on ^{99m}Tc-DTPA permeability, the spreading gel had no significant effect on vaginal mucosal permeability in contrast to a 2,000-fold greater mucosal permeability after a single N-9 dose, a surfactant known to change mucosal integrity.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

SPECT/CT and MRI are complementary imaging modalities for describing the distribution and relative gel concentration in the lower female genital tract over 24 hours. The data from this study suggest that shortly following intravaginal gel administration, relatively higher concentrations of an active pharmaceutical ingredient could be achieved and maintained within the region of the fornices and external surface of the cervix than in the lower vagina. This effect persists over 25 hours, though the concentration gradient lessens over time. If the critical anatomic region to protect against establishment of HIV infection is the exterior surface of the cervix, the ability to deliver relatively higher concentrations of drug to the cervix could be reasonably achieved with this gel formulation. Alternatively, relatively lower concentrations are observed in the lower vagina, suggesting that if adequate coverage of the entire vaginal canal is necessary to prevent HIV transmission, gel formulation and dosing may need to take this into account. The distribution observed for the spreading gel is consistent with prior studies of HIV surrogate distribution within the lower female genital tract when mixed with fluid simulating semen in which both cell-free and cell-associated HIV surrogates migrate to the same peri-cervical location. The spreading gel had very low vaginal mucosal permeability by our measure, which would suggest that it does not have the same effect on disruption of the mucosal barrier as does Nonoxynol-9.

The use of SPECT/CT in combination with MRI can provide complementary anatomic and quantitative imaging data on gel distribution and concentration following intravaginal dosing. Further, the ^{99m}Tc-DTPA radiolabel used for SPECT/CT can also be used as a surrogate measure of vaginal mucosal permeability that can describe the relative effect of different gel formulations on some aspects of structural integrity of the vaginal mucosa. While the significance of alteration of vaginal mucosal permeability for either increasing tissue toxicity or susceptibility to HIV infection remains to be determined, use of this method with other gel formulations may help to further characterize alterations in tissue permeability that have clinical significance. Thus, the use of this combination of imaging modalities along with measuring associated mucosal permeability changes may enhance the process of topical formulation development.

Involvement of Other Donors and Interested Parties:

Craig Hendrix (JHU) used non-CONRAD funding to support some of the basic laboratory techniques.

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USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.5 – Exploratory Studies: 1) Using Indicators of Vaginal Inflammation to Assess New Microbicide Candidates, and 2) Using Explants as Early Indicators of Microbicide Efficacy
CA Activity Number:	PPA-10-074
Primary Country of Activity:	USA
Technical Coordinators:	Christine Mauck, Marianne Callahan
Implementers:	Gustavo Doncel (EVMS); Debra Weiner (FHI 360); Charlene Dezutti (Magee-Womens Hospital), Annie Thurman (EVMS), Sharon Hillier, Raina Fichorova
Period:	9/1/08 – 6/30/13

Objective:

The objective of these studies was to produce information that will facilitate the assessment of microbicide candidate safety and efficacy. They are exploratory in nature, but their ultimate goal is to assist in the development of CONRAD proprietary compounds.

Activity Description:

CONRAD's effort to identify and validate new clinical biomarkers of microbicide safety began with the completed study of new markers of inflammation among users of the universal HEC placebo, cellulose sulfate (CS), and nonoxynol-9 (N-9) – CONRAD Protocol A04-097. The second type of exploratory study is the development of explanted tissue as an early indicator of microbicide efficacy by applying microbicides *in vivo* and then sampling tissue and challenging it with HIV.

Progress:

Results of the completed study of the universal placebo, CS, and N-9 showed that, while the surfactant N-9 was associated with obvious biochemical and histological signs of inflammation, more subtle changes, including depression of SLPI, tissue influx of CD45+ and CD3+ cells and subclinical microflora shifts were associated with CS use and may help to explain clinical failure of non-surfactant microbicides. Results were published in *AIDS Research and Human Retroviruses* and *mBio.*

Explants were incorporated into the pilot UC781 study that was conducted prior to the UC781 PK/PD study. This study addressed the issues of biopsy site, size, and handling and is fully described in the activity entitled, "Studies of Gels – PK, PD and Markers of Mucosal Safety." (#3.1)

Prior to conducting a pharmacokinetic/pharmacodynamics (PK/PD) and safety study of TFV gel a pilot study was undertaken to establish collection, transport and assay methodology for clinical samples. Between December 2010 and March 2011, ten women underwent three genital biopsies at baseline, and again 4 hours post-treatment with 4 ml intravaginal TFV gel, during the same phase of sequential menstrual cycles. These

fresh genital tissue samples were used to develop the infectivity protocol for the HIV explant challenge assay.

The clinical team developed the study design for the PK/PD/safety protocol with input from the preclinical and product development teams. An investigator at the University of North Carolina developed assays for the measurement of drug concentrations in genital tissue and fluids and an investigator at the University of Dartmouth, in conjunction with EVMS, is developing the ex vivo explant HIV infectivity assay to support the PK/PD study of TFV 1% gel using different dosing regimens.

The pilot UC781 data demonstrated the feasibility of infecting biopsy tissue. All baseline biopsies 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.

The PK/PD safety study (Pharmacokinetic and Pharmacodynamic Study of Tenofovir 1% Gel Using the BAT24 Regimen Versus Daily and Pericoital Dosing, Protocol #A10-113) was conducted to assess the impact of different vaginal dosing regimens (BAT24 versus daily and pericoital dosing regimens) on the PK and PD of TFV 1% gel and the impact of these dosing regimens on immune cell activation. In this study HIV inhibition was noted in CVL after exposure to all dosing regimens up to 72 hours. There was a significant negative correlation between TFV-DP concentration in tissue and maximum/cumulative HIV-1 p24 with BAT24 multiple dosing

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This activity allowed development of subclinical safety markers that will facilitate earlier triage of microbicide candidates. The ex vivo HIV-1 infection assay in cervicovaginal explant tissues has been successfully implemented as a surrogate marker of mucosal susceptibility to infection in other recent and ongoing CONRAD studies.

Involvement of Other Donors and Interested Parties:

FHI 360 has provided data management and biostatistical support, initially using USAID funding provided directly to FHI; however, completion of their role has been funded by CONRAD under CONRAD's OHA APS Aim 1 Cooperative agreement, "Completion of Phase III clinical trial, New Drug Application dossier, and implementation studies for tenofovir 1% gel, a first-in-class topical product for the prevention of HIV acquisition by women."

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USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.6 – Phase III Effectiveness Trial of the PATH SILCS Diaphragm plus BufferGel
CA Activity Numbers:	PPA-10-054, PPA-10-055, PPA-10-057 & PPA-10-058
Primary Country of Activity:	USA
Technical Coordinators:	Jill Schwartz, Marianne Callahan
Implementers:	Ron Frezieres (California Family Health Council, Inc. [CFHC]); Mitchell Creinin (University of Pittsburgh/Magee-Womens Research Institute); Lynn Bradley (Johns Hopkins Community Physicians); David F. Archer (Eastern Virginia Medical School/CONRAD); Alfred Poindexter (Advances in Health, Inc.); Kurt Barnhart (University of Pennsylvania)
Period:	9/1/08 – 6/30/13

Objective:

The purpose of this study is to evaluate the contraceptive effectiveness and safety of the SILCS diaphragm, a new contraceptive diaphragm developed by PATH (in collaboration with CONRAD) that can also be used as a microbicide delivery system.

Activity Description:

Four hundred-fifty (450) couples at risk for pregnancy were to be enrolled into a study at six (6) clinical sites to evaluate the contraceptive effectiveness and safety of the SILCS diaphragm that can also be used as a microbicide delivery system.

Women were to be randomized in an overall 2:1 ratio to use the SILCS diaphragm with either BufferGel or Gynol II at 6 sites (approximately 300 couples randomized to BufferGel and approximately 150 couples randomized to Gynol II). At 2 of the sites (University of Pittsburgh Medical Center and Eastern Virginia Medical School), a substudy involving colposcopy and microflora analysis was to be conducted in 80 of these women (approximately 40 women per site). All women were to undergo insertion/removal assessments and fit procedures for the SILCS diaphragm and sized for an Ortho® diaphragm.

This activity includes consultation with PATH on regulatory aspects such as labeling, the over-the-counter indication, and the 510K submission to the FDA.

Progress:

A total of 450 women (300 BufferGel; 150 N-9) were enrolled from February 4, 2008, to February 13, 2009. The last participant visit was on October 22, 2009. Data from this study were presented at the International Conference on Family Planning: Research and Best Practices, November 15-18, 2009, Kampala, Uganda, and

at Reproductive Health 2011, September 15-17, 2011 Las Vegas, Nevada. A manuscript has been accepted for publication by Obstetrics and Gynecology and is currently in press.

421/450 (94%) met inclusion criteria for the Treated Population; of these 397/421 (94%) were included in the Pregnancy Population, with most exclusions due to not having used the single-size diaphragm during intercourse. In the pregnancy analysis, 219/397 (55%) had at least one perfect-use cycle. The vast majority (92%) of Treated Population had never used a diaphragm. About two-thirds would have required a size 70-75 mm standard diaphragm. There were no important differences in baseline characteristics between gel groups. About 54% of Treated Population completed the study. Most early discontinuations were for personal reasons not related to the device, followed by pregnancy. Loss to follow-up was low (approximately 4%).

There were approximately 22,348 reported coital acts with the single-size diaphragm. Exposure to the single-size diaphragm and study gel could be calculated for 375 couples. The per-couple monthly average number of coital acts was 13.3 (SD=12.2, median=10.5).

There were 40 pregnancies, of which 35 met the pre-specified definition of a study event (estimated date of fertilization after enrollment and before the last day in the pregnancy analysis). The Kaplan-Meier cumulative 6-month typical-use pregnancy probability for the single-size diaphragm was 10.4 per 100 women (95% CI= 6.9, 14.0); extrapolated to 12 months 17.8 (95% CI=12.0, 23.6).

Historical control analysis yielded a propensity score-adjusted estimate of the cumulative 6-month typical use pregnancy probability for the single-size diaphragm of 11.3 vs. 10.7 per 100 women for the standard diaphragm ([rounded] difference =0.7, 95% CI = -3.6, 4.9, Table 3). Since the upper 95% confidence bound was less than 6.0, the null hypothesis was rejected, i.e., the single-size diaphragm was non-inferior to (at least as good as) the standard diaphragm for contraception. All three propensity score-adjusted comparisons on safety endpoints showed statistically significantly lower cumulative 6-month probabilities in the single-size diaphragm group: moderate-to-severe urogenital AE (-23.6, 95% CI=-29.1, -18.1), moderate/severe product-related AE (-24.0, 95% CI=-28.3, -19.6), and moderate/severe UTI (- 6.4, 95% CI=-8.9, -4.0) per 100 women. The estimated cumulative 6-cycle typical-use pregnancy probability adjusting for 34 emergency contraception users was about 0.5 (per 100 women) higher than that without adjustment. The 680 perfect-use cycles yielded a cumulative six-cycle perfect-use pregnancy probability of 7.9 (95% CI = 1.7, 14.0); extrapolated to 12 months 14.0 (95% CI=3.0, 23.6).

Colposcopy findings were minimal, consisting of erythema, petechiae, ecchymosis, and grossly white findings; there was no deep epithelial disruption. Most women did not develop an adverse change in microflora. About one-third had a decrease in Lactobacillus H_2O_2+ colony count, one quarter had an adverse change in each of Gardnerella, Ureaplasma, and/or E. coli colony counts, and less than 10% had an adverse change in Candida. There were no important differences between gel groups in colposcopy or microflora.

Almost all [439/450=97.6% (95% CI 95.7-98.8%)] women could be fitted with the single-size diaphragm. On one attempt after reading written instructions only, over three quarters [76% (95%CI 72.0-80.1%)] of participants were able to insert, correctly position, and remove the device, and thought accurately that the position was correct. However, with coaching and up to three attempts, almost all [94% (95%CI 91.9-96.4%)] participants could insert, correctly position, and remove the device. Of the 39% (162/421) who discontinued early, only 12% discontinued for personal reasons related to the single-size diaphragm or gel (data not shown).

Couples reported a high proportion of coital acts with the single-size diaphragm (median=97%). Approximately two-thirds (225/342=66%) reported 573 device or gel problems, most commonly device shift or dislodgment (13%) (data not shown). Most women who completed questionnaires (282/342, 82%) liked the

single-size diaphragm, 40% would choose it as a contraceptive if it was available and effective and 91% would recommend to a friend.

CONRAD supported PATH in the labeling study performed at CFHC and in the preparation of the product label. Kessel Marketing & Vertriebs GmbH of Frankfurt, Germany, will manufacture the SILCS diaphragm under a license from PATH. CONRAD supported PATH and Kessel in their preparations for the regulatory applications for Europe and the United States. Kessel succeeded in securing CE Mark certification for the SILCS diaphragm in Germany and to date has launched the product in over 10 European countries under the name of Caya®. CONRAD worked with PATH and Kessel to submit the 510(k) for the SILCS to FDA which resulted in clearance of the diaphragm in late 2014. .

Additional studies with SILCS and alternative gels, i.e., ContraGel and TFV 1% gel, are being funded through PATH's USAID's HealthTech Cooperative Agreement or CONRAD's new APS Cooperative Agreement.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

The use of diaphragms globally has declined dramatically in recent decades due to lack of marketing and an increase in hormonal contraceptive products. However, hormonal methods and IUDs do not meet the needs of women who desire a pericoital method. The single-size diaphragm has features to improve fit and ease of use vs. the traditional diaphragm and also has been shown to be associated with fewer urinary symptoms than standard diaphragms. Its single size removes the need for a pelvic examination and fit assessment, simplifying provision and access. For all of these reasons, the single-size diaphragm has the potential to be more widely used than traditional diaphragms. The single-size diaphragm also has potential as a Multipurpose Prevention Technology for the prevention of pregnancy and STIs by being a physical barrier as well as a delivery system for microbicidal drugs.

Involvement of Other Donors and Interested Parties:

FHI 360 was a major collaborator in the trial. PATH supplied the SILCS diaphragms and will submit the 510K to the FDA. PATH drafted the proposed product label and performed a labeling study. CONRAD-III USAID/Microbicide and USAID/Pop funds were also used to support the study.

CONRAD

Microbicide Account - Final Summary Report

USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.7 – Prospective Evaluation of the Impact of Bacterial Vaginosis and its Treatment on Endpoints of Cervicovaginal Safety and Mucosal Susceptibility to HIV Infection
CA Activity Number:	NA
Primary Country of Activity:	USA
Technical Coordinator:	Andrea Thurman
Implementers:	Gustavo Doncel, Andrea Thurman (EVMS); Susana Asin (Dartmouth); Sharon Hillier (University of Pittsburgh)
Period:	7/1/10 – 6/30/13

Objective:

The objective of this activity was to perform prospective, collaborative clinical and preclinical studies aimed at characterizing the mechanisms by which bacterial vaginosis (BV) and its subsequent treatment and resolution alters endpoints of cervicovaginal safety and mucosal susceptibility to HIV infection.

Activity Description:

BV is the most common cause of vaginal discharge worldwide. It is highly prevalent among women at high risk for HIV-1 and epidemiologic data clearly implicate BV as an HIV-1 enhancing co-factor. The biologic plausibility of this is supported by data which show that BV induces a subclinical proinflammatory and suppressed innate immune cervicovaginal environment, which is conducive to HIV-1 entry and viral replication. Data on the quantitative interactions between BV flora, unculturable BV-associated bacteria, cervicovaginal immuno-inflammatory mediators, HIV target cells and target cell activation markers, H_2O_2-producing lactobacilli, and vaginal pH and susceptibility to HIV, are missing because in vivo experiments cannot ethically be done. Novel cervical explant assays, developed by CONRAD, offer unique ex vivo methods to investigate the effect of BV and BV treatment on epithelial protection from HIV-1. In vivo validation of cervicovaginal epithelial safety endpoints requires the ability to study a host in the normal state and in a disease/abnormal state with known adverse effects. BV represented an ideal model for validating safety endpoints in the presence of proinflammatory, epithelial-altering conditions. The clinical trial was a collaboration with CDC and funded primarily by CDC through an Interagency Agreement with USAID and had two specific aims:

1. Determination of cervicovaginal tissue protection against HIV infection during acute BV and approximately 1 and 6 weeks after successful BV treatment;

2. Characterization of the association between HIV susceptibility and changes in the cervicovaginal safety endpoints (protective functions of the cervicovaginal epithelium) during BV and throughout BV treatment.

Progress:

The study was initiated at the CONRAD Clinical Research Center at Eastern Virginia Medical School on March 30, 2011. The primary reason for screen failure is that the women did not have BV, as determined by Nugent Scores. The clinical phases of the study were completed in December 2011. Seventy-two (72) women were screened and 35 women were enrolled in the study. Due to the study design, the first visit was a screening/enrollment visit, as genital sampling during an active BV infection was required. Thirty-three (33) women completed all 3 visits, which was the goal of the study.

Tissue biopsies were tested for HIV susceptibility with an *ex vivo* HIV infection assay (Dr. Asin). All 99 study samples were analyzed for Nugent score and semi-quantitative vaginal cultures by Dr Hillier's lab. Tissue samples were analyzed for gene expression by microarray, and differentially expressed genes in samples with BV versus normal flora were identified. Anti-HIV and anti-*E coli.* Activity of the CVL was characterized by the laboratory of Betsy Herold. Lymphocyte phenotype analysis was conducted in Dr. Doncel's laboratory.

All 99 study samples were analyzed for immune cell phenotype and activation status, and cervicovaginal lavage (CVL) was processed for anti-viral and anti-bacterial activity. A subset of tissue samples (16 baseline samples, 8 samples in which the BV resolved and 8 samples in which the BV persisted, 5 controls) was analyzed for gene expression by microarray, and differentially expressed genes in samples with BV versus normal flora were identified. Analysis of cytokines and chemokines in the CVL was completed for a subset of cytokines (IL-1B, IL-8, IL-6, TNF-α, IL-10, IL-12) with evaluation of other cytokines and chemokines ongoing. Unculturable bacterial species were characterized by pyrosequencing with 16S ribosomal RNA primers, with statistical analyses and interpretation ongoing.

The CVL from women with resolved BV (Nugent 0 – 3) had significantly higher anti-HIV activity, secretory leukocyte protease inhibitor (SLPI) and growth related oncogene alpha (GRO-α) levels and their ectocervical tissues had significantly more CD8 cells in the epithelium. Women with persistent BV after treatment had significantly higher levels of interleukin-1β, tumor necrosis factor alpha (TNF-α) and intercellular adhesion molecule 1 (ICAM-1) in the CVL. At study entry, participants had significantly greater numbers of CCR5+ immune cells and a higher CD4/CD8 ratio in ectocervical tissues prior to metronidazole treatment, compared to a racially matched cohort of women with Nugent score of 0 - 3.

We submitted an abstract which included the CVL, semi-quantitative vaginal cultures and tissue immune cell data to the HIV R4P conference in 2014. The data regarding gene expression are still undergoing analysis by Dr. Doncel's laboratory. These unique results will be used for future funding requests, including requests for funding on topics such as the effect of normal and abnormal vaginal microflora on tenofovir activity.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This study demonstrated that common safety endpoints used in phase I clinical trials, such as genital tissue immune cell populations and anti-microbial activity of the CVL is significantly altered by the presence of abnormal vaginal flora. This is important to future microbicide R&D as products are evaluated for safety in healthy women. Going forward, we will also test how BV and alterations of vaginal flora affect the pharmacokinetics and pharmacodynamics of future HIV prevention products.

Involvement of Other Donors and Interested Parties:

The clinical study was funded primarily using CDC Interagency Agreement funds.

CONRAD

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USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.8 – Safety and Pharmacokinetic Studies of Dual-Protection Tenofovir/Levonorgestrel Intravaginal Ring
CA Activity Number:	NA
Primary Country of Activity:	USA
Technical Coordinators:	Jill Schwartz, Andrea Thurman
Implementers:	Gustavo Doncel, David Archer (EVMS); Vivian Brache (Profamilia); Angela Kashuba (UNC); Susan Asin (Dartmouth University); and CONRAD Clinical Staff
Period:	7/1/10 – 9/4/14

Objective:

There is an urgent need for female-controlled products that provide contraceptive and microbicidal activity. CONRAD plans to meet this need by developing a dual-protection product, providing both contraceptive and microbicidal activity, that is safe, highly effective, acceptable and low-cost. CONRAD has developed an intravaginal ring (IVR) that releases levonorgestrel (LNG) and tenofovir (TFV) for at least 90 days. LNG has an established track record of safety and efficacy and is, arguably, the best progestin to be incorporated in a controlled-release device due to its low molecular weight, physical stability and steady micro-dose release rates from IVRs. The proof-of-concept for a microbicide, tenofovir (TFV) 1% gel, to prevent HIV infection was demonstrated by the CAPRISA 004 results.

The purpose of the planned clinical trial was for a Phase II dose-finding study of the safety, pharmacokinetics (PK), pharmacodynamics (PD) and acceptability of two doses of an LNG/TFV IVR in women not at risk of pregnancy. In addition, after a review of the funding priorities of the program, it was decided that the single-entity product (TFV-only IVR, Activity #3.12) would be included as an arm in the clinical study of the TFV/LNG IVR.

Activity Description:

A clinical trial of two doses of an LNG/TFV IVR would be conducted which would be randomized (as to the order of Ring A versus Ring B), double-blind, controlled (there will be a one month control cycle prior to treatment), cross-over (with a one month washout cycle between the use of the two rings) study of ovulatory women who are not at risk of pregnancy. Women would undergo a screening visit to detect the presence of exclusion factors. They would be seen up to two times in a control cycle to confirm that they are ovulatory, measured by appropriate luteal phase progesterone levels. After confirmed ovulatory, women would undergo baseline sampling in the control cycle. Women would then be screened approximately weekly during months 1 and 3 of each IVR use and every other week in the 2nd month. There would be a washout cycle between the use of the two 3-month IVRs. There would be at least 28 visits during an approximate 8 month time period.

The objectives of this trial were to assess and compare the safety, PK and PD of two LNG/TFV IVRs with release of 10 mg of TFV and 10 or 20 ug of LNG. This trial would determine the lowest safe and effective dose of LNG to be used in a larger contraceptive efficacy trial.

The planned endpoints were:

- PK endpoints: Concentration of LNG and TFV in the plasma, CV secretions (aspirate) and genital tissue (biopsies). TFV-DP will be measured in tissue and endocervical cells.
- PD endpoints:
    - Surrogates of contraceptive efficacy: cervical mucus quality, inhibition of sperm migration (assessed by sperm penetration assay), ovulation by serum progesterone
    - Surrogates of microbicidal efficacy: comparison of CV tissue permissiveness to *ex vivo* infection with HIV-1 $_{BaL}$, as measured by p24 antigen concentration (picograms of p24 antigen per mg of CV tissue) and incorporated viral DNA between the control cycle and treatment cycles, CVL antiviral (HIV/HSV) activity.

- Safety endpoints (to be compared between the control cycle and during use of the two IVRs):
    - Adverse events, as reported by the patient
    - Changes in menstrual cycle duration or frequency
    - Changes in lipid, serum glucose, blood count and serum chemistry concentrations
    - Development of CV ulcerations, abrasions or edema, as assessed by colposcopic visualization of the CV epithelium
    - Changes in soluble markers of innate mucosal immunity and inflammatory response
    - Changes in HIV-1 target cells
    - Changes in epithelial integrity, as measured by histology (number of cell layers) and immuno-histochemistry (IHC), anti-HSV-2 and anti-HIV-1$_{BaL}$ capacity of the CVL
    - Semi-quantitative vaginal culture
    - Vaginal pH
    - Nugent Score

Based on the concerns expressed about the proposed 3-month duration for the first-in-human vaginal ring trial for the TFV IVR by FDA in their written responses following review of the pre-IND submission package and their suggestion in 2012 that a shorter duration one-month trial evaluating safety would be a necessary first step before proceeding to longer duration trials, we proposed that strategy for the combination TFV/LNG IVR as well. A randomized, partially blinded, placebo-controlled, parallel study would be conducted. Originally, ninety (90) ovulatory women would be randomized in a 2:2:1 ratio (36 TFV/LNG: 36 TFV-only:18 placebo) to use 1 of the 3 IVRs for approximately 1 month. An additional 6 women at 1 clinical site (EVMS) will be recruited to comprise an unblinded TFV-only arm in order to obtain early data on TFV and TFV-diphosphate (TFV-DP) levels in tissue.

The objectives of this trial would be:

Primary objective:
- Evaluate genital and systemic safety of the TFV/LNG IVR, TFV-only IVR, and placebo IVR

Secondary objectives:
- Evaluate pharmacokinetics (PK) of TFV in users of the TFV/LNG and TFV-only IVRs
- Evaluate PK of LNG in users of the TFV/LNG IVR

Tertiary objectives:

- Evaluate pharmacodynamics (PD) surrogates of contraceptive efficacy of LNG in users of the TFV/LNG IVR
- Evaluate acceptability of the TFV/LNG IVR, TFV-only IVR, and placebo IVR

Exploratory objectives:

- Evaluate PD surrogates of microbicidal efficacy in users of the TFV/LNG and TFV-only IVRs
- Evaluate PD endpoints of LNG in the endometrium in users of the TFV/LNG IVR
- Evaluate genital safety using exploratory markers in users of the TFV/LNG IVR, TFV IVR, and placebo IVR
- Assess the correlation of TFV levels between less-invasive swabs and more invasive PK measures

For budgetary reasons, the sample size was reduced from 90 to 50: 100 ovulatory women would be consented and randomized in a 2:2:1 ratio such that 50 complete the study (20 TFV/LNG: 20 TFV-only: 10 placebo). Each woman will use 1 of the 3 IVRs for approximately 1 month. An additional unblinded TFV-only arm that was to obtain early data on TFV and TFV-diphosphate (TFV-DP) levels in tissue was removed from the protocol due to other changes in the study design.

Progress:

The clinical phases of this study were to be funded primarily through an award from the USAID Annual Program Statements (APS), awarded to CONRAD in September 2010. However, considerable effort was already ongoing by the clinical team to develop a study design for this trial. This preparatory work was expanded into a full protocol with input from the preclinical and product development teams. CONRAD Product Development group undertook optimization of segmented polyurethane-based IVRs capable of releasing TFV at 10-15 mg per day and LNG at ~10 µg and ~20 µg per day. An investigator at the University of North Carolina developed assays for the measurement of TFV drug concentrations in genital tissue and fluids. An investigator at the University of Dartmouth, in conjunction with EVMS, developed the ex vivo explant HIV infectivity assay.

A pre-investigational new drug (IND) application submission for the TFV-only IVR was sent to the FDA in October 2012 and the FDA responded with written comments to our questions in December 2013. Product development completed most activities for the TFV/LNG IVR to support an IND filing and was in the process of performing biocompatibility studies. Several feasibility ring batches were successfully manufactured at DPT, and analytical method validation and equipment qualification activities are ongoing. Manufacture of pilot batches and informal stability studies began in Q3 2013. The clinical study design was finalized for the initial 30-day study. A pre-IND meeting with the FDA was held in July 2013. Two INDs (one for the TFV-only IVR and one for the TFV/LNG IVR) were submitted on April 4, 2014. Letters stating that the clinical trial was safe to proceed were received on May 27, 2014. An investigator meeting for both sites was held at EVMS on June 11 and 12, 2014. The protocol was amended, incorporating comments from the FDA and investigators. IRB-required materials were submitted to both IRBs and the FDA in the summer of 2014.

The study was initiated at the EVMS site in October 2014 and in the Dominican Republic in December 2014. As of April 16, 2015, 59 women had been enrolled across the two sites and 19 completed, out of the targeted 50. A planned interim analysis is underway, evaluating PK of both TFV and LNG, pharmacodynamics of LNG, and product attributes with respect to acceptability. The results of the interim analysis will be available in mid-May 2015. The clinical portion of the study is expected to be complete in January 2016.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

CONRAD's development of a combination IVR capable of releasing both a microbicide and a contraceptive agent has enabling initiation of the first such product to enter clinical trials. This was a major contribution to the field since it is believed that women will be more motivated to use a contraceptive product compared with a microbicide-only product. Thus, an area that has plagued clinical evaluation of microbicides (adherence) may be obviated.

Involvement of Other Donors and Interested Parties:

Implementation of this clinical study, including laboratory and data analysis, will be funded primarily through CONRAD's APS from USAID/GH/PRH.

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USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.9 – Collaboration and Support for Microbicide Trials Network Studies
CA Activity Number:	NA
Primary Country of Activity:	USA and African Countries
Technical Coordinators:	Jill Schwartz, Kim Linton, David Friend
Implementers:	Multiple sites through the Microbicide Trials Network
Period:	7/1/10-9/4/14

Objective:

The objective of this activity is to provide gel product, regulatory support, and collaborative clinical input on project teams to various clinical studies being carried out by the Microbicide Trials Network (MTN) using tenofovir 1% gel.

Activity Description:

Tenofovir (PMPA or TFV) is an adenosine nucleoside monophosphate (nucleotide) analog with potent antiretroviral activity that is manufactured by Gilead Sciences, Inc. (Gilead). CONRAD has a co-exclusive (with the International Partnership for Microbicides [IPM]) licensing agreement with Gilead to develop TFV gel as a microbicide gel and has filed an investigational new drug (IND) application with the FDA (73,382). The National Institutes of Health (NIH) Division of Acquired Immunodeficiency Syndrome (DAIDS) has an agreement with Gilead to evaluate TFV gel as a microbicide gel under IND 55,690. With appropriate authorization, cross-reference is permitted between IND 55,690 and IND 73,382. Some of the MTN studies with TFV gel are carried out under the DAIDS IND and some under CONRAD's IND. For those studies that are submitted under CONRAD's IND, the CONRAD clinical team is part of the study team that reviews the protocol and documents and provides regulatory support and medical review for adverse events that occur during the course of the study. For studies that are not submitted under CONRAD's IND, the clinical team reviews serious adverse events (SAEs) for possible FDA reporting. Product for all of the studies, regardless of the IND used, is supplied by the Product Development group at CONRAD. MTN provides funds to CONRAD for some, but not all, of the clinical supplies for MTN studies.

Progress:

Two rectal safety studies, MTN 006 and 007 were carried out under CONRAD's IND 73,382. MTN 006 was using the standard TFV 1% vaginal gel while MTN 007 used the reduced glycerin (RG) version of the TFV 1% gel. All regulatory submissions for both studies were made by the CONRAD regulatory group.

MTN-006 – This rectal safety study with TFV vaginal gel has been completed and the final Clinical Study Report was submitted to the CONRAD TFV gel IND. The following three publications have resulted from this study:

Anton PA, Cranston RD, Kashuba A, Hendrix CW, Bumpus NN, Richardson-Harman N, Elliott J, Janocko L, Khanukhova E, Dennis R, et al. RMP-02/MTN-006: A phase 1 rectal safety, acceptability, pharmacokinetic, and pharmacodynamic study of tenofovir 1% gel compared with oral tenofovir disoproxil fumarate. AIDS Res Hum Retroviruses 2012; 28(11):1412-21. PMCID:PMC3484811

Yang KH, Hendrix C, Bumpus N, Elliott J, Tanner K, Mauck C, Cranston R, McGowan I, Richardson-Harman N, Anton PA, et al. A multi-compartment single and multiple dose pharmacokinetic comparison of rectally applied tenofovir 1% gel and oral tenofovir disoproxil fumarate. PLoS One 2014;9(10):e106196. PMCID:PMC4211672

Richardson-Harman N, Hendrix CW, Bumpus NN, Mauck C, Cranston RD, Yang K, Elliott J, Tanner K, McGowan I, Kashuba A, et al. Correlation between Compartmental Tenofovir Concentrations and an Ex Vivo Rectal Biopsy Model of Tissue Infectibility in the RMP-02/MTN-006 Phase 1 Study. PLoS One 2014;9(10):e111507. PMCID:PMC4211741

MTN-007 – This rectal safety study with the reduced glycerin (RG) formulation of TFV gel completed accrual in July 2011 and the data analyzed. The final Clinical Study Report was submitted to the CONRAD TFV gel IND.

McGowan I, Hoesley C, Cranston RD, Andrew P, Janocko L, Dai JY, Carballo-Dieguez A, Ayudhya RK, Piper J, Hladik F, et al. A phase 1 randomized, double blind, placebo controlled rectal safety and acceptability study of tenofovir 1% gel (MTN-007). PLoS One 2013;8(4):e60147. PMCID:PMC3616022

New protocols developed by MTN were submitted to the CONRAD IND: MTN 011 (PK/PD of TFV Following Coitus); MTN 021 (Safety of TFV in Adolescent Females) which was subsequently withdrawn; MTN 014 (Rectal/Vaginal Safety Cross-over Study Using the Reduced Glycerin TFV Formulation) and MTN-017 (Phase II Randomized Expanded Safety and Acceptability Study of Rectally Applied RG Tenofovir 1% Gel and Oral Truvada®).

MTN-014 is a Phase 1, 2-arm crossover randomized trial in healthy, HIV-negative, sexually-active women aged 21-45 (inclusive) years, assessing concentrations of TFV in both the rectal and vaginal compartments after the rectal and vaginal application of TFV RG 1% gel. The study completed enrollment and follow-up of 14 women. Data analysis underway; late-breaker for IAS abstract planned

MTN-017 is a Phase II, multi-site, randomized, six-sequence, three-period, open label crossover study, examining the effects of oral FTC/TDF (Truvada®) and reduced-glycerin TFV 1% gel. The primary objectives are to: 1) compare the safety profiles of Truvada®, daily RG TFV 1% gel, and receptive anal intercourse (RAI)-associated RG TFV 1% gel and 2) evaluate and compare acceptability of Truvada®, daily RG TFV 1% gel, and RAI-associated RG TFV 1% gel. The study enrolled participants in the US, South Africa, Thailand, and Peru. The first participant in MTN-017 was enrolled on September 25, 2013.Enrollment is complete and last follow-up anticipated by Q3 2015. A potential late-breaker to IAS of baseline study data analysis is being considered.

In addition to the above studies, the Product Development group has supplied TFV gel and HEC placebo gel to MTN 003 (VOICE), MTN 001, MTN 002, and MTN 008.

The MTN network announced in early 2013 that the MTN-003 trial was stopped due to futility, stating that none of the 3 pre-exposure prophylaxis (PrEP) and microbicide interventions tested—daily oral tenofovir, daily oral tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC) (Truvada®), and daily vaginal TFV 1% gel—provided additional protection against HIV in the study, likely because few of the women in the trial used the

products as directed. This low adherence explains the lack of benefit and is consistent with data from other antiretroviral (ARV)-based prevention trials that found a correlation between higher levels of adherence and protection from HIV.

CONRAD's clinical team will continue to collaborate with the MTN team, and to obtain study and site documents for submission to CONRAD's IND. With the completion of the PPRD agreement, this work is unfunded.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This collaboration between MTN and CONRAD helped progress the clinical testing of TFV gel for use in women as a vaginal microbicide. It also enabled the clinical testing of TFV gel in special populations, such as pregnancy. CONRAD also developed the TFV RG gel for use as a rectal microbicide. Results from MTN-017 may form the basis to move forward with a Phase III rectal microbicide trial.

Involvement of Other Donors and Interested Parties:

MTN provided funds to CONRAD for some, but not all, of the clinical supplies for MTN studies.

CONRAD

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USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.11 – A Phase I Clinical Trial Assessing the Safety, Pharmacokinetics, Pharmacodynamics, and Disintegration Time of Vaginal Tablets Containing Tenofovir and/or Emtricitabine
CA Activity Numbers:	Numerous
Primary Country of Activity:	USA
Technical Coordinators:	Jill Schwartz, Kim Linton
Implementers:	David Archer (EVMS), FHI 360, Gustavo Doncel (EVMS), Marla Keller (Albert Einstein College of Medicine), Angela Kashuba (UNC), Susan Asin (Dartmouth University), Raina Fichorova (Brigham and Women's Hospital), others
Period:	7/1/11-9/4/14

Objective:

The overall goal of this activity was to complete a Phase I trial of vaginal tablets containing tenofovir (TVF) alone, TFV in combination with emtricitabine (FTC), and FTC alone. The trial will assess the vaginal and systemic safety, pharmacokinetics (PK), preliminary and exploratory pharmacodynamics (PD), and disintegration time of tablets inserted vaginally once daily for 14 days by sterilized, sexually abstinent women, with assessments after the 1st, 8th, and 15th doses. The study will be conducted at EVMS and one other site, to be determined.

The specific aims of this activity were to: 1) complete the regulatory work needed to initiate the study, including preparation of 2 new investigational new drug (IND) applications; 2) complete the preparatory clinical work needed to initiate the study; 3) conduct the study, in compliance with Good Clinical Practice (GCP) and other FDA regulations; and 4) analyze the results and prepare a final International Conference on Harmonisation (ICH) report and manuscript.

Activity Description:

Vaginal tablets offer many advantages over vaginal gels. Tablets are designed to be inserted without an applicator which eliminates the cost of the applicators and their environmental impact. In addition, tablets are smaller and lighter than gels in applicators, thereby lowering the cost of shipping and storage at the site of manufacture and in the clinic. The absence of an applicator also allows for more discreet storage and insertion by the user.

This study, entitled "A Phase I Clinical Trial Assessing the Safety, Pharmacokinetics, Pharmacodynamics, and Disintegration Time of Vaginal Tablets Containing Tenofovir and/or Embricitabine," CONRAD A11-117, was the first study of vaginal tablets containing TFV and/or FTC in humans. Vaginal tablets containing TFV would have significant advantages over TFV gel, as described above. In addition, combining a nucleotide reverse transcriptase inhibitor (NtRTI) with a nucleoside reverse transcriptase inhibitor (NRTI) may result in greater activity than either drug alone, with safety comparable to either drug alone.

The study was to be a prospective, double-blinded, randomized parallel cohort study. Up to 100 healthy, HIV-uninfected, sterilized sexually abstinent women at low risk for STIs, aged 18-50 would be consented and undergo assessment procedures to provide study tablets to approximately 48 (12 per group) who meet eligibility criteria. The study would examine the vaginal and systemic safety, PK, preliminary and exploratory PD, and disintegration time of four vaginal tablets: 1) TFV alone; 2) FTC alone; 3) TFV combined with FTC; and 4) placebo. Tablets would be inserted vaginally twice on the first day and then once daily for the next 13 days, with assessments after the 1st, 8th, and 15th doses.

Each woman would be seen in 5-6 visits. Volunteers would be consented at Visit 1 and undergo screening procedures. At Visit 1B, baseline blood and genital tract samples would be taken. At Visit 2, a single tablet would be administered vaginally in the clinic. Blood samples for PK would be taken 30 minutes and 1, 2, 4, 6, and 8 hours afterward. A pelvic exam with colposcopy would be done 30 minutes and 8 hours after tablet insertion. Signs of irritation and the degree to which the tablet appears to have disintegrated (i.e., is no longer visible by naked eye and colposcopy) would be noted. Vaginal aspirate, CVL, gram stain, and endocervical cells would be collected 8 hours after tablet insertion. If the tablet appears to have been well tolerated after 8 hours, the participant would be given 14 more tablets to administer vaginally at bedtime starting that evening and continuing for the next 13 days. She would return the morning after the 8th dose for Visit 3 and again after the 15th dose for Visit 4 for blood and genital tract sampling. Participants in whom the first tablet did not appear to have completely disintegrated within the first 30 minutes after insertion would return for Visit 5 1-30 days after Visit 4, at a time when they do not expect to be menstruating. A tablet would be inserted vaginally in the clinic and a speculum examination would be done every 15 minutes, starting at 30 minutes after insertion until the tablet is no longer visible by naked eye and colposcopy, up to 90 minutes after insertion.

Study endpoints included: 1) safety, genital: changes on physical examination, including colposcopy; soluble markers of mucosal immunity as well as immune cell numbers and characteristics in CVL; genitourinary AEs); 2) safety, systemic: systemic laboratory tests; 3) pharmacokinetics: TFV and emtricitabine concentrations in plasma, vaginal aspirate, and genital tissue; TFV-DP and emtricitabine-triphosphate concentrations in PBMCs, endocervical cells, and genital tissue; 4) pharmacodynamics: preliminary: anti-HIV and anti-HSV activity in CVL; exploratory: inhibition of HIV-1 infection in an explant challenge assay; 5) tablet disintegration time: average and range of time to complete disintegration (i.e., tablet is no longer visible by naked eye examination).

As above, the specific aims of this activity were to: 1) complete the regulatory work needed to initiate the study, including preparation of two new INDs; 2) complete the preparatory clinical work needed to initiate the study; 3) conduct the study, in compliance with Good Clinical Practice and other FDA regulations; and 4) analyze the results and prepare a final ICH report and manuscript. Under Specific Aim #1, a licensing agreement with Gilead was needed to conduct the clinical trial with FTC. In addition, a letter permitting the FDA to cross-reference Gilead's Drug Master File for emtricitabine would be needed. Two new INDs would be prepared and submitted: one for TFV alone in a tablet formulation and a second for the combination of TFV and FTC in a tablet formulation. Under Specific Aim #2, preparatory clinical work included designing and printing case report forms, arranging for various laboratory work, drafting and executing subproject agreements and other agreements (e.g., Materials Transfer Agreement, Clinical Trials Agreement, Transfer of Obligations, Memo of Understanding), finalizing a Data Management Plan, collecting regulatory documents and source documents from the site(s), arranging for study product to be delivered to the site(s), conducting an Investigator Meeting and site initiation visits, finalizing a monitoring plan, and entering the study on clinicaltrials.gov. Specific Aim #3 included overseeing and monitoring the study. Specific Aim #4 included working with FHI to prepare an analysis plan and table shells, reviewing the biostatistical report, preparing an ICH final study report, and writing a manuscript.

Progress:

Two new INDs were prepared and electronically submitted: one for TFV alone in a tablet formulation and a second for the combination of TFV and FTC in a tablet formulation. Preparatory clinical work was completed, including designing and printing case report forms, arranging for various laboratory work, drafting and executing subproject agreements and other agreements (e.g., Materials Transfer Agreement, Clinical Trials Agreement, Transfer of Obligations, Memo of Understanding), finalizing a Data Management Plan, collecting regulatory documents and source documents from the site(s), arranging for study product to be delivered to the site(s), monitoring institutional review board (IRB) submissions, conducting an Investigators' Meeting and site initiation visits, finalizing a monitoring plan, and entering the study on clinicaltrials.gov. The study began at EVMS and Albert Einstein College of Medicine in February of 2013. It was completed in December 2013 and closed out in January 2014. A total of 90 participants were screened/enrolled, 49 were randomized and, of these, 47 completed the study.

The study samples were sent to the appropriate laboratories for endpoint analysis, which were completed in Q3 2014, with results incorporated in to the study database. At the end of the PPRD funding, CONRAD was working with FHI 360 to finalize the analysis plan and table shells. The final biostatistical report is expected from FHI in May 2015.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This activity involved development of and the first clinical testing of vaginal tablets containing microbicides, a formulation that is expected to be cheaper, smaller, less noticeable to use, and easier to store and insert than gel formulations.

Involvement of Other Donors and Interested Parties:

FHI 360 provided data management and statistical support for CONRAD A10-117 through their PTA Cooperative Agreement with USAID. Completion of this work will be funded through CONRAD's new APS Cooperative Agreement (AID-OAA-A-14-00005), "Development of On Demand and Longer-Acting Microbicide Product Leads".

CONRAD

Microbicide Account - Final Summary Report

USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.12 – Tenofovir Intravaginal Ring Studies
CA Activity Number:	NA
Primary Country of Activity:	USA
Technical Coordinators:	Jill Schwartz, Kim Linton
Implementers:	Gustavo Doncel, David Archer (EVMS); Marla Keller (Albert Einstein College of Medicine); Angela Kashuba (UNC); Susan Asin (Dartmouth University); Charlene Dezzutti (Magee-Womens); FHI 360
Period:	7/1/11 – 9/4/14

Objective:

The proof-of-concept for a microbicide, tenofovir (TFV) 1% gel, to prevent HIV infection was demonstrated by the CAPRISA 004 results and has led to shifting priorities for CONRAD and the microbicide field. Registering TFV gel and improving TFV performance to increase protection is now a higher priority. In CAPRISA 004, a trend of higher effectiveness was observed as gel adherence improved; high adherers had a 54% lower HIV incidence rate (compared to a 39% reduction in lower adherers) in the tenofovir gel arm. Adherence estimates of self-reported sex acts in the prior 30 days based on applicator returns demonstrated a mean of 72.2% (median = 60.2%) of sex acts covered by two doses of gel.

Formulation of TFV into an intravaginal ring (IVR) for continuous use over 3 months is a feasible way to increase product adherence and optimize efficacy. The original objective of this activity as to obtain clinical data on the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of the TFV IVR during 3 months of continuous use.

Activity Description:

CONRAD has developed a novel 90-day TFV reservoir-type IVR composed of a hydrophilic polyurethane tubing and semisolid drug loaded core that releases TFV at a rate of 10 mg per day over 90 days. A 2-arm, randomized, double-blind clinical trial that would be conducted in 36 women at 3 sites was originally planned. Volunteers would undergo baseline assessment and then be randomized to the TFV or placebo IVR in a 2:1 ratio and would use the ring for 3 months. Each participant would undergo safety, PK, and PD testing 3 times per week in the first week of use, weekly in months 1 and 3, and biweekly in month 2. Participants would also undergo PK testing after removal of the IVR. After a review of the funding priorities of the program, it was decided that this single-entity product would be included as an arm in the clinical study of our multipurpose preventive technology product, the TFV/levonorgestrel (LNG) IVR (CONRAD Protocol A13-128). An investigator at the University of North Carolina developed assays for the measurement of TFV drug concentrations in genital tissue and fluids, and an investigator at the University of Dartmouth, in conjunction with EVMS, developed the ex vivo explant HIV infectivity assay.

The objectives of the study were:

Primary Objectives:

- To assess safety as measured by genitourinary adverse events after insertion of TFV IVR compared to placebo IVR during 3 months of use
- To assess TFV concentrations in blood plasma, aspirate and genital tissue and TFV-diphosphate (TFV-DP) concentrations in peripheral blood mononuclear cells (PBMCs), endocervical cells and genital tissue during 3 months of use

Secondary Objectives:

- To determine HIV-1 infection in an explant challenge assay at baseline and at selected time points during IVR use
- To characterize anti-HSV and anti-HIV activity of CVLs at baseline and during 3 months of IVR use
- To characterize changes in soluble markers (IL-1 alpha and beta, IL-1 RA, IL-6, IL-8, IL-10, MIP 1 alpha, TNF, RANTES, and SLPI) of mucosal immunity as well as immune cell numbers and phenotypes (e.g., CD45+, CD4+) in CVL

The planned endpoints were:

- PK endpoints: Concentration of TFV in the plasma, CV secretions (aspirate) and genital tissue (biopsies). TFV-DP will be measured in tissue and endocervical cells.

- PD endpoints:
  - Surrogates of microbicidal efficacy: comparison of CV tissue permissiveness to *ex vivo* infection with HIV-1 $_{BaL}$, as measured by p24 antigen concentration (picograms of p24 antigen per mg of CV tissue) and incorporated viral DNA between the control cycle and treatment cycles, CVL antiviral (HIV/HSV) activity.

- Safety endpoints:
  - Adverse events, as reported by the patient
  - Changes in menstrual cycle duration or frequency
  - Changes in lipid, serum glucose, blood count and serum chemistry concentrations
  - Development of CV ulcerations, abrasions or edema, as assessed by colposcopic visualization of the CV epithelium
  - Changes in soluble markers of innate mucosal immunity and inflammatory response
  - Changes in HIV-1 target cells
  - Changes in epithelial integrity, as measured by histology (number of cell layers) and immuno-histochemistry (IHC), anti-HSV-2 and anti-HIV-1$_{BaL}$ capacity of the CVL
  - Semi-quantitative vaginal culture
  - Vaginal pH
  - Nugent Score

Progress:

CONRAD Product Development developed a novel 90-day TFV IVR (reservoir with a rate limiting membrane) composed of a unique polyurethane that releases TFV at a rate of 10-15 mg per day over 90 days.

A request for a pre-IND meeting for the TFV/LNG IVR was sent to the FDA on May 22, 2013, with a study design that included the TFV IVR single-entity ring and the TFV/LNG IVR for a 30-day initial PK/PD study (based on feedback received from FDA on the TFV IVR pre-IND submission). The meeting was held on Tuesday, July 23, 2013. Two INDs (one for the TFV-only IVR and one for the TFV/LNG IVR) were submitted on April 4, 2014. Letters stating that the clinical trial was safe to proceed were received on May 27, 2014. An investigator meeting for both sites was held at EVMS on June 11 and 12, 2014. The protocol was amended, incorporating comments from the FDA and investigators. IRB-required materials were prepared and submitted to both IRBs and the FDA in summer 2014.

It should be noted that the FDA expressed concerns about the proposed 3-month duration for the first-in-human vaginal ring trial for the TFV IVR in their written responses following review of the pre-IND submission package and their suggestion in 2013 that a shorter duration one-month trial evaluating safety would be a necessary first step before proceeding to longer duration trials, the clinical testing strategy was modified accordingly for the combination TFV/LNG IVR, as well.

The study was revised to be an outpatient, randomized, partially blinded, placebo-controlled, parallel study. Up to 100 ovulatory women would be consented such that 50 complete the study in a 2:2:1 ratio (20 TFV/LNG:20 TFV-only:10 placebo). Each woman would use 1 of the 3 IVRs for approximately 1 month. The study is also described in detail in the Activity entitled, "Safety and Pharmacokinetic Studies of Dual-Protection Tenofovir/Levonorgestrel Intravaginal Ring Number." The study was initiated at the EVMS site in October 2014 and in the Dominican Republic in December 2014. As of April 16, 2015, 59 women had been enrolled across the two sites and 19 completed, out of the targeted 50. A planned interim analysis is underway, evaluating PK of both TFV and LNG, pharmacodynamics of LNG, and product attributes with respect to acceptability. The results of the interim analysis will be available in mid-May 2015. The clinical portion of the study is expected to be complete in January 2016.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This activity involved preclinical development and testing as well as clinical testing of a microbicide-eluting vaginal ring that is less dependent on user compliance than pericoital methods.

Involvement of Other Donors and Interested Parties:

Implementation of this study, including laboratory and data analysis will primarily be funded through CONRAD's PRH APS Cooperative Agreement AID-OAA-A-10-00068 (Aim 4, "Development of a Highly Effective Contraceptive with Potential to Prevent HIV Sexual Infection") and CONRAD's OHA APS Cooperative Agreement AID-OAA-A-14-00010 (Aim 2, "Development of Novel On-Demand and Longer-Acting Microbicide Product Leads").

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USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.13 – Interference with Semen Markers Due to Vaginal Product Use
CA Activity Number:	NA
Primary Country of Activity:	USA
Technical Coordinators:	Christine Mauck, Marianne Callahan
Implementers:	Johan Melendez (Johns Hopkins Bayview Medical Center); Maurizio Macaluso (CDC)
Period:	9/1/08 – 6/30/12

Objective:

The general objective of this study was to produce information that will facilitate the assessment of microbicide candidate compliance. They are exploratory in nature, but their ultimate goal is to assist in the development of CONRAD proprietary compounds. This particular study would assess whether the presence of vaginal products, including microbicide candidates, interferes with detection of semen biomarkers.

Activity Description:

A previous CONRAD study of the new SILCS diaphragm suggested that the presence of nonoxynol-9 (N-9) and KY® interfered with the detection of PSA. A plan was developed to assess whether and to what extent the use of microbicides and other vaginal products might interfere with the detection of biomarkers of semen exposure (i.e., PSA, Y-chromosome DNA [Yc-DNA], and possibly semenogelin). Such biomarkers could be used to assess participant compliance with instructions for sexual activity and condom use in future microbicide trials.

Progress:

Laboratory investigators at Johns Hopkins and the CDC, using non-CONRAD funding, studied whether adding various vaginal products to semen samples interfered with their ability to detect semen biomarkers (i.e., PSA and Yc-DNA). The following products were tested: Replens®, Gynol®, KY®, Astroglide®, Surgilube, N-9 in saline, carboxymethyl cellulose (CMC), UC781, HEC placebo, and tenofovir. Serially diluted semen samples were prepared and each of the gels individually added to these samples to achieve a final gel concentration of 10% v/v. Additionally, serial dilutions of each of the lubricants were added to three different semen dilutions (high, medium, low). The main polymer ingredient in Replens® was also investigated against a panel of serially diluted semen samples. The resulting samples were tested for PSA and Yc DNA. PSA and Yc DNA levels from experimental samples were compared to control samples with no gels added. PSA levels in semen samples spiked with Replens® were significantly lower than those of control samples. All Replens®-spiked samples were PCR-negative for Yc DNA. The main ingredient in Replens® (Carbopol) showed the same inhibitory effect as the lubricant gel. Gynol® showed some inhibitory effect for both PSA and Yc, while KY® showed no interference with the assays. The level of inhibition was directly related to the concentration of lubricant gel in the sample.

Detection of PSA was tested using the qualitative ABAcard and quantitative Abbott Architect assays at the CDC. It was found that most of the products have some effect on the ABAcard, interfering, either, causing false positives or causing invalid results, at one or more concentrations of product and/or semen. Tenofovir interfered with the ABAcard, but not the Abbott Architect.

This suggests that caution is warranted when using these assays for detection of PSA and Yc DNA as biomarkers of semen exposure in trials where Replens® might also be used. A clinical trial was planned in which lab results would be confirmed, but those plans were cancelled in May 2010.

Results were published in *Contraception* and presented at the International Society for Sexually Transmitted Disease Research (ISSTDR) conference in 2009 in London, UK.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This activity yielded practical information for designers of clinical trials in which evidence of unprotected intercourse is sought among women using vaginal products, as well as the degree to which the presence of vaginal products, including microbicide candidates, interfered with detection of some semen biomarkers.

Involvement of Other Donors and Interested Parties:

Johns Hopkins and CDC collaborated on the laboratory testing of study endpoints.

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Microbicide Account - Final Summary Report

USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.14 –Biomarkers of Semen Exposure
CA Activity Number:	EVMS 780302
Primary Country of Activity:	USA
Technical Coordinators:	Christine Mauck, Gustavo Doncel
Implementers:	Johan Melendez (Johns Hopkins University); David Archer (EVMS)
Period:	09/01/09 – 6/30/12

Objective:

Biomarkers of semen exposure may be used to verify protocol compliance in contraceptive and STD prevention trials as well as to determine barrier efficacy. Development of new biomarkers with enhanced sensitivity and specificity as well as short and long time of residence in the vagina is necessary to expand their use in clinical studies. In particular, evidence of unprotected intercourse in the context of HIV prevention trials may be useful in assessing condom non-use. The main objective of this activity is to determine the sensitivity, specificity, and time of decay of two new sperm-specific biomarkers, protamine-2 (Prot-2) and a new marker for chromosome Y (SRY), and of semenogelins measured by a new assay, compared with PSA and Yc-DNA in a clinical trial where volunteers are inseminated with known amounts of their partner's semen or undergo unprotected intercourse.

Activity Description:

Candidate biomarkers of semen exposure fall into two broad categories: 1) biomarkers of seminal plasma and 2) biomarkers of spermatozoa and other cells present in semen. Within the first category, PSA is the best characterized semen biomarker. Within the latter category, in addition to microscopic identification of the spermatozoa, Yc-DNA has been reported to be a good biomarker of semen exposure in the vagina. The sperm-specific markers SRY and Prot-2 originally included in this proposal were replaced with a new assay (see below).

Progress:

The clinical study comparing 5 semen biomarkers was conducted between December 2008 and June 2010 at two sites: the Johns Hopkins Bayview Medical Center, under the direction of Roxanne Jamshidi, MD, and at EVMS under David Archer, MD. The clinical study enrolled 32 women, 16 in each of two groups. One group underwent inoculation of the vagina with the partner's semen. In the other group, women engaged in a single act of unprotected intercourse. Both groups abstained from intercourse for at least 72 hours before inoculation or intercourse and had to have a negative result for the markers prior to inoculation or intercourse. Half the women in each group returned for vaginal swabbing 6 hours after inoculation or intercourse and at 24, 48, and 72 hours, and 7, 11 and 15 days. The other half only returned for sampling at 6 and 24 hours, and 7 and 15 days. Data was thus used to construct a "decay curve" for the biomarkers.

A number of protocol amendments were made during 2009, including discontinuation of the semenogelin marker because the buffer required for its assay interfered with assays for the other markers, and the addition of at-home sampling as an option for couples in the hope of reducing their study burden.

PSA and Yc-DNA assays were completed and data analyses conducted at FHI 360. The procedure used to analyze the other markers at the laboratory at EVMS was delayed until a new DNA marker was developed. The new technique was a DNA multiplex PCR test that could detect two Y-chromosome-linked genes, SRY and TSPY4, as well as amelogenin, a gene found on both the X and Y chromosome. The PCR test could distinguish between female-only DNA (a marker for vaginal insertion) and the additional presence of male DNA as a result of semen exposure, which could indicate unprotected intercourse prior to applicator insertion in pericoital and post-exposure regimens. This innovative, non-dye-based biomarker could be used to confirm vaginal insertion of applicators or swabs as well as unprotected intercourse.

We characterized the vaginal decay curve of markers of recent (SRY and PSA) versus distant (TSPY4 and YcDNA) semen exposure. Timed intercourse results in higher concentrations of biomarkers than vaginal inoculation. Biomarker concentrations are similar with frequent versus less frequent and in home versus in clinic sampling. A manuscript is in preparation.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This activity contributed to the development of new biomarkers of unprotected intercourse with potential use in HIV prevention and contraception studies to verify protocol compliance in as well as to determine barrier efficacy

Involvement of Other Donors and Interested Parties:

None

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USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.15 – Behavioral Assessments of Combination Formulations
CA Activity Number:	PPA-09-023
Primary Country of Activity:	USA
Technical Coordinators:	Marianne Callahan, Jill Schwartz, David Friend
Implementer:	Kate Morrow (The Miriam Hospital)
Period:	07/01/09 – 06/30/12

Objective:

CONRAD is currently developing formulations for use in microbicide combination products. Two different types of formulations have been selected from an in vitro screening algorithm and include a spreading gel and a bolus gel. The traditional approach to microbicide gel formulation is to use a spreading gel that will coat the cervicovaginal surfaces to provide maximal protection. Thicker bolus type formulations, in contrast, do not coat the entire cervicovaginal surfaces, but instead act as a reservoir for the drug. They are likely to be associated with less leakage, and therefore, greater acceptability and drug retention. Although these gel formulations have been tested in the laboratory, they have not been studied in the clinic.

The correlation between perceptions and sensations of gel users and the biophysical properties and rheological performance measures of the vaginal gels have been evaluated by Kate Marrow in previous work with the HEC placebo (universal placebo) and Replens and has led to the development of unique behavioral measures that are able to show differences between user perception of the gels tested. The major objective of this project was to further identify the specific semi-solid properties and rheological performance measures that are most influential in determining the perceptual differences experienced by the user and that ultimately relate to user acceptance of, preference for, and willingness to use specific gels. This study will lead to the development and evaluation of behavioral measures of acceptability of vaginal gels, and help determine the correspondence between user perceptions of gel performance and the biophysical and rheological properties and performance measures of those same gels. The ultimate goal is to develop a preclinical framework for co-optimizing acceptability and gel vaginal distribution/retention and drug delivery. This work was an add-on to an already funded NIH Microbicide Innovation Program (MIP) award. It was an expansion in terms of the number of gels being evaluated clinically, the number of participants enrolled, and the number of protocol sessions implemented.

Activity Description:

This study compared behavioral assessments of four gel formulations among 200 participants in a cross-over design. Two of these formulations were obtained from CONRAD (spreading and bolus) and two were obtained through the pre-existing MIP award. The clinical study participants evaluated the gels with respect to gel behavior, user perception, and willingness to use each gel. Completed User Perception Scale Scores representing four primary dimensions of acceptability (e.g., leakage), application process, implications for covert use, and implications for sexual pleasure across all 200 participants will be delivered for the bolus and

the spreading gels (blinded as "Orange" or "Purple") in addition to the other test gels. Interim analyses will be carried out after 50 and after 100 participants have been completed.

Progress:

The first participant enrolled in December 2009. In total, 499 potential volunteers were approached. Four hundred fifty-one (451) were screened, of which 282 were eligible. Excluding those who were not interested in participating and those who otherwise chose to decline enrollment (e.g., unable to locate post-screening), a total of 220 women enrolled in the study. Four (4) were lost to follow-up, 11 withdrew from the study, and 1 participant's data was lost due to a technical error. In total, there are 204 complete data sets evaluating all four study gels.

With respect to the CONRAD gels being evaluated, results were as follows:

Number of participants who completed the study using CONRAD/"Orange" Gels: 210
Number of participants who completed the study using CONRAD/"Purple" Gels: 215
Number of participants who completed the study using both CONRAD Gels: 205

Data analysis was carried out during the summer and fall of 2011. The first step was to conduct full-cohort exploratory dimensional analyses (EDA) on the scales that were developed, followed by confirmatory factor analyses (CFA). After scale psychometrics were completed, analyses of the "willingness to use" data and exploration of how user experience impacts "willingness to use" were completed.

Twenty-plus User Sensory Perception & Experience (USPE) scales were validated using a range of formulations. USPE scale scores differentiated between gels across 3 different activities: manipulation in the hands; applying/ambulating; and simulating intercourse. Each participant (N=204) experienced 4 gels, rated how each felt, and declared a preference for HIV prevention. Out of all 4 gels, the "orange" product received the most endorsements for willingness to use. However there was no significant different (thus far in the sample) between the "orange" and "purple" products in regards to probability of use if the product used during the last session reduced their chance of getting HIV/AIDS, other STIs, or pregnant.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This activity was important in understanding user preferences with respect to vaginal gel physical attributes. Most gel products are developed without much consideration of the end-user in mind. Since this work was performed, it have become increasingly evident the user-preferences are a critical factor in determining if a women will use a product, particularly in a clinical setting.

Involvement of Other Donors and Interested Parties:

This project received a grant from NIAID through their Microbicides Innovation Program (MIP). These MIP grants usually run around $200,000. NIAID supplied the other two gels in the project for comparison.

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USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.16 – Exploratory Studies – Vaginal Taggants for Compliance Monitoring
CA Activity Numbers:	PPC-08-006; PPC-09-040
Primary Country of Activity:	USA
Technical Coordinator:	Christine Mauck, Marianne Callahan
Implementer:	Ariane van der Straten (Research Triangle Institute); Xhale, Inc.; and the University of Florida
Period:	9/01/08-6/30/11

Objective:

This activity was aimed at producing information that will facilitate the assessment of study participant compliance with instructions for use of microbicide candidates. It was exploratory in nature, but its ultimate goal is to assist in the development of CONRAD proprietary compounds.

Activity Description:

This activity would use uniquely tagged, exhaled biomarkers to assess adherence to vaginal product use instructions. The general objectives were to identify two GRAS compounds that have appropriate chemical and kinetic profiles to be used as taggants of vaginal products and to conduct a proof-of-concept study with 24 female volunteers to demonstrate the feasibility of using tagged tenofovir placebo gel and condoms (with tagged hydroxyethylcellulose [HEC] placebo lubricant) to monitor product adherence in clinical trials.

Progress:

In agreement with CONRAD, it was decided that Astroglide was not going to be used in this study for the condom lubricant, as it induced inflammatory responses in in vitro testing (G. Doncel, personal communication). Instead, HEC placebo would be the condom lubricant tested, and tenofovir placebo gel would be the vaginal gel tested.

Prior to the clinical study, the mini-gas chromatograph (mGC) was validated against traditional gaschromatography-mass spectroscopy (GC-MS) in measuring acetates and corresponding alcohols and ketone metabolites in human breath and after oral ingestion of 2-butanol (40 mg). The mGC performed as well as conventional GC-MS.

In the clinical phase, 8 women during 8 different visits randomly received an intravaginal taggant (30 mg of 2-butyl acetate, 2-pentyl acetate, isopropyl butyrate, or 2-pentyl butyrate) formulated in 1 mL hydroxyethylcellusose (HEC) gel or 4 mL tenofovir placebo gel. A second group of 4 women randomly received the same formulations administered dermally on the ulnar forearm. Sequential breath samples were collected using gas-impermeable bags before and after taggant administration for 1 hour. Samples were measured using a miniature gas chromatograph for ester taggant, alcohol, and ketone concentrations. After

vaginal administration, 2-butyl acetate, 2-pentyl acetate and metabolites were observed in breath whereas isopropyl butyrate, 2-pentyl butyrate and metabolites were not present. Some women reported transient, self-resolving, mild burning (24/64 visits) with vaginal administration or a "bubblegum" taste (7/64 visits). No taggants or metabolites were detected in breath following dermal application. It was concluded that 2-butyl acetate and 2-pentyl acetate are potential taggants deserving additional development for measurement of vaginal gel adherence using breath sampling and a miniature gas chromatograph. A "breath test" for adherence to vaginal gel application appears physiologically and technically feasible.

There were no plans to continue funding this project; however, the Investigator suggested four directions in which this work could be expanded:

1. Expand the taggant chemistry program to include other classes of small molecules should be investigated. In particular, carbonate esters make promising alternatives to simple esters in that they: 1) are chemically more stable, but enzymatically labile, 2) tend to have a lower volatilities and less pungent odors, 3) can be stoichiometrically more efficient at generating breath markers and 4) have less potential for irritation.

2. Optimize the breath collection device for sensor performance and usability, within the context of a simple interface for the participant to use. The first two studies have already yielded breath concentration data following the administration of gels containing varying concentrations of taggant. It would be possible to use this data to roughly estimate the concentration of breath markers that would result from a given amount of taggant. This knowledge provides a framework to guide the modifications needed for optimization.

3. Design GMP delivery system to have a simple device which stores the taggant separate from the gel prior to use but delivers both compounds simultaneously. A dual reservoir device will in principle accomplish this goal. However, designing a practical device based on this concept that is essentially foolproof would require some effort.

4. Study the interaction of the taggant with the API: Once the optimal taggant has been identified and its proper concentration established, determine if the presence of that amount of taggant adversely affects the antiviral activity of the API. This can be established through a sequential series of analyses, starting with the determination that tenofovir has not been chemically altered by the presence of the taggant. If these initial tests show that the API is intact, then an in vitro assay for antiviral activity can be performed, followed by efficacy testing in an animal model(s).

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

Compliance with instructions for use of microbicide candidates is critical for demonstrating product effectiveness. This activity demonstrated the feasibility of breath-based taggants for assessment of compliance with topical microbicide use.

Involvement of Other Donors and Interested Parties:

None

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USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.17 – Tenofovir Phase III Trials for Safety and Effectiveness: Support for Mazabuka Site in Zambia
CA Activity Number:	PPA-09-043
Primary Country of Activity:	Zambia, Uganda, South Africa, Mozambique
Technical Coordinator:	Jill Schwartz
Implementers:	Sheena McCormack, Medical Research Council (MRC), London; Anatoli Kamali, MRC/UVRI Uganda Research Unit; Maureen Chisembele, University of Zambia
Period:	7/1/09-6/30/11

Objective:

Tenofovir (TFV) gel is a candidate vaginal microbicide designed to prevent the sexual transmission of HIV. The general objective of this activity is support ongoing and planned clinical trials being conducted by collaborating organizations, in particular, "Top-Up", an Microbicides Development Programme **(**MDP) study to determine the feasibility of conducting a microbicide trial of both daily and coitally-dependent tenofovir gel in a single trial and to inform the way adherence should be assessed. The effectiveness of tenofovir gel when used in a coitally-dependent manner was being studied in South Africa (CAPRISA) and would shortly be studied by the MTN in another IIB trial (VOICE) as a daily regimen. The proposed trial by the MDP will also help fulfill regulatory requirements to obtain US approval for TFV gel should either regimen prove to be effective.

Activity Description:

The Microbicide Development Program (MDP) is a partnership set up to develop vaginal microbicides for prevention of HIV transmission among women. Top-Up was a multicentre open-label study, in which participants were randomized to one of the three methods for monitoring adherence to daily gel use. The study was carried out in six MDP trial sites (Durban in South Africa, Mwanza in Tanzania, Masaka in Uganda and Mazabuka in Zambia, and Manhica and Maputo in Mozambique).

A minimum of 45 participants per site were recruited to the study (approximately 15 per site per randomized group), and received pre-filled vaginal applicators containing a placebo vaginal gel, Hydroxyethyl cellulose (HEC), and were asked to insert one dose of gel intravaginally each day for 12 weeks. All participants were provided with safe sex education, condoms and voluntary counseling and testing (VCT) for HIV were offered to all. The goal of the Top-Up study was to determine the feasibility of conducting a microbicide trial of once-daily use of a vaginal gel and assess different methods for monitoring adherence in order to inform the design of a future trial. The primary outcome was adherence to daily use of gel. Secondary outcomes were consistency of adherence measure, retention of participants at week 12 and reasons for non-adherence.

The MDP study was never initiated but was designed as a multi-centre, randomized, placebo-controlled trial in approximately 6,320 women to assess the safety and effectiveness of TFV 1% gel applied daily or prior to sex,

in the prevention of vaginally acquired HIV infection. Volunteers would be randomized in a 1:1:1:1 ratio to the following four arms: 1) daily vaginal administration of 1% TFV gel; 2) daily vaginal administration of universal placebo gel; 3) pericoital vaginal administration of 1% TFV gel; and 4) pericoital vaginal administration of universal placebo gel. In the daily administration arms, participants will be asked to insert gel into the vaginal once daily (at a similar time each day). In the pericoital arms, participants would be asked to insert gel into the vagina within one hour before intercourse. Four African sites were identified: Mazabuka (Zambia), Mwanza (Tanzania), Johanesburg, and Durban (both in South Africa).

Specific objectives of this preparatory work performed under this project included to:

1. Provide capacity building of health personnel for both MDP and Mazabuka district hospital staff, in terms of training in ART, PMTCT, adherence counselling and other training.
2. Be seconded to the district hospital to help with counselling of patients on ART, with adherence counselling in particular. Hospital staff will be supported in data collection and management.
3. Support pharmacy staff at the district in terms of trainings as well as management of data.
4. Support laboratory staff in managing workload of investigations needed, as well as helping to raise the standard of the lab in terms of helping with expanding the scope of investigations done as well as with accreditation.
5. Conduct the Top-Up protocol at the Mazabuka site in Zambian women.

Progress:

Mazabuka Site, Zambia

Forty-five (45) participants were successfully enrolled into the Top-Up study in December 2010. The final week 12 follow-up visit for the last participant recruited was completed in March 2011.

Retention: Five participants left the study early giving a retention rate of 89%. Reasons for not completing the study were: pregnancy (1), consent withdrawn (2), and lost-to-follow-up (2).

Randomisation: Sixteen (16) women were randomised to the daily collection group, 14 to weekly and 15 to monthly.

Adherence (primary outcome): The main finding from the study was that there was no statistical difference in adherence to daily dosing of gel between randomised groups, as measured by the reconciled proportion of study days when gel was used. Data were reconciled across gel diaries, behavioural data and applicator returns. Data were available in the database for 39 participants at time of analysis.

Adherence to daily use of gel – Mazabuka site

Proportion of sex acts when gel used	Daily gel collection (n=13)	Weekly gel collection (n=12)	Monthly gel collection (n=14)
Mean	0.78	0.92	0.86

Due to social unrest in Mazabuka at the end of the study, support for this site was not extended. An abstract for the whole study was submitted to IAS 2011 and accepted as a poster presentation.

Top-Up (all sites):

The MDP Top-Up study is a randomised study of 249 women from 5 centres in Mozambique, South Africa, Tanzania, Uganda and Zambia. All women were asked to use a placebo vaginal gel every day for 12 weeks

keeping a record of use in gel diaries. They were randomised to one of 3 gel collection methods and asked to return used and unused gel applicators either: (1) daily, (2) weekly or (3) monthly. Gel returns, diary reports and behavioural interview data were reconciled during a monthly interview, producing a final adherence measure. Adherence was measured per participant as the proportion of days where gel was used. The overall mean adherence was about 80% and was similar across randomised groups. More women in the daily contact group left the study early.. There were more inconsistencies between the diary reports, gel returns and behavioural interview reports in the daily contact group: 41% daily, 20% weekly and 20% monthly. The most common reported reasons for not using gel were travelling and forgetting. Adherence to daily dosing in this study is lower than the 92% adherence to a single pre-sex dose observed in these populations in the MDP301 trial. Although increased contact did not significantly affect the overall measured adherence, asking women to return applicators daily led to an increased drop-out rate. The inverse relationship between frequency of contact and consistency of reporting may reflect more accurate real-time self-reporting in gel diaries in the daily contact group.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

The Top-Up study was designed to find out if daily microbicide gel is acceptable to women in Africa. Gel use by an adherence measure combining gel returns, diary and interview was found to be around 80%. We have subsequently learned that self-report in this population is not as accurate as objective markers of adherence and the field has moved toward utilizing biologic measures to evaluate adherence to PrEP.

Involvement of Other Donors and Interested Parties:

UK MRC and the Department for International Development (DFID) are the major funders of MDP. MRC has also received provisional funding from the European and Developing Countries Clinical Trials Partnership (EDCTP) and is seeking support from other European agencies for co-funding.

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Microbicide Account - Final Summary Report
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Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.18 – TFV/MIV-170 Intravaginal Ring (IVR) Studies (originally "Safety and PK of UC781/TFV Combination Ring")
CA Activity Number:	TBD
Primary Country of Activity:	USA
Technical Coordinator:	Jill Schwartz
Implementer:	**CANCELED**
Period:	7/1/10-6/30/11

Objective:

In addition to supporting the development of TFV and UC781 as single-entity microbicides, CONRAD began actively pursuing the development of a combination gel product with these compounds. This combination microbicide candidate was to target HIV replication by combining two drugs, TFV and UC781, from a group of highly active reverse transcriptase inhibitors. In addition to the combination gel project, the two drugs were to be formulated in a polyurethane ring designed to release the drugs and assessed in an initial 7 day safety study.

CONRAD encountered difficulties in the product development of UC781which led to the decision to substitute MIV-170 for UC781 in the combination product. The development work focused on producing an intravaginal ring (IVR) with the two drugs.

Tenofovir (9-[(R)-2-(phosphonomethoxy)propyl] adenine or PMPA) is an adenosine nucleoside monophosphate (nucleotide) analog with potent activity against retroviruses. MIV-170 is a highly potent NNRTI which is active against HIV-1 resistant to both efavirenz and nevirapine and has higher potency than efavirenz and TMC125. It was hoped that combining an NRTI (tenofovir) with an NNRTI (MIV-170) will result in greater activity than either drug alone.

Activity Description:

A clinical study was planned to assess the vaginal and systemic safety, pharmacokinetics (PK), and preliminary pharmacodynamics (PD) of the combination TFV/MIV-170 IVR in comparison to a TFV IVR, MIV-170 IVR, and a placebo IVR.

Progress:

This first study in this activity was to be a randomized, double-blind, placebo-controlled study in 60 healthy, sexually abstinent women to evaluate delivery of UC781 and TFV from a polyurethane ring and to assess local and systemic safety.

Given the difficulties encountered with UC781 development and in light of the CAPRISA results, much of CONRAD's funding was reprogrammed to move tenofovir (TFV) forward as quickly as possible. Issues encountered with UC781 include: degradation of UC781 in rings and films, difficulty achieving adequate

release rates, and degradation of UC781 to UC22 (for which there is currently no good toxicological profile). Given these issues, CONRAD has decided not to proceed with UC781 as a component of a combination product and to substitute MIV-170 for the UC781 component.

The clinical team developed the study design for the combination gel TFV/MIV-170 and was working with CONRAD's Product Development team on a pre-IND regulatory submission package in order to discuss the requirements for the dual active product with the FDA. A decision was made shortly after the study design was developed to stop working on gels containing MIV-170 and to move directly into intravaginal rings (IVRs) for these products. USAID then requested that CONRAD suspend its current effort on MIV-170 to concentrate on the primary product, tenofovir 1% gel. Clinical projects related to MIV-170 alone or in combination were subsequently canceled.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

It was expected that the clinical study would lead to further development of a microbicide product containing MIV-170 that could be used by women to reduce their risk of HIV transmission. However, the planned clinical studies of MIV-170 were canceled before they began.

Involvement of Other Donors and Interested Parties:

None

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Microbicide Account - Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#3 – Clinical Development
Activity Number and Title:	#3.19 – Safety and PK/PD of Combination Gel
CA Activity Numbers:	TBD
Primary Country of Activity:	USA
Technical Coordinators:	Jill Schwartz, Andrea Thurman
Implementer:	**CANCELED**
Period:	7/1/10 – 6/30/11

Objective:

In addition to supporting the development of TFV as a single entity microbicide, CONRAD has actively pursued the development of a combination product. A microbicide candidate that would target HIV replication by combining two drugs from a group of highly active reverse transcriptase inhibitors would endow the resulting microbicide with enhanced HIV replication inhibiting activity, high genetic barrier to resistance, and advantageous pharmacokinetic and pharmacodynamics (PK/PD) properties. The objective of this activity was to carry out clinical studies aimed at characterizing PK/PD and safety of a combination gel drug product.

Activity Description:

Tenofovir (9-[(R)-2-(phosphonomethoxy)propyl] adenine or PMPA) is an adenosine nucleoside monophosphate (nucleotide) analog with potent activity against retroviruses. MIV-170 is a highly potent NNRTI that is active against HIV-1 resistant to both efavirenz and nevirapine and has higher potency than efavirenz and TMC125. MIV-170 has not been studied as a vaginal gel in humans. It is hoped that combining an NRTI (tenofovir) with an NNRTI would result in greater activity than either drug alone. The purpose of the planned study was to assess the vaginal and systemic safety, pharmacokinetics, and preliminary pharmacodynamics of four vaginal gels: 1) 1% tenofovir; 2) 0.1% MIV-170; 3) 1% tenofovir combined with 0.1% MIV-170; and 4) the HEC placebo inserted vaginally once daily for 14 days.

The study was designed to be a prospective, double-blinded, randomized parallel cohort study in healthy, HIV-uninfected, sterilized women at low risk for STIs, aged 18-50 who meet eligibility criteria and are willing to be abstinent during the study. The study would examine the vaginal and systemic safety, pharmacokinetics, and preliminary pharmacodynamics of the four vaginal gels listed above, inserted vaginally once daily for 14 days, with assessments after the 1st, 8th, and 15th doses.

The intended study endpoints were: 1) pharmacokinetics: tenofovir concentrations in plasma, vaginal aspirate, and cervicovaginal tissue; tenofovir-diphosphate concentrations in PBMCs, endocervical cells, and cervicovaginal tissue; MIV-170 concentrations in plasma, PBMCs, vaginal aspirate, endocervical cells, and cervicovaginal tissue; 2) mucosal safety: anti-HIV and anti-HSV activity in CVL; soluble markers of mucosal immunity as well as immune cell numbers and phenotypes (e.g., CD45+, CD4+) in CVL; genitourinary AEs; 3) exploratory: inhibition of HIV-1 infection in an explant challenge assay; immune cell activation in cervicovaginal tissue.

Progress:

The clinical team developed the study design and was working with CONRAD's Product Development team on the pre-IND regulatory submission package to discuss the requirements for the dual active product with the FDA. Shortly after the study design was developed, a decision was made to stop working on gels other than 1% TFV alone and to move directly into intravaginal rings (IVRs) for these combination products. USAID then requested that CONRAD suspend its efforts on MIV-170 to concentrate on the primary product, tenofovir 1% gel. Clinical projects related to MIV-170 alone or in combination were subsequently canceled.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

It was expected that the clinical study would lead to further development of a microbicide product containing MIV-170 that could be used by women to reduce their risk of HIV transmission. However, the planned clinical studies of MIV-170 were canceled before they began.

Involvement of Other Donors and Interested Parties:

None

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Activity Category:	#4 – Other
Activity Number and Title:	#4.1 – Biomarkers of Inflammation, Infection, and Adherence (formerly titled "Conference on Semen Biomarkers for Female Condom Development")
CA Activity Numbers:	N/A
Primary Country of Activity:	USA
Technical Coordinators:	Jill Schwartz, Kim Linton
Implementer:	CONRAD
Period:	9/5/08 – 9/4/14

Objectives:

The objective of this activity was to encourage further development of biomarkers of inflammation, infection, and adherence to condom and product use.

Activity Description:

CONRAD has been active in the development of biomarkers in 4 broad areas:

1) Biomarkers of inflammation: We have been studying vaginal markers of inflammation for years and have published numerous articles on the results. We conducted CONRAD study A10-114 ("Assessing the Effect of Contraception and the Menstrual Cycle on Pharmacokinetics, Pharmacodynamics, and Vaginal Safety in Tenofovir Vaginal Gel Users") which assessed how 2 contraceptive methods and the menstrual cycle affected the vaginal environment. We also collaborated with FHI 360 on a nested case-control analysis of immune biomarkers in cervical swabs from HIV+ and HIV- women in the Hormonal Contraception (HC)-HIV study, to look for a link with contraception. We played an active role in WHO's assessment of recommendations for contraceptive use in women at risk for HIV, which included an evaluation of the possible effect of contraception on inflammation. We have also developed an explant model to explore the effect of microbicide candidates on HIV infectibility.

2) Biomarkers of infection: Dr. Doncel has collaborated with outside researchers to use novel methods of studying the microbiome of participants in CONRAD microbicide trials.

3) Biomarkers of adherence to instructions regarding condom use: CONRAD has convened several conferences on biomarkers of semen exposure, has published several articles on the subject, and has conducted quarterly teleconferences since 2005. We spearheaded the effort to simplify FDA requirements for approval of new female condoms based on prostate-specific antigen (PSA) testing rather than contraceptive efficacy. We have collaborated with Johns Hopkins and the CDC to study how vaginal products may interfere with detection of PSA and other established semen biomarkers. We have supported Deborah Anderson's work on an assay to confirm vaginal insertion of swabs from clinical studies. Dr. Jacot of CONRAD has developed a polymerase chain reaction (PCR) test that can distinguish between a DNA marker for vaginal insertion and the additional presence of male DNA as a

result of semen exposure, which could indicate unprotected intercourse prior to applicator insertion in pericoital and post-exposure regimens.

4) Biomarkers of adherence to product use: CONRAD has supported the development of pharmacokinetic assays in various biological matrices and has conducted and is conducting several clinical studies with pharmacokinetics as an endpoint. CONRAD also supported the development of a breath-based taggant as an objective indicator of vaginal product use. Drs. Mauck and Schwartz were invited to write an editorial for *Sexually Transmitted Diseases* on the subject of vaginal applicator dyes for assessing adherence to use of vaginal products. In addition, CONRAD initiated a study comparing objective biomarkers of protocol compliance and protocol adherence with classical markers of compliance and adherence.

Progress:

1) Biomarkers of inflammation: CONRAD study A10-114 was completed in October 2013. Results showed that elevated RANTES and decreased SLPI correlated with an increased risk of HIV acquisition and shedding, and were published in *JAIDS* in June 2014. We continue our work on explants and have sent a subset of samples from the CONRAD study A10-113 ("Parmacokinetic and Pharmacodynamic Study of Tenofovir 1% Gel Using the BAT24 Regimen Versus Daily and Pericoital Dosing") for additional analyses to obtain preliminary data for a re-submission.
2) Biomarkers of infection: Our collaboration with outside researchers to assess archived cervical swabs from the Cellulose Sulfate (CS) Phase III HIV prevention trial (CONRAD C03-090) with regard to the relationship among seroconversion and microbiome, anti-*E. coli* and anti-HIV activity, cytokines, and innate antimicrobial factors is still in progress. All samples have been assayed and the statistical analysis has been completed by Jacques Ravel at the University of Maryland. Results will be discussed prior to preparation of a manuscript.
3) Biomarkers of adherence to instructions regarding condom use: The acute failure mode study of the PATH Woman's Condom (WC) has been completed, the PSA swabs analyzed, and similar analyses have been carried out on substudy samples from the NICHD contraceptive study of the same device. The PATH WC condom received higher acceptability ratings and caused less female discomfort than the FC2 condom. Moreover, the WC condom was non-inferior to the FC2 condom as measured by self-reports of condom failure, the standard means of evaluating an investigational condom. In addition, the probability of semen exposure during uses of the WC condom was non-inferior to that of the FC2 condom, suggesting that PSA testing can contribute critical information to the evaluation of experimental female condoms. Results ae pending from the NICHD study. See also the Activity 4 summary for "Integration of Biomarkers in Comparative Studies of Barrier Devices: Application to New Female Condoms."
4) Biomarkers of adherence to product use: CONRAD completed the CONRAD study D13-125 ("Comparison of Objective Biomarkers of Protocol Compliance and Product Adherence with Classic Markers of Compliance and Adherence"), " in August 2013. The biomarkers studied were close to 100% sensitive and specific. A manuscript was submitted to *STDs* on June 25, 2014. CONRAD submitted a manuscript detailing the methodology of the vaginal insertion and semen biomarkers to *PLOS ONE* on July 14, 2014. CONRAD continues to conduct clinical studies with pharmacokinetics as an endpoint, and plans to to continue evaluating objective adherence measures in collaboration with other researchers.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This activity has allowed further development of biomarkers of inflammation and infection that will be useful in the safety evaluation of new vaginal products. Biomarkers of adherence to condom and vaginal product use

will make results of trials more interpretable given variable adherence seen in trials to date.

Involvement of Other Donors and Interested Parties:

The completion of CONRAD study A10-114 will be funded through CONRAD's Cooperative Agreement AID-OAA-A-14-00011, "Completion of Phase III Clinical Trial, New Drug Application Dossier, and Implementation Studies for Tenofovir 1% Gel, a First-in-Class Topical Product for the Prevention of HIV acquisition by Women".

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Activity Category:	#4 – Other
Activity Number and Title:	#4.2 – Integration of Biomarkers in Comparative Studies of Barrier Devices: Application to New Female Condoms
CA Activity Numbers:	PPC-09-047, PPC-11-111
Primary Country of Activity:	USA
Technical Coordinators:	Jill Schwartz, Kim Linton
Implementers:	Ron Frezieres (California Family Health Council); Maria Gallo (CDC); Deborah Anderson (Boston University School of Medicine)

Period:

Activity Category:
Activity Number and Title:

CA Activity Numbers:
Primary Country of Activity:
Technical Coordinators:
Implementers:

Period: 7/1/08 – 8/31/14

Objectives:

The objectives of this activity were to develop benchmark criteria for evaluating newly designed female condoms by integrating self-report with the detection of prostate-specific antigen (PSA) and possibly other DNA-based biomarkers of exposure to semen; and to compare the frequencies of self-reported problems and exposure to semen during use of a new female condom developed by PATH, the Woman's Condom (WC), with those during use of the FDA-approved FC2 female condom (developed by the Female Health Company).

Activity Description:

The field of biomarkers of semen exposure has grown rapidly during the past decade, and PSA is clearly the most tested and probably the most accurate biomarker currently available. The main limitation of PSA is that it is a protein, which degrades in the vagina to undetectable levels approximately 24 – 48 hours after exposure. Therefore, CONRAD is developing DNA biomarkers of semen exposure. Preliminary data indicate that DNA biomarkers are detectable in the vagina between 7 – 15 days after semen exposure. Integration of self-report with PSA and or DNA biomarker detection offers the opportunity to strengthen the evidence that a particular self-reported problem indeed represents failure of the female condom; and, used together, self-report and biomarkers may provide the right criteria for female condom evaluation that does not require a biological endpoint such as pregnancy or infection.

Prior to initiation of CONRAD's PPRD Cooperative Agreement, CONRAD convened a 1-day meeting entitled

"Critical Next Steps for Female Condom Research - A Meeting/Workshop," in July 2008. Investigators in the fields of female condoms and semen biomarkers were charged with devising a study design that would validate a biomarker against a biological endpoint, such as pregnancy or a sexually transmitted infection (STI), so that the validated marker could be used to augment a slippage and breakage study for approval of new female condoms, eliminating the need for the currently required contraceptive effectiveness trial. The meeting participants determined that a study to validate a semen biomarker against a clinical outcome was not feasible for either pregnancy or STI transmission. Since semen exposure following a condom failure does not always result in either pregnancy or infection, given the extreme variability in the occurrence of these outcomes due to differences in fertility, timing of sex, partner infection, participant compliance, and self-reporting of compliance, these biologic endpoints provide limited sensitivity and evidence that a condom has failed as a barrier. Detection of semen (or biomarkers to include DNA and protein [PSA]) in the vagina following the use of a female condom is a significantly more sensitive measure to indicate that the female condom has failed. The meeting participants agreed that PSA would be the best biomarker for evaluating female condom performance, providing even stronger evidence for the barrier function of a new female condom than either pregnancy or STIs. The proceedings of this meeting were published in *Contraception* in May 2009 and included a suggested detailed study design for incorporating PSA into upcoming acute failure mode studies.

The working group's study design was developed into an acute failure mode protocol , D09-108 ("A Randomized Cross-Over Study of Vaginal Semen Exposure and Clinical Failure Comparing the PATH Woman's Condom and the FC2 Female Condom") for an open-label, randomized, crossover clinical study to be conducted in couples not at risk for pregnancy or STIs, comparing the WC with the FC2. The primary study objective of this study was to compare the performance of the WC to the FC2, as assessed by self-reported total clinical failure and its components (clinical breakage, slippage, misdirection, and invagination). Secondary objectives included: comparing the ability of the WC and the FC2 to prevent vaginal exposure to semen, as indicated by detection of PSA within the vagina; calculating the sensitivity and specificity of reported failures using PSA as the gold standard for the presence of semen; and comparing acceptability of the WC and FC2. This CONRAD acute failure mode study comparing the WC and FC2 at two centers, Los Angeles and Berkeley, under the direction of CFHC, California Family Health Council (CFHC) enrolled 425 couples, with 375 couples completing the study. Each couple was asked to use 4 condoms of each type and to collect a precoital vaginal swab, postcoital vaginal swab, and postcoital swab from inside the condom. The acute failure mode study comparing the WC and FC2 exited its last couple in June 2011.

In addition, NICHD conducted a single-arm contraceptive efficacy study of the WC in collaboration with CONRAD, CCN008 (A Multicenter, Open-Label, Non-Comparative Study of the Safety and Contraceptive Efficacy of the Woman's Condom). The NICHD study enrolled 511 couples at risk for pregnancy, with 244 couples completing the 6 months of study participation. Precoital vaginal, postcoital vaginal, and postcoital condom swabs were collected from the first 4 uses of the WC by 150 couples. The study exited its last participant in August 2012. It was hypothesized that the regulatory decision regarding approval of the condom would be the same regardless of whether data from the contraceptive efficacy study was considered.

Progress:

A working group of clinical researchers identified PSA as the most promising marker to pursue for this application because of the extensive, previous work. However, the idea of correlating PSA results with the biological endpoint of pregnancy in a contraceptive efficacy study was considered and dismissed because of the high rate of non-compliance with condom use in such studies. Using an STI endpoint was also dismissed due to the need for treatment of partners which, when combined with a low reinfection rate in the female condom group, would result in a sample size that would be prohibitively large.

Detection of semen (or biomarkers to include DNA and protein [PSA]) in the vagina following the use of a female condom was felt to be a significantly more sensitive measure to indicate that the female condom has

failed as a barrier, than either pregnancy or infection. The working group concluded that this type of evidence is actually *stronger* than that provided by a contraceptive effectiveness study or STI reinfection study, given the extreme variability in fertility, timing of sex, partner infection, participant compliance, and self-reporting of compliance expected in those studies.

A pre-IDE meeting with the FDA was held in early 2010 to discuss linking the two studies so that future female condoms will only need to undergo an acute failure mode study with a semen biomarker to obtain FDA approval. The FDA indicated that CONRAD only needed to undergo an acute failure mode study with a semen biomarker and requested additional clarification on the use of PSA in linking the CONRAD and NIH studies.

The goal of the acute failure mode study was to complete 1,500 uses of each condom type in order to meet the power requirements of the study. The study was completed in August 2012. The swabs were tested for PSA using a highly sensitive method with a lower limit of detection no higher than 1 ng/mL. Vaginal swabs with no detectable PSA were assessed for exposure to the vagina by testing for glycogen with epithelial cells using periodic acid-Schiff (PAS) staining. Both tests were run on swabs at the CDC under Dr. Macaluso. CFHC prepared the report, which included final GEE analysis and biostatistics input. The PATH Woman's Condom (WC) received higher acceptability ratings and caused less female discomfort than the FC2 condom. Moreover, the WC was non-inferior to the FC2 condom as measured by self-reports of condom failure, the standard means of evaluating an investigational condom. However, the probability of semen exposure during uses of the WC was non-inferior to that of the FC2 condom, suggesting that PSA testing can contribute critical information to the evaluation of experimental female condoms.

Data from the NICHD study and the acute failure mode study were assessed. Testing of PSA swabs from the NICHD study was completed by the CDC in April 2013 and specimens were received by Deborah Anderson for PAS/glycogen testing in May 2013. The NICHD contraceptive study was submitted to the Woman's Condom IDE by CONRAD in March 2011. Extensive negotiation with the FDA was needed to come to an agreement on preclinical condom testing. The study was completed in August 2012. As of April 16, 2015, CFHC had just received PSA data from the NICHD study, and was preparing a comparative report. Health Decisions, Inc (HDI), the CRO managing the contraceptive trial for NICHD with regards to product needs, was to perform the statistical analysis of the rest of the data from the NICHD study.

In addition to maintaining the IDE, CONRAD remained the main liaison between PATH and HDI. CONRAD also arranged for the USAID-required QA testing of the products used in both studies. This QA testing was carried out according to ISO standards by FHI.

CDC analyzed the swabs for PSA and Deborah Anderson's laboratory at Boston University adapted an assay for soluble glycogen to demonstrate vaginal exposure of the swabs. When applying it to the swabs obtained in the acute failure mode study, however, the results using the new assay did not completely agree with the established (but labor-intensive) PAS method. This may have been due in part to the repeated thawing and refreezing of the samples. As a result, the initial statistical analysis did take into account glycogen testing, but subsequent exploratory analyses would.

The decision on the part of USAID not to support submission of a PMA but rather to use WHO prequalification to procure PATH Woman's Condoms for women in developing countries led to a "gap analysis" effort on the part of NICHD to determine requirements to file a PMA without USAID support. In the course of that analysis, the notion of petitioning the FDA to reclassify female condoms as Class II devices which could be approved via the less onerous 510(k) route was discussed. In June 2014, PATH informed CONRAD that the National Female Condom Coalition (NFCC) had scheduled a meeting with the FDA in July to discuss changes to the female condom part of the FDA website and the FC2 labeling. The NFCC was also

interested in the possibility of reclassifying female condoms as class II devices in order to facilitate approval of new designs. If the FDA was receptive and if the study data support reclassification, plans will be made to pursue this course, both for approval of the PATH WC and for future designs. Funding for this effort is needed.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

Incorporation of PSA testing within functionality studies of experimental female condoms may provide a viable alternative to contraceptive efficacy studies which are expensive, lengthy, and vulnerable to selection bias, poor compliance, and high dropout rates. This easing of regulatory requirements would likely encourage more research leading to improved female condom designs.

Involvement of Other Donors and Interested Parties:

CDC contributed technical expertise (in-kind) for up to 1 FTE/year and matching funds at an estimated rate of $100,000/year (depending on availability of funding). NICHD provided $425,000 in Interagency Agreement funds to CONRAD to co-support the acute failure mode study as well and the Female Health Company donated 3,000 FC2s for use in this study.

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Activity Category:	#4 – Other
Activity Title:	#4.3 – Public Relations Services
CA Activity Numbers:	PPC-09-003, PPC-08-024
Primary Country of Activity:	USA
Technical Coordinator:	Doug Colvard
Implementers:	Annette Larkin; Firmseek (Website developer)
Period:	10/1/08 – 9/4/14

Objective:

The main objective of this activity was to provide outside professional and supporting services across all of CONRAD. Such activities included media consultation, message development, media outreach, website development, technical support services relating to public relations and other internal and external communication needs. In particular, public affairs and strategic media counsel would be needed related to trial results announcements such as CAPRISA 004 in 2010, and FACTS 001, planned for 2015. Media relations support for FACTS 001 would include media training for site staff, preparing for each DSMB meeting, and working with other organizations to keep them informed. Support for an adaptive trial workshop, submission of a satellite session at HIVR4P, and day to day management of the website.

Activity Description:

CONRAD has played key roles in the development of microbicides for HIV prevention, facilitated extensive collaborations between investigators, other development organizations, for-profit partners, and funders, as well as played key roles in promoting the microbicide field. In lieu of having in-house public relations staff, CONRAD contracts with knowledgeable PR organizations on an "as needed" basis for professional assistance in communications, message development, media outreach, and development of strategic materials is critical for CONRAD to remain a leader in the microbicide development field.

AJL Consulting was to provide CONRAD with their experience in dealing with the media, as spokespeople in situations, as day-to-day proactive communicators and as media trainers and to improve CONRAD's web presence and other media relations.

Progress:

The PR consultant:

1) Assisted CONRAD in its message development, in particular, helping CONRAD to adopt focused messaging, proactively educate key reporters, and strategically target media outlets;

2) Provided issue expertise, serving as CONRAD's representative on the Microbicides Media and Communications Initiative (MMCI) to communicate developments related to CONRAD to the entire microbicides network;

3) Provided website development assistance to enhance its value as a tool for reporters, to potentially use it as a strategy to attract donors, and to provide up-to-date information for the general public;

4) Raised awareness of CONRAD's work in developing microbicides and contraceptive methods, and provided support to respond to media queries and/or in the event of a crisis;

5) Assisted the organizers of M2012 with media coverage and arranged a co-sponsored event with the Global Campaign for Microbicides at M2012.

6) Assisted in strategic material development, including annual reports and reports to the field.

7) Provided guidance and counsel on communications around the ongoing FACTS 001 trial. This includes attending the annual FACTS meetings and working with site staff on messaging and issues related to communications. At trial end, whether that is due to a DSMB recommendation or successful completion of the trial, Annette Larkin is responsible for all external and internal audience communication

8) Prepared messages and media strategy based on possible outcomes of the FACTS 001 Data and Safety Monitoring Board (DSMB).

9) Attended the FACTS 001 annual meeting and provided media training to all of the principal investigators and site staff.

10) Provided strategic communications/messaging during the VOICE trial – at trial end and during CROI to manage public perception of tenofovir gel and clinical trials in general.

11) Revised the website to focus more on MPTs, tenofovir products and currently working to expand information on CONRAD's work in biomarkers and adherence.

12) Initiated an effort to host an adaptive trial workshop and provided support before and during the event.

13) Represented CONRAD on the MMCI and attended all calls and meetings.

14) Worked with a videographer to create a short film on the TFV LNG ring in development by CONRAD.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

Public commiunicaitons and messaging is vital for broad understanding and advocacy of biomedical HIV prevention research and development, including media staregies for the field's challenges as well as successes. By managing announcements of key developments, including the Phase 1 initiation of the TFV/LNG IVR and the FACTS 001 trial results, and working closely with investigators, funders and advocates to minimize negative or unwarranted press coverage, continued support for the microbicide field was strengthened. The consultant organized two events/workshops on adherence, an issue that is currently the biggest barrier to successful HIV prevention studies in young, African women.

Involvement of Other Donors and Interested Parties:

Some website development costs were co-funded with CONRAD's private foundation funds.

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Activity Category:	#4 – Other
Activity Number and Title:	#4.4 – Regulatory Support for 1% Tenofovir Gel Investigational New Drug (IND) Application and New Drug Application (NDA) Submissions
CA Activity Numbers:	PPC-12-147, PPC-12-148, PPC-12-153, PPC-12-162
Primary Country of Activity:	USA
Technical Coordinator:	Jill Schwartz (Archana Reddy, Marianne Callahan)
Implementers:	Accenture, Inc.
Period:	6/1/12 – 9/4/14

Objectives:

The objectives of this activity were to complete the regulatory activities required for tenofovir (TFV) gel licensure. To accomplish this goal, CONRAD needed the additional services of an outside CRO for preparation of the electronic New Drug Application (NDA) submission. Accenture has built a strong reputation in the marketplace as a leader in the implementation of Clinical Data Interchange Standards Consortium (CDISC) standards and electronic regulatory submissions by continuously working with global regulatory health authorities and industry committees. Accenture is a CDISC corporate sponsor and a registered solution provider. Members of Accenture's Data Strategies and Standards team lead and participate in CDISC development, testing, and training initiatives. Their Regulatory Affairs and Operations team is comprised of recognized leaders with experience in implementation of electronic Common Technical Document (eCTD) and other electronic submission standard issues.

Activity Description:

In order to complete the regulatory activities required for TFV gel licensure, CONRAD needed the additional services of an outside CRO for preparation of the NDA. Also, CONRAD acquired Accenture's Quantum™ Submission/Document Publishing software to make the transition to the eCTD and electronic submission for FDA reporting, as eCTD submission was to become an FDA requirement for regulatory reporting for the pharmaceutical industry by 2015. This software ensures that CONRAD is FDA-compliant by enabling us to have document version control and accountability, and meet industry standards when submitting our investigational new drug (IND) application submissions and the then-anticipated TFV NDA.

During the "End-of-Phase 2" meeting for TFV gel in October 2012, the FDA suggested that CONRAD meet with the Agency's biostatistical group to discuss the formatting of the datasets for the TFV NDA, which was Accenture's particular area of expertise. CONRAD requested Accenture assistance in several areas, two of which were funded under this activity:

1. Support for eCTD Publishing and Content Management Software as a Service
2. Regulatory Affairs & Clinical Data Management/Statistical support Consulting Services

Progress:

CONRAD acquired Accenture's Quantum™ Submission/Document Publishing software to make the transition to the eCTD and electronic submission software. The first INDs submitted electronically using this system were for the TFV tablet and the TFV/emtricitabine (FTC) combination tablet in August 2012, with Amendment #1 submitted in March 2013. Submissions to the TFV 1% gel IND continued to be made under the paper system, with 19 being made in 2012 alone, however plans were put in place for electronic submission of the TFV 1% gel NDA.

Accenture began Study Data Tabulation Model (SDTM) data conversion in early 2013 of the 17 clinical studies that were to be part of the NDA dossier. This new combined database being built by Accenture was to be used to prepare the Integrated Summary of Safety / Integrated Summary of Effectiveness (ISS/ISE) based on the statistical analysis plan to be developed by Accenture with input and approval from CONRAD. CONRAD transferred documents from FHI 360 (CONRAD 095 and CONRAD 099), CAPRISA (CAPRISA 004), and SCHARP (HPTN 050, HPTN 059, MTN 001, MTN 002, MTN 003, and MTN 006) for data conversion and organized several teleconferences with SCHARP in preparation for other MTN datasets to be sent later in the year.

CONRAD oversaw Accenture's work to submit the Type C (Biostatistical Guidance) meeting request and associated briefing package to the Center for Drug Evaluation and Research (CDER) Review Division of the Division of Antiviral Drug Products (DAVP). CONRAD and Accenture also began drafting the overall ISS/ISE plan. The meeting request was submitted in June 2014 and DAVP provided their written response in July 2014. This response provided recommendations from the FDA on the way the datasets from FACTS 001 and other ongoing studies were to be formatted for the NDA submission.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

Given that the FACTS 001 study was unable to confirm the CAPRISA 004 study results, licensure of tenofovir gel is not currently being pursued.

Involvement of Other Donors and Interested Parties:

Completion of this work will be funded through CONRAD's APS Cooperative Agreement, AID-OAA-A-14-00011, "Completion of Clinical Studies Required for TFV Gel Licensure." This overall activity was co-funded by the Gates Foundation.

CONRAD
Microbicide Account - Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#4 – Other
Activity Number and Title:	#4.5 – General Support Services
CA Activity Numbers:	EVMS 750999
Primary Country of Activity:	USA
Technical Coordinator:	Doug Colvard
Implementers:	Numerous vendors
Period:	10/1/08 – 9/4/14

Objective:

This activity includes expenditures related to the CONRAD projects and activities which did not readily fit CONRAD's typical subagreement or contract mechanism. Thus, they were typically costed to general Extramural Support Services.

Activity Description:

This activity included goods and services directly related to ongoing projects and activities that were not readily covered under CONRAD's typical subagreements and contracts.

Progress:

General Extramural Support Services included many items, such as:

- Purchase and shipping charges for supplies used in clinical studies
- Peer review of clinical supply packaging and clinical labels
- Storages fees for toxicology studies of microbicides
- Add-ons to stability studies
- - cGMP stability storage for elvitegravir drug substance
- Occupational health recategorization of TFV API and development of a revised MSDS
- Purchase of cynomolgus monkey plasma and vaginal tissue to support bioanalytical method dev/validation for MIV-170 at Huntingdon Life Sciences
- Purchase of cynomolgus and pigtail macaque reproductive tracts for method dev/validation at MPI Research
- Purchase of clinical supply bags and labels
- Shipping CONRAD materials from Azopharma to DPT to continue stability studies
- Micronization of MIV-170 API
- Co-share of clinical liability insurance
- Destruction of materials
- Release testing for GMP batch of micronized UC781
- Lab analysis of samples from biomarkers clinical study

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention

Programs:

The work funded under this Activity was important in moving many different development projects along in a timely and effective manner. As a result, larger activities were able to move ahead, leading to in some cases to the clinical evaluation of new products.

Involvement of Other Donors and Interested Parties:

None

CONRAD
Microbicide Account - Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#4 – Other
Activity Number and Title:	#4.6 – Manufacturing and Packaging of Tenofovir 1% Gel and Clinical Oversight of a Phase I Safety Study of the PATH Applicator
CA Activity Numbers:	PPC-09-027
Primary Country of Activity:	USA
Technical Coordinator:	David Friend (and Marianne Callahan)
Implementers:	DPT Laboratories; CONRAD clinical staff
Period:	01/01/10 – 12/31/12

Objective:

The objective of this activity was to prepare tenofovir (TFV) 1% gel for a Phase I clinical safety study of the PATH paper applicator for vaginal products and to provide oversight for the study.

Activity Description:

CONRAD has been responsible for the manufacture of GMP TFV 1% gel clinical supplies for several microbicide trials. All TFV 1% gel supplies for clinical studies implemented during the period of PPRD were prepared initially at Patheon Pharmaceuticals and then at DPT Laboratories under the direction of CONRAD. For this activity, DPT was to manufacture and package TFV 1% gel for use in a Phase I clinical safety study to be conducted at Profamilia in the Dominican Republic and sponsored by PATH. DPT was to produce and package a single batch of 200 kg TFV 1% gel and perform microbiology, chemistry, and stability testing on the finished product.

The primary objective of the PATH clinical study was to compare the effects of the pre-filled plastic applicator and the user-filled paper-applicator on symptoms and signs of irritation of the external genitalia, cervix, and vagina as seen on colposcopy after 7 days of twice daily use. The secondary objectives were to evaluate and compare the dosing accuracy and precision of the user-filled applicator compared to the pre-filled applicator. It was anticipated that the study results would support the delivery of TFV with the user-filled, paper applicator, in addition to the pre-filled plastic applicator that was currently being used in clinical trials.

Progress:

DPT manufactured and packaged a single batch of 200 kg TFV 1% gel and shipped the supplies to the study site. DPT performed microbiology, chemistry, and stability testing on the finished product throughout the clinical portion of the study.

PATH, CONRAD and Profamilia conducted a randomized comparative study in the Dominican Republic to assess the safety, acceptability and dose delivery of the user-filled, paper applicator compared to the plastic, pre-filled applicator for delivery of TFV gel. The study was submitted to CONRAD's TFV IND #73,382 with PATH as the study sponsor and CONRAD as the IND sponsor. A Transfer of Sponsor Obligation was

prepared to document the CONRAD IND responsibilities assumed by PATH. As CONRAD's responsibilities included the collection of critical regulatory documents, CONRAD monitored the clinical study to assure compliance with Good Clinical Practice.

In this cross-over study, 25 participants were randomized to use either the paper or plastic applicator to deliver two 4.0 ml doses of TFV gel into the vagina each day for 7 consecutive days. They delivered one dose of TFV gel at the clinic under observation each day, and one dose at home each evening. For measurement of applicator safety, the primary study outcome, colposcopy was conducted at two time points in each study arm. Results from this study suggested that the paper applicator was safe, with no colposcopic findings or AEs attributable to the paper applicator or the plastic applicator. To assess participants' ability to use the paper applicator as directed (fill and dispense target dose of 4.0 ml of TFV gel), dose delivery data was collected at three time points in each study arm for a total of 75 dose delivery measurements for each paper applicator. The data suggested that participants were able to fill the paper applicator according to instructions, delivering an average dose of 3.83 ml of TFV gel, representing 96% of the target 4.0 ml dose. With regard to dosing precision, 85% of doses expressed were within +/-10% of the target 4.0 ml dose. With regard to paper applicator acceptability, 100% of participants found the applicator easy to fill and were confident /very confident that they had inserted the correct amount of gel into the applicator. The paper applicator rated similarly to the plastic applicator with regard to ease of use, insertion, dispensing and comfort. The paper applicator was preferred over the plastic for disposal, while the plastic applicator was found preferable for storage. In relation to future willingness to use the paper applicator for delivery of TFV gel for HIV prevention, 96% (24/25) of participants expressed willingness to use the paper applicator and 100% indicated they would recommend it to other women if it came with a gel that helped prevent HIV. Overall, when asked to select a preferred applicator, 60% preferred the paper, user-filled applicator to the plastic, pre-filled.[1]

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

The results of this Activity support the delivery of a microbicide gel by user-filled paper applicator. The high cost associated with providing microbicide gel in the plastic, pre-filled applicator could be a barrier to widespread access and use. Specifically, the production and filling costs of the plastic, pre-filled applicator are the major proportion of the overall product cost. The user-filled paper applicator provides significant cost-reduction in the overall cost per dose. As such, the paper applicator provides an important component of the introduction strategy for microbicide gel delivery in low-resource settings.

Involvement of Other Donors and Interested Parties:

PATH provided financial support for a CONRAD study monitor and fully supported the clinical study site using its USAID funds.

[1] Cohen J, Brache V, Foster J, et al. A randomized, comparative safety study of a prefilled plastic and user-filled paper applicator with candidate microbicide Tenofovir 1% gel. American Sexually Transmitted Diseases Assocation. 2013; 40(6): 476-481.

CONRAD
Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#5 – Support for Other Organizations
Activity Number and Title:	#5.1a – Regulatory Support and Trial Oversight and Management for FACTS 001
CA Activity Numbers:	PPA-11-124, and numerous others
Primary Country of Activity:	South Africa
Technical Coordinators:	Jill Schwartz, Susan Ju, Kim Linton, others
Implementers:	FACTS, ACRO, Logenix, Ramon Dempers, Michele Commins, Tracey Webster
Period:	7/1/11 – 9/4/14

Objective:

The proof-of-concept for a microbicide, tenofovir (TFV) 1% gel, to prevent HIV-1 and Herpes Simplex Virus-2 (HSV-2) infections was demonstrated by the CAPRISA 004 results and led to shifting priorities for CONRAD and the microbicide field. Registering TFV gel and making it available in South Africa and other African countries became top priorities. CONRAD provided study product, regulatory support, and oversight and management for clinical trials designed to enable registration in South Africa and other African countries. This activity covered the first of these trials, FACTS 001, "Support for FACTS 001: A Phase III Multi-Centre, Randomised Controlled Trial to Assess the Safety and Effectiveness of the Vaginal Microbicide 1% Tenofovir Gel in the Prevention of Human Immunodeficiency Virus Type 1 Infection in Women, and to Examine Effects of the Microbicide on the Incidence of Herpes Simplex Virus Type 2 Infection."

Activity Description:

The Follow-on African Consortium for Tenofovir Studies (FACTS) designed the FACTS 001 study to confirm the results of the CAPRISA 004 study to determine whether the study findings could be generalized and to support licensure and implementation in South Africa. This multi-center trial was conducted at 9 sites in South Africa. It was a Phase III, 2-arm, double-blind, randomized, placebo-controlled trial of the safety and effectiveness of TFV 1% gel applied intravaginally in preventing sexually transmitted HIV-1 infection in women and to examine the effects of TFV on the incidence of Herpes Simplex Virus (HSV-2) infection.

This was an event-driven trial designed to randomize sexually active women ages 18-30 at high risk for sexually transmitted HIV to use vaginal TFV 1% gel or placebo gel using the BAT24 regimen (within 12 hours before and after a coital event). All women were evaluated for adverse events (AEs) and the rate of HIV seroconversion. In addition, the study was designed to evaluate several secondary endpoints that bear directly on potential risks and benefits of TFV 1% gel use. In the final version of the protocol, Version 5.0, an estimated 2,900 participants (N=1450 to use TFV 1% gel and N=1450 to use placebo gel) were planned for enrollment in the trial overall; approximately 2,600 women aged 18-30 years of age were to be enrolled to achieve the required 118 incident infections needed to detect a product effectiveness of 45%, and up to 300 additional women aged 31-40 years were to be enrolled to collect safety information in this older age group, although the target endpoints were reached before older women were enrolled.

Progress:

As sponsor of the study, CONRAD provided regulatory support and oversight and management of the implementation of FACTS 001. In partnership with the South African FACTS team (CORE), CONRAD

developed and implemented quality assurance and management systems to ensure FACTS 001 was conducted and documented to the highest good clinical practice (GCP) standards, is able to deliver high quality data promptly, and will lead to an International Conference on Harmonisation (ICH)-compliant clinical study report as soon as practical after study completion. The African Clinical Research Organisation, Ltd. (ACRO) was hired to act as the local regulatory applicant and study contract research organization (CRO) and Triclinium, Ltd, a second CRO, assisted with study monitoring. Ramon Dempers from Umthunzi and Michele Commins provided consultant services to manage the oversight of this trial, and an in-country Project Manager (Tracey Webster) provided oversight on the ground. The CONRAD Product Development group supervised the manufacturing and shipping of study product.

In addition to reviewing monitoring reports at all sites, CONRAD engaged Tracey Webster and Retha Britz (Triclinium) to perform quality control mentoring visits to all nine sites (October – December 2013) with a focus on Standard Operating Procedures and adherence processes. Tracey Webster subsequently performed follow up visits (May - July 2014). In November 2013, Michele Commins reassessed both ACRO and CORE to ensure proper follow up of issues identified in her previous assessment in 2012. In March 2014, Ramon Dempers reassessed processes and queries from his initial visits (November – December 2012) with Logic Trials, Clinical Laboratory Services (CLS), Perinatal HIV Research Unit Data Management Centre (PHRU DMC) and ACRO; in addition, he ensured that the In-Depth Interview process was sound with regard to electronic transmissions and processes.

The FACTS 001 trial began enrolling in October 2011; 3,846 volunteers were screened and 2,059 women enrolled. All active recruitment and enrolment ended March 1, 2014, and the protocol-specified number of endpoints was achieved by the end of March 2014. Product discontinuation visits commenced April 1, 2014 and study exit visits began the following July. The last participant's last study visit took place August 29, 2014. Due to the endpoints having been achieved, the older cohort was not enrolled.

To enforce CONRAD's sponsor oversight responsibilities, the CONRAD FACTS 001 team developed a study project management plan in conjunction with CORE and ACRO. Monthly study and monitoring reports produced by ACRO and Triclinium were evaluated by CONRAD. The CORE team was responsible for the overall protocol development and implementation of the study. The Clinical Safety Committee consisted of representatives from CORE and ACRO, and the CONRAD Medical Director, and held weekly calls to review all safety events, pregnancies, and to provide guidance on the management of trial safety-related issues. The Escalation and Corrective Action Plan, Data Quality Management Plan, and Communication Plan were included in the overall oversight plan. Monthly data reports were generated from the PHRU DMC by CORE. These reports tracked key trial performance indicators including recruitment, retention, adherence, safety events, data quality and transmissions rates. In addition, monthly monitoring of product adherence included a calculation of the percentage of applicators used, as assessed by visual inspection of returned applicators (VIRA) and self-report. The sites were provided with a line listing of participants with multiple missed visits and low adherence along with monthly priority emails and were asked to provide specific corrective action. CONRAD provided oversight by working closely with CORE and ACRO and by reviewing the information contained in the monthly data reports and the monitoring reports.

CORE performed adherence workshops at all sites in August-September 2013 to emphasize the importance of adherence to product and to further strengthen related processes at sites.

After product use ended in the study, the FACTS study team focused on obtaining adherence data; cervicovaginal lavage (CVL) samples were analyzed by a U.S. laboratory as an objective measure of adherence to tenofovir gel use. The primary objective of this analysis was to examine the association between detectable TFV concentrations in cervicovaginal fluid and the risk of HIV acquisition. In this case-cohort study, CVL samples (collected quarterly) in all participants in the treatment group who seroconverted (cases)

and in 3-5 controls per case for a total of about 1,500 samples were evaluated. A substudy (FACTS 001A) was also implemented at all sites to collect applicators from active participants at one visit to assess for markers of vaginal insertion, semen and the presence of tenofovir.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

After this reporting period, the funding for this activity continues under Cooperative Agreement AID-OAA-A-14-00011. The results of FACTS 001 were presented at the Conference on Retroviruses and Opportunistic Infections (CROI) in late February 2015, and trial close out activities are currently in progress.

Involvement of Other Donors and Interested Parties:

The completion of the trial is being be funded through Cooperative Agreement AID-OAA-A-14-00011, "Completion of Phase III Clinical Trial, New Drug Application Dossier, and Implementation Studies for Tenofovir 1% Gel, a First-in-Class Topical Product for the Prevention of HIV Acquisition by Women."

Helen Rees, (Protocol Chair), Glenda Gray (Protocol Co-Chair), Carl Lombard from Medical Research Council (statistical support), and ACRO (regulatory and operational support) provided support for FACTS 001. Gates Foundation funding to CONRAD was used to manufacture and label the TFV 1% gel supplies. Although Gates funding was intended to cover all of the manufacturing, PPRD funds were used for a small portion of the manufacturing and the shipping of drug. Gates funding was also used to support regulatory activities and for the independent sponsor audits of the clinical sites performed by Triclinium.

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Microbicide Account - Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#5 – Support for Other Organizations
Activity Number and Title:	#5.1b – Regulatory Support and Trial Oversight and Management for TFV gel Adolescent Studies
CA Activity Numbers:	Numerous
Primary Country of Activity:	South Africa
Technical Coordinator:	Jill Schwartz, Kim Linton, Sarah Littlefield and others
Implementers:	AECOM, University of Utah, Agility, FACTS, ACRO, Logenix
Period:	7/1/11 – 9/4/14

Objective:

The proof-of-concept for a microbicide, tenofovir (TFV) 1% gel, to prevent HIV-1 and herpes simplex virus-2 (HSV-2) infection was demonstrated by the CAPRISA 004 results and led to shifting priorities for CONRAD and the microbicide field. Registering TFV gel and making it available in South Africa and other African countries became top priorities. CONRAD would provide study product, regulatory support, and oversight and management for clinical trials designed to enable registration in South Africa and other African countries.

Data on the use of TFV 1% gel in adolescents are an essential component of registration of the gel. Young SA women are at particularly high risk for HIV infection because they initiate sexual activity at an early age, have multiple partners, and use condoms inconsistently. Although it is critical to obtain data on young women in Africa, the FDA also requires safety data in US adolescents. CONRAD pursued studies to obtain data in both populations of adolescents.

Activity Description:

Young women bear the brunt of the HIV epidemic in southern and eastern Africa. A clinical study, FACTS 002 ("A Phase II, Randomised Controlled Trial to Assess the Safety and Acceptability of the Vaginal Microbicide 1% Tenofovir Gel in Healthy, HIV-Uninfected, Sexually-Active Women 16 and 17 Years of Age"), was planned to study the safety of tenofovir gel exclusively in 16- and 17-year old women. This trial would be an essential component on the pathway to registration of the gel for use by adolescents and critical to public health implementation. These data are required both because of the high acquisition rate of HIV in this population and the likelihood that adolescents would use this product once it is on the market. FACTS 002 was originally planned to be a Phase II, 2-arm, double-blind, placebo-controlled trial of the safety and acceptability of tenofovir 1% gel administered intravaginally daily for up to 6 months by 60 healthy, sexually active, HIV-uninfected adolescent women aged 16-17 years. Participants were to be randomly assigned to TFV 1% gel or placebo in a ratio of 2:1and to be evaluated for local and other adverse events, and have TFV concentrations measured in serum and vaginal fluid obtained by aspirate. In addition, the study was to evaluate several secondary endpoints that bear directly on potential risks and benefits of vaginal TFV gel use, including the acceptability of gel use. In February 2012, the US FDA recommended that daily dosing with a second dose on days of sexual intercourse be considered and that the sample size be increased to at least 100 subjects.

Thus, the study would have 2 parts. In Part 1, 30 female participants (20 assigned to TFV and 10 to placebo) will use the gel once daily (with an additional application after coitus) for 4 weeks. In Part 2, 70 female participants (50 assigned to TFV and 20 to placebo) will use gel for 24 weeks before and after coitus (BAT24 regimen). Participants will be instructed to use a minimum of 2 BAT24 regimens (4 doses of gel) per week, regardless of sexual frequency. This would provide intense safety data and the dosing regimen intended for safety in adolescents as well as the dosing regimen which was currently under investigation in the FACTS 001 HIV prevention trial and intended for licensure, namely coitally driven BAT24.

The clinical study FACTS 002 was originally submitted to the South African Medicines Control Council (MCC) in May 2011. In July 2012, in response for approval MCC, they responded that the review of the FACTS 002 protocol could only proceed after it had reviewed and analyzed the following: 1) preliminary data of FACTS 001; 2) clinical study report of CAPRISA 004, and 3) clinical study report of VOICE tenofovir gel arm. Since FACTS 001 data would not be available until after this Cooperative Agreement ended, alternative safety studies in adolescences were planned.

In the first of these, CONRAD made a request to NIH/NIAID/DAIDS to reconsider their decision to place MTN-021 on hold. MTN-021 was a clinical trial planning to enroll 90 15-17 year olds for 3 months of TFV gel exposure through the Adolescent Medicine Trials Network (ATN). This was discussed at the MTN Executive Committee Meeting on August 3, 2012 and there was general enthusiasm for this study, with the ATN contributing site costs, but no funds available for SCHARP's data management costs. However, on December 10, 2012, CONRAD was informed by DAIDS that they had reconsidered their decision due to their organizational priorities and withdrew support.

CONRAD also designed a safety study to be conducted in US adolescents which would be conducted at two centers: the Albert Einstein College of Medicine and the University of Utah. It would be a two-arm, double-blind, placebo-controlled trial of the safety and acceptability of TFV or placebo gel use by 60 healthy sexually experienced HIV-uninfected 15-17 year olds over 12 weeks of use. Participants will be instructed to use a minimum of 2 BAT24 regimens (4 doses of gel) per week, regardless of sexual frequency.

Progress:

With regard to the FACTS 002, a revised protocol was submitted to the MCC in Q1 2013 to explain that no significant safety findings have been observed in the TFV 1% gel studies to date and that this study was on a critical pathway. The MCC responded that they would wait for the first Data and Safety Monitoring Board (DSMB) interim review of FACTS 001 to conduct their review. Although it was important to get data on young women in Africa, we sought to pursue other options to acquire these data prior to a planned Q1 2016 new drug application (NDA) submission.

Since the FDA also requires safety data in U.S. adolescents, we planned a study to be conducted at the Albert Einstein College of Medicine and other collaborating adolescent centers for a potential 2-arm, double-blind, placebo-controlled trial of the safety and acceptability of TFV or placebo gel use by 90 healthy HIV-uninfected 15-17 year olds over 12 weeks of use. Participants would be instructed to use gel before and after coitus (BAT24) and to use a minimum of 2 doses per week, regardless of sexual frequency. In addition to safety and acceptability, cervicovaginal (CV) fluid would be assessed for drug and biomarkers of genital mucosal innate immunity, since mucosal responses differ between adolescents and adult women.

Active protocol development for the US adolescent study began in January 2014. In April 2014, CONRAD began work with Sybil Hosek, PhD to develop the acceptability and adherence measurement tools for this study. Dr. Hosek has extensive experience working with adolescent populations in the context of PrEP clinical development. The study would utilize text message technology and secure internet-based survey to collect

adherence data throughout the 12 weeks of use, as well as ACASI interviews at follow up visits to collect acceptability information. CONRAD will develop the ACASI system via Questionnaire Design System (QDS), supplied by Nova Research Company. ACASI would be implemented at the sites via windows-based tablets.

The Protocol A14-130, Phase I Safety and Acceptability of Tenofovir 1% Gel in Adolescent Females, Version 1.0, was submitted to the Albert Einstein College of Medicine IRB on August 19, 2014, with subsequent submission to University of Utah IRB. After this reporting period, the funding for this activity continued under Cooperative Agreement #AID-OAA-A-14-00011. CONRAD submitted the protocol to the FDA on October 8, 2014 and received comments on October 27, 2014. Agility was to provide data management and analysis and implement electronic data capture (EDC) for this study and began work December 17th 2014. Based on the FDA comments CONRAD amended the protocol and submitted the revised protocol, Version 2.0, along with responses to FDA comments on December 23, 2014. The investigator go-to-meeting was held on March 2, 2015. Given the FACTS results the decision was made to not move forward with this study. The sites were notified in writing of this decision on April 2, 2015.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

Given the decision to not move forward with this study, data on the safety of TFV gel in adolescents will not be acquired.

Involvement of Other Donors and Interested Parties:

Continued funding for this activity continued through Cooperative Agreement AID-OAA-A-14-00011, Completion of Phase III Clinical Trial, New Drug Application Dossier, and Implementation Studies for Tenofovir 1% Gel, a First-in-class Topical Product for the Prevention of HIV Acquisition by Women.

CONRAD
Microbicide Account - Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#5 – Support for Other Organizations
Activity Number and Title:	#5.1c – Regulatory Support and Trial Oversight and Management for CAPRISA 008
CA Activity Numbers:	PPA-12-143, PPA-12-144
Primary Country of Activity:	South Africa
Technical Coordinator:	Jill Schwartz, Marianne Callahan, Kim Linton, others
Implementers:	CAPRISA, Logenix
Period:	7/1/11 – 9/4/14

Objective:

The proof-of-concept for a microbicide, tenofovir (TFV) 1% gel, to prevent HIV-1 and herpes simplex virus-2 (HSV-2) infection was demonstrated by the CAPRISA 004 results and has led to shifting priorities for CONRAD and the microbicide field. Registering TFV gel and making it available in South Africa and other African countries became top priorities. CONRAD provided study product, regulatory support, and oversight and management for clinical trials designed to enable registration in South Africa and other African countries. This activity covered the first of these trials, CAPRISA 008, "Open-Label Randomized Controlled Trial to Assess the Implementation Effectiveness and Safety of 1% Tenofovir Gel Provision through Family Planning Services in KwaZulu-Natal, South Africa."

Activity Description:

CAPRISA 008 was a follow-up study to CAPRISA 004. It was designed as a Phase IIIb, 2-arm, open-label, randomized, controlled trial. Approximately 700 sexually active HIV-uninfected CAPRISA 004 participants aged 18 years and older were to be enrolled from the CAPRISA eThekwini and Vulindlela clinics and their neighboring public sector family planning services in KwaZulu-Natal, South Africa. Participants were randomized to receive TFV 1% gel through either: 1) public sector family planning services with 2-3 monthly provision and monitoring of TFV 1% gel and the use of quality improvement methodology to promote reliable service delivery (intervention arm), or 2) the CAPRISA research clinics with monthly provision and monitoring of tenofovir 1% gel (control arm). Participants in both study arms were instructed to use TFV gel within 12 hours before and after a coital event. The primary endpoint of gel use would be assessed as the mean number of returned used applicators per participant per month. The sample size was estimated to provide 90% power to demonstrate whether gel use in women attending family planning services is similar, but no more than 20% lower than gel use among women attending the CAPRISA research clinics, stratified by study population and adjusted for 10% loss to follow-up. Safety was assessed by the frequency of adverse events using laboratory and clinical markers. The acceptability of family planning services for tenofovir provision was assessed using self-report and clinic attendance rates. The anticipated total study duration was approximately 30 months, with accrual requiring approximately 12 months and follow-up continuing for approximately 18 months after the end of the accrual period.

CONRAD provided study product, regulatory support, and oversight and management for this clinical study. Manufacture of the clinical supplies was supported using CONRAD's Gates Foundation funding.

Progress:

CAPRISA 008 received Medicines Control Council (MCC) approval on May 21, 2012. CAPRISA 008 was initiated and started screening in November 2012 and was conducted at 2 sites; Vulindlela and eThekwini. By Q3 2014, enrollment was complete. A total of 448 participants were screened and 382 of those participants were enrolled. After this reporting period, the funding for this activity continues under Cooperative Agreement #AID-OAA-A-14-00011. CAPRISA 008 starting exiting participants February 1, 2015 and all participants will exit by April 30, 2015. Quality Assurance checks will be performed and the database will be locked by Q2 2015. CAPRISA expects to have data by Q3 2015.

CAPRISA and CONRAD also began working with PATH on the acceptability study of the PATH paper applicator used with multi-dose tubes of TFV 1% gel (CAPRISA 070) under this agreement. The study was submitted to the MCC and BREC for review on July 8, 2014. Since the FACTS 001 study was unable to confirm the CAPRISA 004 study results, hindering the path to licensure of tenofovir gel, CONRAD (in consultation with CAPRISA and PATH) decided to defer the CAPRISA 070 study indefinitely.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

CAPRISA 008 will provide data on the feasibility and effectiveness of integrating microbicide provision into existing family planning services. In addition, CAPRISA 008 seroconversion data can be compared to a counterfactual group to provide additional effectiveness data on tenofovir gel.

Involvement of Other Donors and Interested Parties:

The completion of the trial is being funded through a new Cooperative Agreement AID-OAA-A-14-00011, entitled "Completion of Phase III Clinical Trial, New Drug Application Dossier, and Implementation Studies for Tenofovir 1% Gel, a First-in-class Topical Product for the Prevention of HIV Acquisition by Women."

CONRAD
Microbicide Account - Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#5 – Support for Other Organizations
Activity Number and Title:	#5.2 – Support for AVAC
CA Activity Numbers:	PPA-11-139
Primary Country of Activity:	USA
Technical Coordinators:	Marianne Callahan, Doug Colvard
Implementer:	AVAC
Period:	1/1/12 – 6/30/13

Objective:

As of 2012, the HIV prevention narrative was complicated, with mixed results from microbicides and PrEP trials, new microbicide trials beginning, planning in progress for potential implementation of tenofovir gel, and emerging issues, such as the use of hormonal contraceptives in prevention trials. Developing messages and materials that clearly explain the state of the field and address emerging issues was never been more important. AVAC uses education, policy analysis, advocacy and a network of global collaborations to accelerate the ethical development and global delivery of AIDS vaccines, male circumcision, microbicides, PrEP and other emerging HIV prevention options as part of a comprehensive response to the pandemic. AVAC and the Microbicides Media and Communications Initiative (which is coordinated by AVAC) are well-placed to help the field communicate the intricacies of microbicides and HIV prevention research.

Activity Description:

The workplan focused on developing a solid suite of materials and messages to explain the HIV prevention field and key issues and conducting outreach to key media and civil society partners to ensure that they are well informed and have accurate information about the state of the field, particularly tenofovir gel, and the promise of microbicides.

The first general objective was to provide support for AVAC to act as the secretariat for the Microbicides Media and Communications Initiative (MMCI), providing the technical leadership, administrative functions, and financial resources needed to support MMCI operations. The MMCI works to support integrated communications strategies across the microbicide field and in conjunction with the broader HIV prevention research field. The MMCI addresses the special communications challenges and opportunities of the current state of the field, with special focus on effectiveness trials in resource-limited settings, preparing for results, crisis communications and, now, issues regarding rollout and implementation.

The second general objective was to support implementation of the USAID Proposal for a Shared Vision and Strategic Plan for Microbicide Introduction, in particular, Strategy Element #3, which is to develop and implement a comprehensive communication and advocacy strategy for the introduction of microbicides, especially tenofovir gel. Experience with other HIV/AIDS prevention products has demonstrated the crucial need for effective product advocacy and for engaging civil society and the media as critical elements of a comprehensive approach.

Progress:

MMCI worked to provide communications support to in-country trial site staff and advocates through communications training focused on preparing for the VOICE results, and providing support to MMCI member organizations and others in the field through the development and dissemination of scenarios for the VOICE results and messages about both the results and the broader field. In addition, MMCI continued support for African reporters ahead of the VOICE results to help ensure accurate and supportive media coverage.

Group oversight and communications was a core activity. MMCI served as a conduit of information in the field and a platform for information-sharing between members. MMCI provided regular updates and forums for discussion for members and the broader field through conference calls and emails, including research updates and media analysis for ARV-based prevention news.

MMCI and AVAC worked closely with the Microbicides Trial Network (MTN) and other partners to prepare messages, FAQs and other materials in advance of the announcement of the VOICE results in early March and to ensure that MMCI members were kept up-to-date on plans and issues around the announcement. This included developing and sharing scenarios for the results and messages around each scenario

A special update session on microbicides research including an explanation of the VOICE results was included in the AVAC Fellows Orientation & Close-out meeting held in Cape Town in mid-March 2013. This meeting brought together current AVAC Fellows who are working on 1-year prevention research advocacy projects, and the alumni network working on broader HIV prevention issues. Finally, two roundtable discussions on Women's HIV Prevention Research were held in Cape Town and Johannesburg in mid-March. The advocates' presentation reinforced their support for research continuing and for looking for additional ways for community groups to partner with trial sites to support clinical research in the future.

MTN conducted VOICE (Vaginal and Oral Interventions to Control the Epidemic), a large clinical trial testing the safety and effectiveness of two different HIV prevention approaches among 5,029 women in Uganda, South Africa and Zimbabwe: daily use of an antiretroviral (ARV) tablet (tenofovir or Truvada) or daily use of a vaginal gel (tenofovir gel). AVAC prepared for the VOICE trial results and other microbicide trials in the following ways:

- AVAC/MMCI convened several conference calls with MTN communications officers and other key partners, including a confidential pre-embargo call to discuss the results with communications officers from microbicide and PrEP trials and a joint Communications Working Group call ahead of CROI to discuss potential stories, including the VOICE results.

- Key AVAC staff attended the FACTS Consortium annual meeting in February 2013 in Johannesburg, conducting a media training session for the 150 meeting attendees, focusing on the upcoming VOICE results. Staff also worked with CONRAD's Annette Larkin to provide individualized media training sessions for each of the FACTS 001 9 trial sites focused on responding to the VOICE results and potential impact on FACTS 001 sites.

- AVAC developed a document with possible VOICE results scenarios and shared with MMCI, the PrEP Communications Working Group, and key advocacy partners to help organizations plan their communications for the results.

- AVAC/MMCI disseminated and updated the Communications Handbook. The Communications Handbook is a valuable resource for trials site staff and others working on HIV prevention research communications. AVAC/MMCI worked with FHI 360 to disseminate workbooks and train trial site

staff and others on using the handbook, including providing Handbooks for the FACTS Consortium sites as part of a communications training program in February.

- Microbicides and HIV prevention research have been prominently highlighted in the news in the last 2 years and will continue to be newsworthy in the foreseeable future. To help ensure accurate and fair coverage, MMCI nurtured a core cadre of African reporters and expanded the group of reporters who are well-informed and able to report intelligently on HIV prevention research. AVAC's work in this area ahead of and around the VOICE results helped ensure several accurate and well-written articles in key African media outlets.

- AVAC worked with key reporters in Kenya, Uganda, South Africa, and in the U.S. (in individual meetings and through email and phone conversations) ahead of the VOICE results to ensure that they were prepared for the news and understood the possible scenarios and shared AVAC's statement on the results, MTN materials, and other resources with reporters after the announcement.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

AVAC uses education, policy analysis, advocacy, community mobilization and a network of global collaborations to accelerate ethical development and global delivery of biomedical HIV prevention options. Developing messages and materials for international stakeholders that clearly explain the state of the field and address emerging issues is critical for the success of and sustainability of HIV prevention programs.

Involvement of Other Donors and Interested Parties:

NA

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Activity Category:	#5 – Support for Other Organizations
Activity Number and Title:	#5.3 – Support for the Alliance for Microbicide and CAMI
CA Activity Numbers:	MSA-08-494; PPA-09-038
Primary Country of Activity:	USA
Technical Coordinator:	Marianne Callahan
Implementers:	Polly Harrison, Alliance for Microbicide Development; Bethany Holt (Public Health Institute/California Microbicides Initiative [CaMI])
Period:	**7/**1/08 – 12/31/10

Objective:

Founded in 1998, the Alliance for Microbicide Development employs monitoring, research, communication, convening, and evidence-based advocacy to speed development of microbicides to prevent HIV and other sexually transmitted infections. The Alliance would engage in the following activities: (1) meeting and planning management; (2) constitution and coordination of Working Groups required for implementing the objectives of the Microbicide Donor's Committee (MDC) and related initiatives; and (3) information, reporting, and dissemination of reports from MDC activities, as well as provision of regular streams of information to serve needs identified by the Committee.

The Public Health Institute/California Microbicides Initiative (CaMI) will collaborate with the Alliance and PATH to create a multi-disciplinary vehicle through which the advancement of multipurpose sexual and reproductive health prevention technologies and strategies can be achieved and to provide a multi-disciplinary vehicle through which existing and new efforts can be coordinated to leverage support (funding, marketing, testing and public policy).

Activity Description:

The Microbicide Donors Committee (MDC) was convened by the Gates Foundation and the Alliance in early 2004 as a forum for key microbicide researchers and funders to discuss next steps in microbicide development, and to facilitate and coordinate activities designed to reach consensus on critical portions of the R&D pathway. As Secretariat of the MDC, the Alliance will provide a steady, neutral, inclusive platform for dialogue on key science and policy issues by convening meetings of the MDC, the Quick Working Group (QWG), and Preclinical Working Group (PWG). The Alliance also works to link the MDC to the NIH/OAR Microbicide Research Working Group, and implementing projects that further the goals of both groups. The QWG was established as a mechanism for facilitating exchange of knowledge, experience, data, and ideas among the investigators involved in the first wave of microbicide effectiveness trials. The Alliance would convene regular meetings of the MDC and a range of scientific roundtables/workshops to discuss and harmonize pre-clinical and clinical research agendas and to address critical issues as they arise. The Alliance's website houses the *Microbicide Research and Development Database (MRDD)* and the new *Clinical Trial Site Capacity Database (CTSCD)*. The *MRDD* provides comprehensive and current information on microbicide candidate products, clinical trials, and supportive research.

A separate agreement was developed with CaMI to carry on with some of the tasks identified at the symposium on advancing prevention technologies for sexual and reproductive health held in Berkeley, CA, that were to be completed under their subcontract with the Alliance. The general objectives included: 1) dissemination of Berkeley Symposium Proceedings to appropriate audiences; 2) coordination of the New and Current Technologies working groups in managing data collection, work outcomes and communication; 3) promotion of awareness and advocacy around multipurpose prevention technologies; 4) identification of the next steps for advancing multipurpose prevention technologies both domestically and internationally, in collaboration with the Think Tank members; and 5) facilitation of conversations around funding and integration with key stakeholders.

Progress:

The MDC met in June 2008 in New York City and addressed updates from the QWG and the Microbicide Research Working Group, and led a general discussion of strategies for funding microbicide Phase 3 trials. The 8th meeting of the QWG took place in August 2008 in Arlington, Virginia. The meeting focused on reports from planned or recently-completed activities, such as the NIAID Phase 1 workshop, the PWG, the Adherence Meeting, the Alliance Annual Meeting, and select time-sensitive topics. The QWG again met in February 2009, addressing critical issues around ARV-based microbicides, activities of relevance to pre-exposure prophylaxis (PrEP) approaches. The PWG had planned to hold its first meeting following the NIAID Phase 1 design meeting in February 2009. Due to delays by NIAID, it was held in conjunction with the Alliance Annual Meeting in May 2009. That Annual Meeting focused on "The Changing Terrain for Microbicides," underscoring key issues in the microbicide field through a comprehensive retrospective of critical meetings, activities, and findings in the past year and an evaluation of current microbicide efforts to support the field's future growth.

The Alliance performed ongoing maintenance, troubleshooting, and quality checks to ensure that the website is working properly and is easy to navigate. In addition to quarterly and ongoing updates, in December 2008, the Alliance began work to improve the quality and user-friendliness of the MRDD, to power up its search capacity, and enhance user control over report generation. The Clinical Trial Site Capacity Catalogue was transitioned from PDF format to an interactive, more user-friendly database.. This database will be expandable for new fields of information and prevention options, and the Alliance is in discussion with key groups regarding potential collaboration in this effort.

Various staff members distributed publications, presented and gathering information, and/or reported on microbicide-related research and activities at several conferences, including AIDS 2008, Reproductive Health 2008, a WHO Nanjing Regulatory Meeting, AIDS 2031 Consultation, and ICASA 2008.

During 2009-2010, the Alliance continued engaging in similar activities to advance the field of microbicide develop by:

1) Convening 1 meeting of the MDC, 2 QWG and PWG meetings and a "What Next Think Tank."
2) Continuing to update, improve and expand the Alliance website and its two databases (the MMRD and CTSC).
3) Convening the Annual Alliance Meeting and sponsoring and organizing a microbicide-specific poster session at *Microbicides 2010.*
4) Participating in numerous domestic and international conferences to keep Alliance staff abreast of microbicide R&D and relevant discussion surrounding that research in order to fulfill the Alliance's unique roles of monitoring, tracking, and reporting on the field.

In 2010, primarily due to funding constraints, all of the Alliances resources and assets were transferred to

AVAC, and Dr. Harrison continued her role in microbicide development as a consultant to AVAC.

CaMI oversaw the dissemination of Berkeley Symposium Proceedings through various formats and venues such as a website, white paper, peer-reviewed article and other means that will reach target audiences. CaMI also coordinated the New and Current Technologies working groups, managing data collection, work outcomes and communications. This involved mapping, systematically tracking, reporting on, and promotion of evidence-based advocacy for the development of multi-purpose methods for the prevention of HIV, non-HIV STI's, RTIs and unintended pregnancies. In addition, they identified and coordinated input from other key stakeholders and experts in the field of SRH for the mapping of current and new technologies which was used to develop a Funding Case and other tools for advancing multipurpose prevention technologies for SRH. CaMI planned to continue outreach and education by attending and presenting at appropriate conferences (e.g., ARHP, M'2010) to raise awareness about multipurpose SRH prevention technologies. CaMI continued the scope of work described in the current activities, but submitted a plan for its own FY10 funding directly to USAID.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

The Alliance played a significant and leading role in bringing together and coordinating interactions by many of the stakeholders and decision makers in the microbicide development field and contributed greatly to the establishment of microbicide funding to USAID by Congress. That alone increased the possibility of improved, effective and sustainable responses to reduce HIV transmission.

Involvement of Other Donors and Interested Parties:

The Gates Foundation co-funded some of the Alliance's activities. Since the USAID support through CONRAD needed to start before CONRAD's PPRD award was available, CONRAD provided $240,000 of its CONRAD-III/microbicide funding to the Alliance.

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Activity Category:	#5 – Support to Other Organizations – CDC Collaboration
Activity Number and Title:	#5.4a – CDC Collaborations: Support of CONRAD staff
CA Activity Numbers:	N/A
Primary Country of Activity:	USA
Technical Coordinator:	Jennifer Legardy-Williams, MPH
Implementers:	CONRAD/CDC
Period:	7/01/08 – 06/30/10

Objective:

The objective of this activity was to provide funds for CONRAD Staff at CDC to develop, manage, monitor and evaluate the ongoing and planned microbicide activities supported by USAID.

Activity Description:

USAID and the CDC established an Interagency Agreement in 1990 for the purpose of conducting research on the relationship between steroid hormones and HIV. This research would be conducted through CONRAD/EVMS and has been amended to include other forms of contraception, HIV disease progression, condom acceptability, prevalence of OC use among HIV-infected women, and other aspects of HIV manifestation and reproductive health services. Beginning in FY01, collaborative activities between CONRAD and CDC were included in CONRAD's activities under this Cooperative Agreement. Included in the CDC collaborative component of CONRAD's workplan was cost-sharing of CONRAD staff at CDC for microbicide activities of mutual interest.

Progress:

CONRAD staff provided technical support to CDC to manage microbicide activities of mutual interest. This support included development of contracts, overseeing the budgets, and managing study activities. The main activity was preparing for a Phase III randomized, controlled trial of the diaphragm with a candidate microbicide to prevent sexually transmitted infections in Madagascar. CONRAD/CDC staff provided support to the randomized controlled trial by assisting with the development of the protocol, overseeing the budget, coordinating study activities, and conducting site visits to Madagascar and University of North Carolina-Chapel Hill.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

Background research on the effects of cervical application of various vaginal microbicides with a mechanical barrier remains limited, however, and suggests the need for further evaluation. Ideally, a highly effective vaginal microbicide could be combined with the diaphragm as a possible woman-controlled way to decrease transmission of sexually transmitted infections, and particularly of HIV. Even a moderately effective

diaphragm/microbicide combination may have a substantial public health benefit because it would provide an additional means of protection for women with otherwise limited options. Unfortunately, the planned randomized, controlled trial in Madagascar was not initiated due US Government decisions to suspend all funded activities in Madagascar in 2009 and the need to identify an alternative microbicide than was originally planned.

Involvement of Other Donors and Interested Parties:

CDC provided IAA funds to CONRAD's USAID Cooperative Agreements for co-support of CONRAD staff at CDC. USAID also provided $163,000 in FY07 Microbicide funds through FHI to CONRAD for this activity.

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Activity Category:	#5 – Support for Other Organizations
Activity Number and Title:	#5.4b – CDC Collaborations: Diaphragm plus microbicide: Expansion of ongoing clinical study to prevent STDs
CA Activity Numbers:	PPA-08-005, MSA-04-392, PPA-09-028
Primary Country of Activity:	Madagascar
Technical Coordinator:	Jennifer Legardy-Williams, MPH
Implementer:	University of North Carolina, Chapel Hill
Period:	**7**/1/08 – 6/30/12

Objective:

The purpose of this activity was to determine if the diaphragm and a microbicide used separately or together are effective at preventing Neisseria gonorrhea, Chlamydia trachomatis, and Trichomonas vaginalis infections in women. The candidate microbicide selected is BufferGel. The intended population to be studied was a demographically representative sample of women who are at high risk of sexually transmitted infections (STIs).

Activity Description:

This multi-site, randomized clinical trial (RCT) would assess the effects of the diaphragm and candidate microbicide (BufferGel) use on the acquisition of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* among women in five cities in Madagascar (Antananarivo, Antsiranana, Mahajanga, Toliara and Toamasina). This MIARADIA study was powered to detect effectiveness of 33%. High risk women (sample size to be determined) would be recruited from the public clinics when they seek care for STIs or through community outreach by peer educators and followed for six months. Women would be randomly assigned to one of four study arms: (1) Ortho All-Flex® diaphragm used continuously with BufferGel applied once daily in the dome of the diaphragm and intravaginally before every coital act (main intervention arm); (2) Ortho All-Flex diaphragm used continuously with inert hydroxyethylcellulose placebo gel (HEC) applied once daily in the dome of the diaphragm and intravaginally before every coital act; (3) BufferGel applied intravaginally before every coital act; and (4) HEC applied intravaginally before every coital act (control arm). All women would receive STI, HIV, and contraceptive counseling, male condoms at no charge, hormonal contraception at no charge, and diagnosis and treatment of sexually transmitted infections.

Progress:

During the first year leading up to initiation of the planned clinical trial, the informed consents and data collection forms were finalized. Prior to finalization, the behavior questionnaires were pretested. The protocol was approved by the CDC, UNC-Chapel Hill (UNC-CH), and UNC-Madagascar's (UNC-Mad) IRBs. The FDA IND application was submitted in July 2008 and FDA cleared the protocol to proceed in August 2008. The data collections forms were translated into French and version Malagasy dialect. In September, a team from CDC, CONRAD and UNC-CH made a site visit to work with the UNC-Madagascar team to discuss procedures for the Manual of Operations (MOO), which was then translated into French, and to establish the

processes for the central lab at the INSPC and the planned training for central lab staff.

Staff from CDC UNC-Madagascar made a site visit to the BAN study in Malawi to observe their data management process, which, like this RCT, uses Teleforms. Lessons learned from the site visit were incorporated into the data management process for the RCT.

The placebo gel (HEC) was manufactured, and packaged, and the microbicide gel (BufferGel) was being tested and manufactured for packaging.

In February 2009, the results from HPTN 035 study (NIH/NIAID sponsored) was a Phase 2/2B trial on the safety and effectiveness of BufferGel and PRO2000 in preventing HIV transmission to women were released. Although shown to be safe, BufferGel did not demonstrate effectiveness against the acquisition of HIV, nor against the secondary outcomes of GC or CT acquisition. In light of these recent findings, the MIARADIA investigators began revising the current study design, continuing site development, and conducting a prevalence study of gonorrhea and Chlamydia infections among women eligible for inclusion into the RCT.

The clinical study was to be conducted in a well-established cohort of high risk women in five cities in Madagascar; however, due to the suspension of US Government-funded projects in Madagascar following the April 2009 US State Department directive, an alternative setting would need to be found for this clinical trial. This was not accomplished under this activity.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

Background research on the effects of cervical application of various vaginal microbicides with a mechanical barrier remains limited, however, and suggests the need for further evaluation. Ideally, a highly effective vaginal microbicide could be combined with the diaphragm as a possible woman-controlled way to decrease transmission of sexually transmitted infections, and particularly of HIV. Even a moderately effective diaphragm/microbicide combination may have a substantial public health benefit because it would provide an additional means of protection for women with otherwise limited options. Unfortunately, the planned clinical trial of the ability of a diaphragm with a candidate microbicide to prevent sexually transmitted infections was not initiated.

Involvement of Other Donors and Interested Parties:

CDC provided funding to CONRAD through the Interagency Agreement with USAID which covers some of salaries of the CONRAD personnel which would oversee this project as well as for clinical supplies and the UNC/Madagascar site budget (up to $2M). NIAID was prepared to provide $4M towards the clinical study.

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Activity Category:	#5 – Support for Other Organizations
Activity Number and Title:	#5.4c – CDC Collaboration: Overcoming barriers to adherence (OBA): Qualitative research to identify strategies for high-risk women to achieve use of the diaphragm with candidate microbicide during sexual intercourse
CA Activity Numbers:	N/A
Primary Country of Activity:	Madagascar
Technical Coordinator:	Jennifer Legardy-Williams, MPH
Implementer:	University of North Carolina, Chapel Hill
Period:	10/1/08 – 6/30/10

Objective:

The primary objective of the proposed research was to generate knowledge that would allow for more effective encouragement adherent diaphragm and gel use in the MIARADIA trial (a Phase III randomized clinical trial that will assess the effectiveness of the diaphragm and candidate vaginal microbicide in preventing infection with GC/CT in Madagascar). The secondary objective was to generate knowledge that may be generalizable to other settings (research or clinical) in which women at high STI risk may be encouraged to use the diaphragm and/or a candidate vaginal microbicide. The intended populations to be studied were the peer counselors and clinicians who were employed at the MIARADIA study sites.

Activity Description:

This qualitative research will assess viable strategies for achieving adherent use of the diaphragm with a candidate microbicide during and following the MIARADIA trial. The study will be conducted in approximately three to four of the five MIARADIA clinical sites (the cities of Antananarivo, Antsiranana, Mahajanga, Toamasina, and Toliara). At each site, approximately 5-8 peer counselors and/or approximately 4 clinicians will be enrolled in the study. Peer counselors are all women at least 18 years of age. Clinicians are a mix of men and women, all of whom are at least 18 years of age. The only inclusion criterion is employment as a peer counselor or clinician at the study sites where the study will be conducted. There are no exclusion criteria. First qualitative activity will be semi-structured individual interviews (i.e., problem-solving sessions) with approximately 5-8 peer counselors per site. Interviews will be of 0.5 – 1.5 hours' duration. During these individual problem-solving sessions, each participant will be presented with several vignettes related to adherent diaphragm and gel use. The second qualitative assessment will be one semi-structured group interview (problem-solving session) per site. Interviews will be of 1 to 2 hours' duration with approximately 4 participants per group. During each problem-solving session, participants will be presented with several vignettes related to diaphragm and gel adherence. In both activities, participants will respond to descriptions of challenging circumstances that clinical trial subjects may encounter and will be asked to generate strategies that women could use to increase adherent diaphragm and gel use in MIARADIA.

Progress:

The MIARADIA protocol was submitted to CDC and UNC-Chapel Hill (UNC-CH) IRBs and received approval. The manual of operations, data collection instrument, and training materials were developed. A site visit to Madagascar was conducted in September to train the supervisors in qualitative data collection methods and analysis. The study was conducted at the Antananarivo site: 8 peer counselors and 4 clinicians were interviewed. Data files from these interviews were transmitted to CDC.

A comprehensive list of strategies for adherence in MIARADIA was being developed from the OBA data. The strategies for adherence will be incorporated into the study product counseling that MIARADIA participants receive at enrollment and throughout the study. Depending on the timing of the start of MIARADIA, there may be the opportunity to conduct additional interviews. The data from any additional interviews would be incorporated into the list of strategies and into the study product counseling.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This Activity provided better understanding of barriers to achieving diaphragm and microbicide adherence, thus allowing development of strategies to promote adherence in the MIARADIA trial, the intent of which was to determine if the diaphragm and a microbicide used separately or together are effective at preventing Neisseria gonorrhea, Chlamydia trachomatis, and Trichomonas vaginalis infections in women. Unfortunately, the planned MIARADIA trial in Madagascar was not initiated due US Government decisions to suspend all funded activities in Madagascar in 2009 and the need to identify an alternative microbicide that was originally planned.

Involvement of Other Donors and Interested Parties:

CDC provided funding to CONRAD through an Interagency Agreement with USAID which covered some of salaries of the CONRAD personnel overseeing this activity.

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Microbicide Account - Final Summary Report
USAID - GPO-A-00-08-00005-00 (PPRD)

Activity Category:	#5 – Support for Other Organizations
Activity Number and Title:	#5.4d – CDC Collaboration: Cross-sectional study to measure gonorrhea and chlamydial infection among women at high risk in Madagascar
CA Activity Numbers:	N/A
Primary Country of Activity:	Madagascar
Technical Coordinator:	Jennifer Legardy-Williams, MPH
Implementer:	University of North Carolina, Chapel Hill
Period:	11/1/08 – 6/30/10

Objective:

The purpose of this activity was to measure the prevalence of gonorrhea and/or chlamydial infection (GC/CT) among women attending the five public clinics selected as study sites for MIARADIA, a Phase III randomized clinical trial (RCT) planned to assess the effectiveness of the diaphragm and candidate vaginal microbicide in preventing infection with GC/CT. This information would be used to refine the plans for the upcoming RCT.

Activity Description:

A multi-site, cross-sectional study to measure the prevalence of gonorrhea and/or chlamydial infection (GC/CT) among women at high risk for sexually transmitted infection (STI) would be conducted in five cities in Madagascar (Antananarivo, Antsiranana, Mahajanga, Toliara, and Toamasina). This prevalence pre-study would be conducted among women attending the same five public clinics selected as study sites for the MIARADIA trial before the initiation of the MIARADIA trial with the goal of using the study findings to refine the plans regarding enrollment and study site selection for the larger trial.

Women 15-55 years of age attending the study clinics for care would be recruited until approximately 200 women at each site (1,000 total) were enrolled. While participants undergo routine (non-study) speculum examination according to national guidelines, the study clinicians would collect two vaginal swabs to be evaluated for GC/CT using nucleic acid amplification testing (NAAT) at the central laboratory at the Institut National de Santé Publique et Communautaire (INSPC) in Antananarivo, Madagascar. The UNC STI core laboratory in Chapel Hill, NC would perform quality assurance (QA) testing on a subsample of specimens (n=630). Study staff also would administer a short questionnaire to collect data on demographics, reproductive health, and sexual behaviors. Participants who did not receive risk-based treatment for GC/CT based on the national guidelines for care at the time of their enrollment visit and who are positive for GC/CT by NAAT would be contacted to return to the clinic for treatment.

Progress:

The informed consent, data collection forms, and protocol were finalized and the protocol was submitted to the CDC IRB and received approval.

The RCT study was to be conducted in a well-established cohort of high risk women in five cities in Madagascar; however, due to the suspension of US Government-funded projects in Madagascar following the April 2009 US State Department directive, an alternative setting would need to be found for this clinical trial. Thus, this prevalence study was also not initiated.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

This study would contribute to microbicide R&D in that Neisseria gonorrhoeae and Chlamydia trachomatis are known co-factors in HIV infection. Prevention of these infections may be an effective strategy for preventing HIV infection. Unfortunately, neither this prevalence pre-study, as well as the planned RCT study of the ability of a diaphragm with a candidate microbicide to prevent sexually transmitted infections, was not initiated.

Involvement of Other Donors and Interested Parties:

CDC provides funding through the Interagency Agreement with USAID which covers some of salaries of the CONRAD personnel which would oversee this project.

Final Summary of Research Conducted by CONRAD

Under NICHD HIV/AIDS IAA #Y1-HD-0083

Under USAID - GPO-A-00-08-00005-00 (PPRD)

I. Intramural Preclinical Research Program (IPR)

CA Activity Number: EVMS 3253
Primary Country of Activity: US
Technical Coordinator: Gustavo Doncel
Implementer: Gustavo Doncel

Period: 09/5/08 – 09/04/14

Objectives:

CONRAD has focused on the development of new and improved technologies for voluntary family planning and prevention of HIV/AIDS and other sexually transmitted infections (STIs). This includes dual-protection technologies displaying anti-HIV/contraceptive and anti-HIV/anti-STI properties. The CONRAD Intramural Preclinical Research (IPR) Program, led by Dr. Doncel, has focused on: (1) identification, screening and preclinical characterization of new vaginal microbicides and contraceptives, (2) development and validation of new methods and biomarkers of vaginal product-induced mucosal damage, and (3) assessment of mucosal inflammatory markers in Phase I clinical trials. CONRAD IPR has become a center of reference for the screening and characterization of microbicides, particularly those with contraceptive properties. As such, it provided CONRAD, USAID, NICHD and the microbicide research community at large with a comprehensive evaluation service unavailable from any other government or private institution.

Activity Description:

With NICHD and USAID funding, the CONRAD IPR program has pioneered testing of spermicide/ virucide candidates leading to the discovery of dual-activity microbicides, some of which have demonstrated contraceptive efficacy in clinical trials. It has also contributed to the development and implementation of a comprehensive testing algorithm to characterize the preclinical profile of those compounds. The benefits of such a complete evaluation of microbicide and spermicide candidates have been evident to many national and international institutions which have used this service. New, more predictive safety endpoints, both at preclinical and clinical levels, would provide a more efficient way to identify drug candidates, which may cause cervicovaginal alteration and increased susceptibility to HIV-1 infection. Importantly, this would apply to topical microbicides as well as vaginal contraceptives and dual-protection technologies

Progress:

Almost 400 compounds and/or formulations were evaluated within the microbicide screening (drug discovery) program, especially against multidrug resistant virus, of selected candidates, alone and in combination. Although several lead compounds with distinct mechanisms of action have been characterized, the most promising ones belong to the reverse transcriptase inhibitor (RTI) category, such as fatty-acid nucleoside derivatives, integrase inhibitors, non-nucleoside reverse transcriptase inhibitors (NNRTIs), and one CCR5 antagonist, with limited or no cytotoxicity. Through collaboration with ImQuest and University of Rhode Island, a group of these compounds has been submitted for an SBIR grant to support further product development. Additional nucleosides evaluated included a novel derivative of emtricitabine that showed higher cellular uptake and antiviral activity that its parent molecule, a prodrug of tenofovir (GS-7340) which is

more potent and stable than TFV and TDF (Viread). Combinations of antiretrovirals included TFV, dapivirine, elvitegravir, and MIV-170.

Early on, new formulations of UC781 and tenofovir, alone and in combination, were also evaluated. Carbopol containing formulations have been assessed for spermicidal and anti-HIV properties. We have also made significant progress in the identification of new molecular mechanisms and biomarkers of microbicide-mediated mucosal damage. Through a genomic approach, we have identified a set of discriminatory genes, which, if validated, they may represent the next generation of microbicide safety biomarkers. Within this activity and in addition to the preclinical experiments, characterizing the immunophenotype and activation status of vaginal biopsies, cervical (cytobrush) and vaginal (CVL pellet) cells taken from women who underwent a repeat Phase I study on cellulose sulfate were characterized.

CONRAD expanded the identification of new biomarkers of cervicovaginal mucosal alteration. In collaboration with the Bioinformatics Institute of Virginia Tech, we selected a set of gene products capable of identifying proinflammatory microbicide and contraceptive candidates in reproductive tract cell-based and tissue-based preclinical assays. We have also pursued the identification of similar biomarkers in rabbit cervicovaginal tissues with the goal of improving the objectivity and predictive value of the rabbit vaginal irritation model. We developed a quantitative nuclease protection assay, which enables to the evaluation of mRNA gene expression in formalin-fixed, paraffin-embedded rabbit vaginal mucosa. We have also continued with the characterization of new primary safety endpoints in tissue samples obtained from CONRAD clinical studies.

As part of the quest for dual-protection technologies, we evaluated ContraGel (6% lactic acid) and a reformulated TFV 1% gel displaying acid-buffering, spermicidal and antiviral properties. These compounds are not intended as stand-alone contraceptive products but as adjuvants to the contraceptive activity of cervicovaginal devices such as SILCS. In turn, SILCS would serve as a delivery system for TFV gel. Next we plan to investigate the in vitro and in vivo potential of compounds (from Hydra Biosciences), which specifically block calcium channels controlling sperm hypermotility.

CONRAD also made efforts to reformulate the tenofovir 1% gel with the objective of endowing this gel with contraceptive properties to add to those displayed by the SILCS diaphragm. Relatively minor modifications of the formula aimed at increasing its acid buffering capacity failed to produce significant sperm immobilization or functional inhibition, thus additional reformulation was initiated. On a different category of compounds, two of Hydra Biosciences' CatSper inhibitors were tested for human sperm motility, viability and hyperactivation. One of them showed inhibition of sperm activation at concentrations that did not impair standard motility. We have also completed in vitro sperm, rabbit contraceptive and rabbit vaginal irritation studies of ContraGel, a lactic acid based gel to be marketed with SILCS in Europe.

Towards the end of the period, there was renewed interest in non-antiretroviral (ARV) compounds for the topical prevention of HIV. One of their main advantages is the fact that they are not used or in development for treatment purposes; therefore, resistance to these compounds does not jeopardize treatment options. Non-ARVs may also display other activities and thus be part of multipurpose prevention technologies (MPTs). Five compounds showed potent anti-HIV activity and sperm inhibition. Evaluation of anti-HSV properties was planned.

We also completed the in vitro and in vivo evaluation of ContraGel, a lactic acid based gel to be marketed with SILCS in Europe. This combination product will be tested by CONRAD for contraceptive efficacy (post-coital test) and safety in Phase I clinical studies (with funding from PATH/USAID).

New biomarkers of semen exposure are needed to be used as surrogates of contraceptive efficacy or HIV exposure. We identified TSPY4 as a specific marker of sperm exposure, with a significantly longer vaginal decay than PSA. We have developed a multiplex PCR for SRY and TSPY4, and have optimized the methodology to detect traces of sperm/semen exposure from swabs and gel applicators.

II. Evaluation of New Female Condoms

CA Activity Number:	PPA-09-047
Primary Country of Activity:	US
Technical Coordinator:	Marianne Callahan
Implementer:	Ron Frezieres
Period:	7/1/09 – 6/30/14

Objectives:

CONRAD and its collaborators in the fields of female condom and semen biomarker research seek to address the current challenges and limitations of female condom research and to explore alternative approaches to evaluating new female condom products. The main goal of this activity is to work with the FDA to update the premarket evaluation paradigm for female condoms by using PSA as a biomarker of semen exposure.

Activity Description:

The goal of this activity was to work with the FDA to update the premarket evaluation paradigm for female condoms by using PSA as a biomarker of semen exposure. Results from the comparative study of female condom functionality, entitled, "A Randomized Cross-Over Study of Vaginal Semen Exposure and Clinical Failure Comparing the PATH Woman's Condom and the FC2 Female Condom (CONRAD Protocol #D09-108)," will be compared with results from a sub-study being carried out as part of the recently completed NICHD contraceptive trial of the Woman's Condom. In this sub-study, samples were collected from 150 couples during their first 4 acts of intercourse with the Woman's Condom in exactly the same way as the samples were collected in Protocol #D09-108. Condom performance information was collected using the same self-reporting questionnaires. PSA and glycogen/PAS were analyzed by the same laboratories. Through comparison of the results of these two studies, the potential to modify the current algorithm for female condom approval would be explored with the FDA.

Progress:

The acute failure mode study comparing the PATH Woman's Condom and FC2 enrolled 490 couples at two centers, Los Angeles and Berkeley, under the direction of California Family Health Council (CFHC). The goal of the study was to complete 1,500 uses of each condom type in order to meet the power requirements of the study. Both sites were closed out by August 2011, with Berkeley having completed 163 couples and LA 236, for a total of 399 (81% of those enrolled). There were 1,588 uses of the PATH Woman's condom and 1,598 uses of FC2. CDC completed analysis of the swabs for PSA and sent the data to CFHC on 10/14/12. The acute failure mode study was carried out under a pre-IDE.

Debra Anderson's laboratory at Boston University completed the validation study of the glycogen/PAS assays and submitted a manuscript to *STDs* in April 2012. In addition, all the vaginal swabs from the acute failure mode study that were negative for PSA were assayed at Dr. Anderson' lab for glycogen/PAS. Glycogen results using swabs from the study were not as interpretable as those used during development of the assay and described in the manuscript, however. One reason for this may have been that acute failure mode samples

were subjected to more freeze/thaw cycles. It was decided in May 2012 that the primary analysis would not use the glycogen/PAS data and instead rely on randomization to equally distribute unused vaginal swabs between the two arms. Exploratory analyses would take into account the glycogen/PAS results using various thresholds for glycogen and including/excluding the indeterminate/insufficient PAS results. In the future, the number of times the substudy samples are thawed and refrozen would be minimized in an effort to keep cells intact and to minimize PAS signal deterioration.

The results of the CONRAD study showed that the PATH Woman's Condom had higher acceptability ratings than FC2, was associated with less female discomfort than FC2, and was non-inferior to FC2 as measured by self-reports of condom failure. However, the probability of semen exposure during uses of the WC condom was not non-inferior to that of the FC2 condom, possibly due to a manufacturing defect, which has since been corrected, and which also may have affected the NICHD study. The final report for the CONRAD study was submitted to the FDA on October 23, 2014. A manuscript is in preparation.

The NICHD study enrolled 511 couples with the last participant exiting on August 29, 2012. A total of 240 couples completed the study and 271 discontinued early for various reasons. Of the 511 couples enrolled in the main study, 151 were enrolled in the sub-study with about 70 couples completing all 4 uses with the condom and returning the swabs for analysis. Colin Pollard, formerly with the FDA Office of Devices, was hired by Health Decisions as a consultant to oversee the analysis and preparation of the final report. A pregnancy review panel was convened at NICHD on April 4, 2013 and attended by CONRAD. The overall lifetable pregnancy rate was 5.3%, the typical use rate was 4.5%, and the perfect use rate was 2.6%. As of April 22, 2015, the final analysis of the main study and the substudy were still underway at HDI.

In addition to maintaining the IDE, CONRAD served as the main liaison between PATH and Health Decisions (HDI), the CRO managing the contraceptive trial for NICHD, with regards to product needs. A total of 30,550 Woman's Condoms and lubricant were shipped from China in six separate shipments, 5,550 more than original ordered. CONRAD arranged for the USAID-required QA testing by FHI of the products used in both studies. This QA testing was carried out according to ISO standards.

On February 4, 2014, the FDA asked for an update regarding our expected timeframe for submission of the PMA for the Woman's Condom. CONRAD had no additional funding for USAID funding for a PMA submission. We have participated in a series of calls with NICHD, PATH, and Health Decisions, regarding a gap analysis initiated by NICHD of what would be needed to file a PMA. With the help of Colin Pollard, the possibility of asking the FDA to reclassify female condoms as Class II devices was being explored at the end of this funded activity.

Two reports are being finalized: 1) NIH Sub-Study Report; and 2) NIH Sub-Study Report comparing CONRAD results with NIH Sub-Study Contraceptive Study (CCN008) results.

With those in hand, CONRAD will request a meeting with the FDA to discuss the data and their implication for future requirements for female condom approval, including using PSA as a Medical Device Development Tool. We will continue to explore means to file a PMA and/or reclassify female condoms to the extent that funding is available.

Involvement of Other Donors and Interested Parties:

FHI performed QC of the newly manufactured PATH condoms (approximately $3,000) and Female Health Company donated FC2 supplies, both for CONRAD's acute failure mode study.

III. Dual-Protection Technologies

CA Activity Number(s):	PPC-09-017, PPC-09-019, PPC-09-031, PPC-10-099
Primary Country of Activity:	US
Technical Coordinators:	Gustavo Doncel, Dave Friend, Meredith Clark
Implementers:	Patrick Kiser (Univ Utah), Thomas Smith (Oak Crest Inst of Science), Janet Halliday (Controlled Therapeutics)
Period:	5/1/09 – 09/30/14

Objectives:

To develop dual-protection products offering multiple reproductive health benefits. At least one that offers protection against HIV and unwanted pregnancy, and one that offers protection against HIV and other STIs, such as the highly prevalent genital herpes.

Activity Description:

CONRAD is seeking to develop one or more new dual-protection technologies (contraceptive microbicides and dual-activity microbicides). The lead product is a vaginal ring delivering levonorgestrel (contraceptive) and tenofovir (TFV; microbicide). In addition, the contraceptive diaphragm could be a safe and inexpensive delivery system for TFV while also providing contraceptive protection. Specifically, CONRAD envisioned using the SILCS device as an improved delivery system for TFV 1% gel or other microbicide gels with significant advantages over the currently used plastic applicators. The device could work as a reservoir from which the gel can be released for longer periods of time (gel deposited by an applicator can more readily leak from the vagina) and be a safe and reusable carrier. These factors will increase convenience, discretion, and thus adherence while reducing environmental waste and potentially reducing cost.

Intravaginal Ring

CONRAD is developing an intravaginal ring (IVR) that releases levonorgestrel (LNG) and TFV. LNG is a 19-nortestosterone derivative available in the market in different dosage forms, including an LNG-releasing intrauterine system. LNG has a long track-record of safety and efficacy. Preliminary data indicate that release rates of LNG from an IVR are adequate to achieve contraceptive efficacy. The controlled release of LNG and TFV from a vaginal ring device will protect women against sexual acquisition of HIV and unplanned pregnancies.

Reformulated TFV Gel

TFV 1% vaginal gel itself has no known spermicidal activity, although two studies of men taking oral tenofovir (Viread®) showed a reduction in progressively motile sperm in their semen. Furthermore, an analysis of pregnancy rates and contraceptive choices in the CAPRISA004 cohort showed decreased pregnancy rate in the TFV arm, although not statistically significant (Sibeko et al., 2011). Thus, a potential future enhancement to the SILCS/TFV combination product to boost its contraceptive effectiveness would be to modify the TFV gel formulation to increase its acid-buffering ability. The strategy would include modification of the TFV gel formulation to contain acid buffering substances such as polycarbophils and/or lactic acid. A reformulated gel would display high acid buffering capacity and decreased osmolality (compared to the current clinical formula), thereby increasing the contraceptive efficacy of the TFV gel/SILCS combination and reducing the potential effects of high gel osmolality on mucosal tissues.

Formulation optimization followed by physicochemical characterization, stability and in vitro release testing would be necessary before commencing with biological evaluation. A stable, reformulated gel containing acid buffering ingredients would then need to be tested for spermicidal and antiviral activity in vitro. If the results are positive, the reformulated gel would undergo in vitro safety testing using human cervicovaginal tissue constructs and explants. After it is proven safe and efficacious in vitro, the reformulated gel would be tested in animal models (e.g., rabbits) for safety and contraceptive efficacy prior to moving on to clinical testing.

Dr. Patrick Kiser's group (then at University of Utah) would reformulate TFV gel with acid buffers and prepare several gels for preclinical, in vitro testing at Dr. Doncel's lab at EVMS and other laboratories. Based on the results from the first round of reformulation testing, a second round of formulation and testing may be needed. The Utah group would manufacture sufficient quantity of the non-GLP gel for the animal testing: a rabbit contraceptive study, a RVI, and a rabbit pharmacokinetic study.

Once a formulation optimized for potential contraceptive activity has been selected, a Phase I PCT study would be conducted to determine whether or not reformulation of the TFV gel is necessary to significantly increase the contraceptive effectiveness of the combination product. An expanded safety study would follow. All of these activities would be supported after the end of this Cooperative Agreement using other funds.

Progress:

<u>TFV/LNG Intravaginal Ring</u>

In collaboration with CONRAD, Dr. Patrick Kiser (formerly at University of Utah, now at Northwestern University), used polyurethanes (PUs) to create a dosage form capable of releasing TFV and LNG. Matrix-based IVRs were first evaluated, however based on in vitro and in vivo release data, both drugs demonstrated an initial burst release followed by a period of time when dissolution diminished substantially. A constant release rate of about 10 mg/day TFV and 20 µg/day LNG was targeted. Moreover, a pharmacokinetic study in rabbits suggested that delivery of TFV may only provide effective doses for less than 2 months, not the 90-day target duration. Thus, a reservoir-based IVR was developed in which TFV and LNG are formulated in separate ring segments such that the release rate from each segment can be optimized independently. Reservoir-based IVRs, similar to NuvaRing®, contain an outer polymer coating that minimizes the burst release of loaded drug and provides better control over daily release rates.

Pharmacokinetic and vaginal irritation studies were performed in rabbits on TFV and LNG reservoir IVR segments that were vaginally implanted for up to 90 days. No test article-related safety issues were noticed. A 90-day pharmacokinetic study on full sized TFV/LNG IVRs was completed in sheep. Pharmacokinetics of TFV from the IVR were similar to daily dosing with gel in sheep. A 1- and 3-month safety study in sheep was also completed; no significant safety concerns were observed.

Technology and analytical transfer to contract manufacturing organizations (Foster Corporation and DPT) was undertaken for the cGMP manufacture of TFV/LNG IVR subparts (e.g., extruded polyurethane tubes and coextruded LNG-polyurethane segments) and final product assembly, respectively.

All IND-enabling clinical (e.g., protocol development), nonclinical (e.g., biocompatibility testing), and CMC (e.g., pilot batch manufacture and initial stability testing) activities were completed. The electronic IND was filed in early April 2014, along with a separate electronic IND for the TFV-only IVR (to be tested in the same clinical study) and subsequently approved. The initial 30-day Phase I study was initiated in Q4 2014. This study, ongoing at the time of this report, will evaluate the safety, pharmacokinetics, pharmacodynamics, and acceptability of the TFV/LNG IVR (20 µg/day LNG dose), TFV-only IVR and placebo IVR in 50 women (blinded, randomized 2:2:1). If successful, this study will be followed by an expanded, 90-dayPhase I study.

To support this and other future clinical activities, work has already begun to improve the efficiency of manufacturing and testing clinical IVR supplies. Ongoing activities include optimization of the LNG-polymer compounding process and development of accelerated in vitro release testing methods.

At the end of the funding period, ongoing activities included optimization of the manufacturing process for the TFV/LNG IVR and scale-up manufacturing feasibility activities, including initial assessment of automated and/or continuous manufacturing processes. This latter activity is a critical step to help facilitate a cost of goods analysis of the TFV/LNG IVR and other IVR products using a similar technology.

TFV/ACV Intravaginal Ring

A second dual-protection product was designed to deliver TFV and ACV intravaginally for 3 months. Two different approaches were initially funded: PU-based IVRs and the use of proprietary technology owned by the Oak Crest Research Institute (OCRI). In collaboration with Controlled Therapeutics (CT; Scotland), CONRAD developed a PU matrix-based IVR for TFV/ACV. CT manufactured and performed in vitro testing on several ring formulations, and lead prototype rings were selected for further testing in rabbits and sheep. These rings demonstrated similar drug release kinetics as Utah's matrix-based rings described above and were found to provide sustained release of TFV and ACV for less than 2 months. OCRI demonstrated similar progress, having evaluated several prototypes in vitro and in vivo. The daily release rate from OCRI's ring was very constant in vitro, however these rings are limited by the drug loading capacity and may not be capable of delivering milligram quantities of TFV and ACV daily for the target 90 day duration. CONRAD next intended to redesign the TFV/ACV IVR using the polyurethane reservoir technology developed for the TFV and TFV/LNG IVRs, as this technology is expected to better enable achieving the targets of milligram-level release rates of TFV and ACV for at least 3 months. However given the anticipated cuts in funding and a need to focus the program's priorities, this next phase of TFV/ACV/ IVR development was not initiated.

Reformulated TFV Gel

TFV 1% vaginal gel itself has no known spermicidal activity, although two studies of men taking oral tenofovir (Viread®) showed a reduction in progressively motile sperm in their semen. Furthermore, an analysis of pregnancy rates and contraceptive choices in the CAPRISA004 cohort showed decreased pregnancy rate in the TFV arm, although not statistically significant (Sibeko et al., 2011). Initial efforts to reformulate the TFV gel in order to increase its sperm immobilizing properties were performed at the University of Utah, including modification of the current TFV gel formulation with addition of acid-buffering agents (e.g., lactic acid, Carbopol 974P) and decreasing the pH. Prototype gels were evaluated for their physicochemical properties and stability, as well as in vitro safety and TFV tissue permeability, and sperm immobilization activity (at EVMS; see above). These initial reformulation efforts did not produce the desired increase in contraceptive capability and/or resulted in physical instability (i.e., precipitation) of TFV in the gel. Therefore, a second round of efforts was initiated which expanded the reformulation effort to include a broader compositional range. Optimization of these new prototype formulations included evaluating whether changes to the manufacturing process (e.g., order of addition, process temperatures, mixing times) would further improve the stability profile. The viscosity of gels were increased somewhat compared with the current clinical gel. Evaluation of these gels included physicochemical characterization, stability testing, in vitro safety, TFV permeability and contraceptive efficacy. CONRAD also considered incorporating TFV into an Amphora-based gel formulation and comparing this product to the reformulated gel described above. At the end of the funding period, planned activities included continued reformulation development and in vitro testing of prototype reformulated TFV gels, stability testing on lead reformulated gel(s), preclinical in vivo (rabbit) studies for safety, pharmacokinetics and contraceptive efficacy of lead reformulated gel.

Involvement of Other Donors and Interested Parties:

The TFV/LNG activities have been supported primarily using non-IAA from USAID funding. TFV gel reformulation was supported almost exclusively using NICHD IAA funds.

Final Summary of Research Conducted by CONRAD

Under Interagency Agreement between NIH/NIAID/DAIDS and USAID/OHA
NIAID Y1-AI-1756-01

Under USAID - GPO-A-00-08-00005-00 (PPRD)

I. Identification and validation of biomarkers of cervicovaginal mucosal alteration

CA Activity Number:	EVMS 780306
Primary Country of Activity:	US
Technical Coordinator:	Gustavo Doncel,
Implementer:	Gustavo Doncel, Sharon Anderson
Period:	7/1/11 – 12/31/13

Objectives:

The purpose of the Interagency Agreement was to enable NIAID to contribute funding to USAID to provide continued support and conclusion of a biomarker discovery project that is being conducted by CONRAD. The main goal of this project was to identify gene-expression signatures or profiles that enable the preclinical and clinical characterization of the proinflammatory and mucosal altering potential of candidate microbicides and other vaginal health products, both in the form of active pharmaceutical ingredients (APIs) and formulations. To achieve this goal, experiments would be carried out to identify and validate biomarkers of mucosal alteration in 1) human cell- and tissue-based systems, 2) the rabbit vaginal irritation (RVI) model, and 3) human cervicovaginal biopsies. The gene expression responses of cervicovaginal (CV) cells and tissues, first in vitro and then in vivo, using microarray technology, and confirmatory assays based on RNA and protein expression would be studied.

Progress:

1. Identification and validation of biomarkers of mucosal alteration in human cell and tissue based systems.

   A panel of discriminatory gene markers in a vaginal cell model were successfully identified and validated. The new biomarker sets (gene expression profiles) will be used to identify microbicide and vaginal products with potential mucosal altering properties. Further gene enrichment/network analysis will yield possible individual markers or convergent transcriptional pathways, which could enhance the discriminating profile (or perhaps replace it). In addition to selecting discriminatory biomarkers, mining the datasets, we have identified cross-reactive genes with potential HIV transmission/pathogenesis implications.

2. Identification and validation of biomarkers of mucosal alteration in the rabbit vaginal irritation model (RVI).

   Using a refined version of the RVI model, the predictive value of epithelial markers in rabbit tissues to ascertain which ones best identified mucosal alteration was characterized. Two novel, quantitative gene expression assays were designed to quantify mRNA levels of 25 genes, including immune function, epithelial repair, and microbicidal markers, in formalin-fixed paraffin embedded tissue sections. Ultimately, a combination of newly identified, RNA-based biomarkers and the above-validated IHC-based

markers will yield a more robust and objective assessment of rabbit vaginal mucosal alteration to be used in the pre-clinical evaluation of microbicides and vaginal products.

3. Identification and validation of biomarkers of mucosal alteration in human CV biopsies (clinical study).

   The CONRAD clinical trial, "Identification of Novel Biomarkers of Cervicovaginal Mucosal Inflammation" (Protocol D11-119), was, to our knowledge, the first clinical study to investigate mucosal gene expression in CV tissue biopsies obtained after in vivo treatment with known pro-inflammatory compounds (PICs) and non-inflammatory compounds (NICs), and to link microarray data to changes in markers of safety and pharmacodynamics. A common profile of genes dysregulated by PIC compounds was characterized, which can serve as biomarkers of vaginal inflammation, immune activation and compromised barrier function in the safety evaluation of HIV prevention interventions. Importantly, this gene panel derived from microarray analysis and validated by PCR is independent of significant differences in HIV-1 infection and immune cell targets with PIC and NIC treatment, suggesting that it may be a sensitive measure of subclinical mucosal inflammation.

Involvement of Other Donors and Interested Parties:

None.

Final Summary of Research Conducted by CONRAD

Under Interagency Agreement between CDC and USAID

98FED06136

Under USAID - GPO-A-00-08-00005-00 (PPRD)

I. Diaphragm plus microbicide: Clinical studies to prevent STDs

CA Activity Number:	MC-08-507, MSA-04-392
Primary Country of Activity:	US, Madagascar
Technical Coordinator:	Jennifer Legardy-Williams
Implementer:	UNC-CH (Behets), DPT, Jennifer Legardy-Williams
Period:	9/5/08 – 09/04/14

Objectives:

The HIV epidemic is increasingly affecting women of reproductive age and their infants. Heterosexual transmission has always been the primary mode of transmission worldwide and is increasingly important among U.S. women. The purpose of the Interagency Agreement was to provide funding to USAID for co-support of microbicide research. As described in summary for PPRD activity #5.4b, the purpose of this project was to determine if the diaphragm and a candidate microbicide (BufferGel) used separately or together are effective at preventing Neisseria gonorrhea, Chlamydia trachomatis, and Trichomonas vaginalis infections in women. The intended population to be studied was a demographically representative sample of women who were at high risk of sexually transmitted infections (STIs) in Madagascar.

Progress:

The MIARADIA protocol, approved by the CDC, UNC-Chapel Hill (UNC-CH), and UNC-Madagascar's (UNC-Mad) IRBs, was submitted to the FDA IND in July 2008 and approved. The placebo gel (HEC) was manufactured, and packaged, and the microbicide gel (BufferGel) was being tested and manufactured for packaging.

In February 2009, the results from HPTN 035 study (NIH/NIAID sponsored) was a Phase 2/2B trial on the safety and effectiveness of BufferGel and PRO2000 in preventing HIV transmission to women were released. Although shown to be safe, BufferGel did not demonstrate effectiveness against the acquisition of HIV, nor against the secondary outcomes of GC or CT acquisition. In light of those findings, the MIARADIA investigators revised the study design, continued site development, and conducted a prevalence study of gonorrhea and Chlamydia infections among women eligible for inclusion into the larger trial.

The clinical study was to be conducted in a well-established cohort of high risk women in five cities in Madagascar; however, due to the suspension of US Government-funded projects in Madagascar following the April 2009 US State Department directive, an alternative setting would need to be found for this clinical trial. This was not accomplished under this activity.

Involvement of Other Donors and Interested Parties:

USAID provided co-funding to CONRAD through the PPRD Agreement. NIAID was prepared to provide $4M towards the clinical study.

II. Evaluation of New Female Condoms

CA Activity Number: PPA-09-047
Primary Country of Activity: US
Technical Coordinator: Marianne Callahan
Implementer: Ron Frezieres

Period: 7/1/09 – 6/30/14

Objectives:

CONRAD and its collaborators in the fields of female condom and semen biomarker research seek to address the current challenges and limitations of female condom research and to explore alternative approaches to evaluating new female condom products. The main goal of this activity is to advance the premarket evaluation of new female condoms through the integration of biomarkers in functionality studies, in particular, using PSA as a biomarker of semen exposure.

Activity Description:

Advancing the evaluation of new female condoms through the integration of biomarkers in functionality studies. Biological markers of exposure to semen are increasingly recognized as sensitive and specific indicators of vaginal intercourse and of condom failure during use. Considerable experience has been achieved with determining the presence of prostate-specific antigen (PSA) or Y-chromosome DNA in vaginal fluid after inoculation of measured amounts of semen, after unprotected intercourse, and after use of male latex condoms or female condoms. In male condom functionality studies, PSA correlates well with established failure modalities such as breakage and slippage, and its absence under such circumstances can be traced to corrective behavior (e.g., immediately halting intercourse and replacing a condom after breakage). In evaluating new female condoms, the type of user-reported events (slippage, invagination, penis misrouting) are not well-established as failure modalities (i.e., instances in which the condom is believed fails as a barrier to sperm and microorganisms). Studies that have employed PSA to evaluate female condom performance show a variable degree of correlation self-reported use problems, suggesting that certain events (e.g., slippage without breakage) may not necessarily represent failure of a female condom. Identifying real failure modalities would be important for streamlining regulatory approval of new female condoms. USAID and CDC co-sponsored a study of newly designed female condoms in which standardized questionnaires would be employed to obtain self report of events potentially indicating condom failure, and vaginal fluid systematically collected before and after using a condom during intercourse, to sharpen the characterization of female condom failure modalities.

Results from the comparative study of female condom functionality, entitled, "A Randomized Cross-Over Study of Vaginal Semen Exposure and Clinical Failure Comparing the PATH Woman's Condom and the FC2 Female Condom (CONRAD Protocol #D09-108)," would be compared with results from a sub-study being carried out as part of the recently completed NICHD contraceptive trial of the Woman's Condom. In this sub-study, samples were collected from 150 couples during their first 4 acts of intercourse with the Woman's Condom in exactly the same way as the samples were collected in Protocol #D09-108. Condom performance information was collected using the same self-reporting questionnaires. PSA and glycogen/PAS were analyzed by the same laboratories. Through comparison of the results of these two studies, the potential to modify the current algorithm for female condom approval would be explored with the FDA. This activity is also described under PPRD summary #4.2 ("Integration of Biomarkers in Comparative Studies of Barrier Devices: Application to New Female Condoms").

Progress:

Data from the NICHD study and the acute failure mode study were assessed. Testing of PSA swabs from the NICHD study was completed by the CDC in April 2013 and specimens were received by Deborah Anderson for PAS/glycogen testing in May 2013. The study was completed in August 2012. As of April 16, 2015, CFHC had just received PSA data from the NICHD study, and was preparing a comparative report. Health Decisions, Inc (HDI), the CRO managing the contraceptive trial for NICHD with regards to product needs, was to perform the statistical analysis of the rest of the data from the NICHD study.

In addition to maintaining the IDE, CONRAD remained the main liaison between PATH and HDI. CONRAD also arranged for the USAID-required QA testing of the products used in both studies. This QA testing was carried out according to ISO standards by FHI.

CDC analyzed the swabs for PSA and Deborah Anderson's laboratory at Boston University adapted an assay for soluble glycogen to demonstrate vaginal exposure of the swabs. When applying it to the swabs obtained in the acute failure mode study, however, the results using the new assay did not completely agree with the established (but labor-intensive) PAS method. This may have been due in part to the repeated thawing and refreezing of the samples. As a result, the initial statistical analysis did take into account glycogen testing, but subsequent exploratory analyses would.

The decision on the part of USAID not to support submission of a PMA, but rather to use WHO prequalification to procure PATH Woman's Condoms for women in developing countries led to a "gap analysis" effort on the part of NICHD to determine requirements to file a PMA without USAID support. In the course of that analysis, the notion of petitioning the FDA to reclassify female condoms as Class II devices which could be approved via the less onerous 510(k) route was discussed. In June 2014, PATH informed CONRAD that the National Female Condom Coalition (NFCC) had scheduled a meeting with the FDA in July to discuss changes to the female condom part of the FDA website and the FC2 labeling. The NFCC was also interested in the possibility of reclassifying female condoms as class II devices in order to facilitate approval of new designs. If the FDA was receptive and if the study data support reclassification, plans will be made to pursue this course, both for approval of the PATH WC and for future designs. Funding for this effort is needed.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

Incorporation of PSA testing within functionality studies of experimental female condoms may provide a viable alternative to contraceptive efficacy studies which are expensive, lengthy, and vulnerable to selection bias, poor compliance, and high dropout rates. This easing of regulatory requirements would likely encourage more research leading to improved female condom designs.

Involvement of Other Donors and Interested Parties:

CDC contributed technical expertise (in-kind) for up to 1 FTE/year and matching funds at an estimated rate of $100,000/year. NICHD provided $425,000 in Interagency Agreement funds to CONRAD to co-support the acute failure mode study, as well, and the Female Health Company donated 3,000 FC2s for use in this study.

II. Support for CONRAD Personnel at CDC

CA Activity Number:	NA
Primary Country of Activity:	US
Technical Coordinator:	Jennifer Legardy-Williams
Implementer:	Jennifer Legardy-Williams

Period: 7/1/09 – 6/30/14

Objectives:

The purpose of the Interagency Agreement was to provide funding to USAID for co-support of microbicide research. This included providing funds for CONRAD Staff at CDC to develop, manage, monitor and evaluate the ongoing and planned microbicide activities supported by USAID and the IAA.

Activity Description:

As also described in PPRD activity summary #5.4a ("CDC Collaborations: Support of CONRAD staff ") USAID and the CDC established an Interagency Agreement in 1990 for the purpose of conducting research on the relationship between steroid hormones and HIV. This research would be conducted through CONRAD/EVMS and has been amended to include other forms of contraception, HIV disease progression, condom acceptability, prevalence of OC use among HIV-infected women, and other aspects of HIV manifestation and reproductive health services. Beginning in FY08, collaborative activities between CONRAD and CDC were included in CONRAD's activities under this Cooperative Agreement, which included cost-sharing of CONRAD staff at CDC for microbicide activities of mutual interest under this IAA.

Progress:

CONRAD staff (Jennifer Legardy-Williams) provided technical support to CDC to manage microbicide activities of mutual interest. This support included development of contracts, overseeing the budgets, and managing study activities. The main activity was preparing for a Phase III randomized, controlled trial of the diaphragm with a candidate microbicide to prevent sexually transmitted infections in Madagascar. CONRAD/CDC staff provided support to the randomized controlled trial by assisting with the development of the protocol, overseeing the budget, coordinating study activities, and conducting site visits to Madagascar and University of North Carolina-Chapel Hill.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

Background research on the effects of cervical application of various vaginal microbicides with a mechanical barrier remains limited, however, and suggests the need for further evaluation. Ideally, a highly effective vaginal microbicide could be combined with the diaphragm as a possible woman-controlled way to decrease transmission of sexually transmitted infections, and particularly of HIV. Even a moderately effective diaphragm/microbicide combination may have a substantial public health benefit because it would provide an additional means of protection for women with otherwise limited options. Unfortunately, the planned randomized, controlled trial in Madagascar was not initiated due US Government decisions to suspend all funded activities in Madagascar in 2009 and the need to identify an alternative microbicide than was originally planned.

Involvement of Other Donors and Interested Parties:

USAID provided funds for co-support of CONRAD staff at CDC. USAID also provided $163,000 in FY07 Microbicide funds through FHI to CONRAD for this activity.

Final Summary of Research Conducted by CONRAD

Under AID-South Africa Field Support

Under USAID - GPO-A-00-08-00005-00 (PPRD)

I. Regulatory Support and Trial Oversight and Management for CAPRISA 008

CA Activity Number: PPA-12-143

Primary Country of Activity: South Africa

Technical Coordinator: Jill Schwartz

Implementer: CAPRISA

Period: **7**/1/12 – 09/04/14

Objectives:

The proof-of-concept for a microbicide, tenofovir (TFV) 1% gel, to prevent HIV-1 and herpes simplex virus-2 (HSV-2) infection was demonstrated by the CAPRISA 004 results and led to shifting priorities for CONRAD and the microbicide field. Registering TFV gel and making it available in South Africa and other African countries became top priorities. CONRAD provided study product, regulatory support, and oversight and management for clinical trials designed to enable registration in South Africa and other African countries

The purpose of AID-South Africa Field Support funds was to provide co-support of the clinical study CAPRISA 008, "Open-Label Randomized Controlled Trial to Assess the Implementation Effectiveness and Safety of 1% Tenofovir Gel Provision through Family Planning Services in KwaZulu-Natal, South Africa." The $1.5M was provided to CAPRISA for co-support of the conduct of the clinical study.

This study is further described in PPRD activity summary #5.1c, "Regulatory Support and Trial Oversight and Management for CAPRISA 008".

Progress:

CAPRISA 008 was a follow-up study to CAPRISA 004. It was designed as a Phase IIIb, 2-arm, open-label, randomized, controlled trial. Approximately 700 sexually active HIV-uninfected CAPRISA 004 participants aged 18 years and older were to be enrolled from the CAPRISA eThekwini and Vulindlela clinics and their neighboring public sector family planning services in KwaZulu-Natal, South Africa. Participants were randomized to receive TFV 1% gel through either: 1) public sector family planning services with 2-3 monthly provision and monitoring of TFV 1% gel and the use of quality improvement methodology to promote reliable service delivery (intervention arm), or 2) the CAPRISA research clinics with monthly provision and monitoring of tenofovir 1% gel (control arm). Participants in both study arms were instructed to use TFV gel within 12 hours before and after a coital event. The primary endpoint of gel use would be assessed as the mean number of returned used applicators per participant per month.

CAPRISA 008 received Medicines Control Council (MCC) approval on May 21, 2012. CAPRISA 008 was initiated and started screening in November 2012 and was conducted at 2 sites; Vulindlela and eThekwini. By Q3 2014, enrollment was complete. A total of 448 participants were screened and 382 of those participants were enrolled. After this reporting period, the funding for this activity continues under Cooperative Agreement

#AID-OAA-A-14-00011. CAPRISA 008 starting exiting participants February 1, 2015 and all participants will exit by April 30, 2015. Quality Assurance checks will be performed and the database will be locked by Q2 2015. CAPRISA expects to have data by Q3 2015.

Contribution of this Activity to Microbicide R&D and Implications for Future HIV Prevention Programs:

CAPRISA 008 will provide data on the feasibility and effectiveness of integrating microbicide provision into existing family planning services. In addition, CAPRISA 008 seroconversion data can be compared to a counterfactual group to provide additional effectiveness data on tenofovir gel.

Involvement of Other Donors and Interested Parties:

The completion of the trial is being funded through a new Cooperative Agreement AID-OAA-A-14-00011, entitled "Completion of Phase III Clinical Trial, New Drug Application Dossier, and Implementation Studies for Tenofovir 1% Gel, a First-in-class Topical Product for the Prevention of HIV Acquisition by Women."

Appendix B. Patents Issued and Filed

Title	Countries	Patent Number	Issue Date
PATENTS ISSUED			
SURAMIN AND DERIVATIVES THEREOF AS TOPICAL MICROBICIDE AND CONTRACEPTIVE Suramin is a an antimicrobial drug ($C_{51}H_{40}N_6O_{23}S_6$; Chemical name: 8, 8 ' -(carbonyl two (imino group -3, 1- phenylene carbonyl imino (4- methyl -3, 1- phenylene) carbonyl imino)) two -1, 3, naphthalene trisulfonic 5- acid)). The invention describes that the topical application (including the vaginal method) of Suramin or its derivatives inhibit sexually - transmitted diseases by acting as antimicrobial agents. Additional use as contraceptives (inhibit sperm function) has also been described	Luxembourg, Ireland, Germany, France, European Patent Convention Canada	1487432 2477734	06-Mar-2013 28-May-2013

Title	Countries	Patent Number	Issue Date
TOPICAL ANTIVIRAL FORMULATIONS The invention describes formulations of nucleotide reverse transcriptase inhibitor (NRTIs), namely Tenofovir ([2-(6-Amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid), or its derivatives thereof, as topical agents for preventing HIV infection.	African Regional Industrial Property Organization	AP2666	17-May-2013
	Albania, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Cyprus, Denmark, Estonia, European Patent Convention, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Monaco, Netherlands, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, United Kingdom	1773296	04-Nov-2009
	Indonesia	0024769	17-Dec-2009
	Hong Kong	1103026	19-Mar-2010
	Macedonia	903448	10-Dec-2010
	Australia	2005272032	20-Oct-2011
	Japan	5121451	02-Nov-2012
	Korea	10-1225707	17-Jan-2013
	Singapore	154468	28-Feb-2013
	Armenia, Azerbaijan, Belarus, Eurasian Patent Organization, Kazakhstan, Kyrgyz Republic, Moldova, Russian Federation, Tajikistan, Turkmenistan	017763	29-Mar-2013
	China	ZL200580023210.1	N/A

Title	Countries	Patent Number	Issue Date
SUBSTITUTED NUCLEOSIDE DERIVATIVES WITH ANTIVIRAL AND ANTIMICROBIAL PROPERTIES The invention describes fatty acid and fatty alcohol substituted nucleoside derivatives as having potent anti-HIV activity. Some of them also display activity against other sexually transmitted pathogens such as HSV, HPV and CT. Additional use as Contraceptives for the said compounds has also been described.		200880101864.5	27-Aug-2014

Title		Application Number	Filed Date
PATENTS FILED			
LOW-GLYCERIN FORMULATIONS FOR HIV TREATMENT AND PREVENTION		61/793745	March 15, 2014
TOPICAL ANTIVIRAL FORMULATIONS		US 12/893,516	Sep 29, 2010
TOPICAL ANTIVIRAL FORMULATIONS		US 12/890,337	Sep 24, 2010

Appendix C. Publications

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