

LUMINAL PHARMACOKINETICS, PHARMACODYNAMICS, AND  
ACCEPTABILITY OF FOUR RECTAL MICROBICIDE VEHICLE  
CANDIDATES IN THE DISTAL COLON

by  
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## ABSTRACT

### Background

Men who have sex with men (MSM) have a high risk of HIV infection, accounting annually for 53 % of annual new HIV infections in the U.S. Because of an increase in unprotected sex among MSM, there is interest in developing a rectal microbicide (RM) as pre-exposure prophylaxis (PrEP). Topical PrEP requires the selection of a drug vehicle that delivers the active drug to the desired distribution without negative impact on mucosal integrity that could favor HIV infectivity. This clinical study evaluated luminal pharmacokinetics (distribution and clearance), pharmacodynamics (including rectal mucosal permeability, histology integrity, and susceptibility to HIV infection *ex vivo*), and acceptability of four formulation candidates to be used as RM vehicles.

### Methods

Eight male participants were recruited for this randomized, partially-blinded, comparative study. All formulations -- aqueous fluid (AF), aqueous gel (AG), lipid fluid (LF), and lipid gel (LG) -- were evaluated in inpatient and outpatient phases. In the inpatient phase, formulations were mixed with  $^{99m}\text{Tc}$ -DTPA (technetium 99m-labelled diethylene triamine pentaacetic acid), and intra-rectally administered. Blood was collected over 24 hours to assess permeability to DTPA. One hour after dosing, flexible sigmoidoscopy was performed and colonic biopsies and luminal brushes were obtained. Samples were evaluated for histology and HIV explant infection. SPEC/CT imaging studies evaluated formulation in the colon over 24 hours (2, 4, and 24 hours). Distribution was described by the use of a novel concentration-distance parameters within the colon:  $D_{\text{max}}$ , distance associated with most proximal signal migration. Participants completed surveys to evaluate product acceptability.

## Results

Histology evaluation of colonic mucosa found no differences in epithelial denudation. HIV colonic explant infectibility when dosed with LF showed a statistically significant increase of 5.3 times ( $\beta$  coefficient of 5.35,  $p < 0.05$ ) in comparison with a no-drug baseline when analyzed using a multilevel analysis adjusted by distance from the anal sphincter. Same multilevel analysis comparing luminal concentrations of AG and LF and tissue concentrations of LF showed a statistically significant decrease of 1.5, 7.1, and 2.2 times ( $\beta$  coefficients of 0.63, 0.14, and 0.44 respectively, all  $p < 0.05$ ) when compared with AF adjusting by distance from the anal sphincter. Product permeability was analyzed using Wilcoxon paired analysis and the lipid products' permeability was significantly higher statistically than aqueous ones ( $p < 0.05$ ).  $T_{\max}$  of the LF formulation (0.6 hr) was analyzed using Wilcoxon paired analysis, and was significantly lower statistically than the other three products (1.7, 2.2, and 1.8 hr for AF, AG, and LG respectively). Product colonic distribution analysis using multilevel analysis showed a  $D_{\max}$  at 4 hours statistically significantly shorter than at 2 hours after dosing (mean difference = -4.12 cm,  $p = 0.014$ ) adjusting by formulation. The LF formulation had a statistically significantly shorter  $D_{\max}$  when compared with AF ( $p = 0.009$ ). Product acceptability scores (from 1 to 4, low to high) of gel products was higher than for fluid products, mean was 3.75 and 3.25 respectively. However since there was a lack of statistical power it was not possible to come to conclusions about acceptability.

## Conclusions

This study has demonstrated that AF, AG, and LG formulations do not damage the colonic epithelium, and distribute similarly in the colon. All formulations have shown a decrease in colonic distribution in time. Lipid formulations have higher permeability compared to

aqueous ones. Gel formulations trended toward greater acceptability, but low statistical power renders any conclusions about acceptability tentative.

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## INTRODUCTION

### HIV Epidemic

Still today the HIV epidemic continues causing global impact in morbidity and mortality. By the end of December 2009 UNAIDS reported an estimated 33.3 million (31.4 million – 35.3 million) of adults living with HIV and during the same year 2.6 million (2.3 million – 2.8 million) new HIV infections occurred in adults (Joint United Nations Programme on HIV/AIDS. 2010). By July 2010, the Centers for Disease Control and Prevention (CDC) estimated that in the United States more than one million people are infected with HIV and 21% are unaware of being infected. Approximately 56,300 Americans become infected with HIV each year. Furthermore, men who have sex with men (MSM) are the population with the highest risk of infection accounting 53% of all new HIV infections and 48% of all HIV infected people living in the US (CDC 2010), and an increasing rate of transmission is occurring among African-American and Hispanic MSM (Harawa et al. 2004). This high HIV transmission rate in MSM has also been described in several sub-Saharan settings and other places around the world (Baral et al. 2012; Beyrer et al. 2012).

### Rectal Microbicide Need

It is known that the use of condoms reduces the risk of HIV transmission among MSM groups (UNAIDS. 2004). However, several surveys have also reported that condom use is inconsistent in receptive anal intercourse (RAI) (Franssens, Hospers, and Kok 2009; Hill, King, and Smith 2009; Mendoza-Perez and Ortiz-Hernandez 2009) and that there is an increase in sexually transmitted infections among MSM in the US and abroad (Stolte et al.

2006). Due to this increase in unprotected sex, a special interest in the development of a rectal microbicide (RM) to be used for pre-exposure prophylaxis (PrEP) and that could be administrated as a rectal douche and lubricant has brought attention to this issue since the use of douches and lubricants are acceptable and frequently used by MSM (Carballo-Diequez et al. 2008; Schilder et al. 2010; Baral et al. 2012, , Kinsler, 2012). Unlike development of oral PrEP, topical PrEP requires the selection of a drug vehicle that delivers the active drug to a desired location and distribution without having a negative impact on mucosal integrity, causing HIV infection more readily. Previous studies using vaginal microbicides (VM) have shown the safety of these products on vaginal mucosa and their efficiency in reducing HIV transmission (Abdool Karim et al. 2010). However, the outcomes of these products when used as vaginal microbicides are not translatable to use as RM. First, colonic mucosa has only a single columnar epithelium and second, it has a higher number of CD4-cells which are the main target for HIV infection. Both conditions explain the high transmission risk when comparing anal with vaginal intercourse (Yu and Vajdy 2010). Since any condition that affects the columnar epithelium and/or increases the number of CD4-cells in colonic mucosa may increase the risk of HIV transmission, it is possible that a previously established safe vaginal formulation, when intra-rectally administrated as RM, could damage the colonic mucosa and increase the risk of HIV transmission. Therefore it is necessary to develop a formulation that is safe and can be used for RM and VM.

#### Factors Involved in the Development of Microbicide

It is very important to understand the essential elements involved in the interaction between microbicide and virus. Both of them are linked in a series of interrelated processes from

exposure to HIV infection. As seen in Figure 1(Hendrix, Cao, and Fuchs 2009), adherence summarizes the variables of dose, route, and frequency of use and HIV exposure includes HIV dose, route, and frequency variables related to sexual exposure. Pharmacokinetics and viral kinetics describe the movement of drug and virus particles respectively in space and time. The whole dynamic processes include: 1) Pharmacodynamics which is limited to drug-virus interactions directed to prevent virus targeting to susceptible cells. 2) Toxicodynamics, mainly influenced by drug concentration that captures changes in gut or vaginal mucosa that may enhance cell and tissue susceptibility to HIV infection independent of drug-mediated antiviral effects; and 3) Viral dynamics which involves viral interaction with susceptible cells followed by viral replication. Synthesizing all these elements, it is possible that microbicide toxicity, poor adherence, and the likelihood of risk reallocation due to microbicide use (e.g. unprotected sex) may be responsible for the failure of previous clinical trials directed toward demonstrating microbicide efficacy.

### Specific Properties Required for Microbicide Formulation

The development of a microbicide candidate vehicle should involve several key specific properties for the formulation: 1) high acceptability to maximize adherence; 2) optimal pharmacokinetics that match or exceed HIV distribution in space and time; and 3) minimal toxicity, to prevent an increase in local tissue susceptibility to HIV infection.

#### 1. Acceptability

Acceptability of a medication directly impacts adherence to proper use of the product. Factors influencing adherence to prescribed drug regimens is highly complex, however it is

not hard to predict what is the impact of poor adherence on drug's efficacy. In 2008 the results of a clinical trial evaluating the efficacy of a vaginal microbicide called Carraguard were published (Skoler-Karpoff et al. 2008). Results of the Carraguard clinical trial could not demonstrate any difference in HIV seroconversion rate between placebo and active agent. This study also showed discordance between the self-reported dosing adherence rate (96%) and a biological measurement estimating the number of applicators used (42%), suggesting that actual adherence in this study was quite low. This low adherence could be the main reason of failure in demonstrating product efficacy.

## 2. Pharmacokinetics

The ideal topical microbicide needs to cover the mucosal surface exposed to HIV and remain there for a sufficient amount of time without modifying tissue characteristics that could increase the risk of HIV infection. In a previous study aimed at determining the distribution of radiolabeled HIV surrogates and potential microbicide gels, direct comparison of imaging (scintigraphic with MRI) and endoscopic tissue samples was performed following product administration (Hendrix et al. 2008). A simulated intercourse model that replicates the mechanical and physiological effects that anal intercourse could have on gel distribution and gut motility was used. A microbicide gel surrogate (OTC sexual lubricants) labeled with gadolinium was used for MRI visualization and as a surrogate for a small-molecule microbicide the radionuclides  $^{99m}\text{Tc}$  or  $^{111}\text{Indium}$  chelated to DTPA (technetium 99m-labelled diethylene triamine pentaacetic acid). Simulated ejaculation through an artificial phallus, using viscosity-adjusted OTC sexual lubricants or autologous semen radiolabeled with HIV-sized sulfur colloid, allowed the study of surrogates of microbicide and HIV-infected semen interactions in situ. Single photon-emitted computed

tomography (SPECT) coupled with computed tomography (CT) followed immediately by MRI and repeated up to four times within the 24-hour post-dose period allowed nearly simultaneous comparison of the two methods in the same individual. MRI provided high resolution detail of gel distribution in the rectum and lower sigmoid, but gel migration more proximally resulted in a large concentration decrease in the upper sigmoid with degradation of signal-to-noise ratio. SPECT/CT provided superior signal sensitivity, though poorer spatial resolution, allowing visualization of gel migration as far, in one case, as the cecum. SPECT showed signal migrating proximally through the rectosigmoid, mostly within four hours after dosing, with distribution to the descending colon and splenic flexure in 25 and 12% of research participants, respectively (Hendrix, Cao, and Fuchs 2009) (Figure 2). To provide concentration data, a novel three-dimensional curve-fitting algorithm was developed based on the principal curve algorithm of Hastie and Stuetzle (Hastie and Stuetzle 1989) that incorporates operator-defined constraints and image intensity into the fitting; signal intensity (mass) and voxels with signal (volume) were used to estimate concentration along the path of the colon (Cao et al. 2012; Goldsmith et al. 2011). Plotting concentration, distance, and time values describes microbicide surface distribution for each condition studied. New pharmacokinetic parameters that incorporate distance into concentration and time parameters are needed. This will allow quantitative assessments of differences in gel distribution in the presence of different factors such as changes in formulation, coital simulation, presence of ejaculate, and use of preparatory enemas.

The direct sampling of luminal content along the exposed mucosal surface using endoscopic brush sampling and mucosal tissue biopsies provide information bridging macroscopic and microscopic microbicide distribution. Previous studies showed that luminal and biopsy

samples follow similar concentration profiles as expected, however luminal samples show concentrations one to two logs higher than the biopsies (Hendrix et al. 2008).

While microbicide surrogates, especially hyperosmolar ones, were seen to migrate as far as the splenic flexure, distribution of HIV distribution in the colon after sexual exposure had not been known. To assess HIV distribution, Louissaint, et al. studied cell-free and cell-associated HIV surrogates in autologous seminal plasma using SPECT/CT colon imaging after simulated RAI with ejaculation of these radiolabeled HIV surrogates (Louissaint, Nimmagadda, et al. 2012). These studies consistently found that drug sized particles demonstrate the same distribution and clearance as cell-free HIV sized particles. Similarly, cell-free HIV sized particles and autologous lymphocytes (the cell-associated HIV surrogate) have a similar pattern of distribution and clearance (Louissaint, Fuchs, et al. 2012; Louissaint, Nimmagadda, et al. 2012).

### 3. Toxicity

Early identification of drug-induced toxicity is essential, and for microbicides perhaps local toxicity could be as important as systemic toxicity. Despite temporal local mucosa toxicity apparently will have neither clinical relevance nor impact on drug safety, colonic mucosa toxicity could disrupt the mucosal barrier and increase the risk for HIV infection which will hamper with the microbicide efficacy. This observation was previously seen in a randomized clinical trial with nonoxynol-9 (N9) vaginal gel (Van Damme et al. 2002). Assessment of molecular or cellular changes leading to toxicity goes well beyond histology and visual inspection for tissue disruption and inflammation, so the real challenge is to select assays that predict increases in HIV infection susceptibility. A previous *in vitro* study using N9 and cellulose sulfate showed epithelial disruption secondary to loss of tight junction and

adherens junction proteins (Mesquita et al. 2009). Animal studies showed that N9-related inflammatory cell infiltration, epithelial disruption, and increased susceptibility to viral infection can be detected by a variety of methods, including visual and colposcopy inspection, histopathology, measurement of transepithelial electrical resistance, and HSV-2 challenge (Hillier et al. 2005). Previous studies have shown consistently evidence of correlation among epithelial disruption, inflammatory infiltrate, and increased inflammatory cytokine expression of IFN-gamma (McGowan et al. 2004), altered vaginal microflora, and increased susceptibility to ex vivo HIV infection in human cervical and colonic explant tissue (Abner et al. 2005; Dezzutti et al. 2004; Hillier et al. 2005).

The interpretation of microbicide induced toxicity results requires consideration of confounding sources of toxicities, including the microbicide vehicle and para-sexual activities to avoid incorrect attribution to the active microbicide ingredient. Coitally dependent use of microbicide gel vehicles and sexual lubricants are of particular concern because application occurs prior to and during coitus. Sexual lubricants have been associated with rectal epithelial surface disruption within 1.5 hours of application, and vaginal application of hyperosmolar gels results in leakage from the introitus in a dose-dependent manner (Fuchs et al. 2007; Lacey et al. 2010). Transudation of fluid due to hyperosmolar gels can dilute microbicide concentration and potentially reducing effectiveness. Moreover some common formulation excipients have shown to enhance mucosal permeability, sometimes resulting in relaxation of tight junctions and rectal epithelial disruption in human research participants (Aungst 2000; Muranishi 1990).

Epidemiologic studies suggest both vaginal and rectal douching (with enemas) increase the risk of HIV infection (Coates et al. 1988; Moss et al. 1987; Myer et al. 2006). It has been

reported that approximately two thirds of MSM who practice RAI use rectal enemas in preparation for sex, most commonly with tap water (hypo-osmolar) and Fleet<sup>TM</sup>-type enemas (hyperosmolar) (Carballo-Diequez et al. 2008; Hylton, Fuchs, and Hendrix 2004). Different studies have shown that hyperosmolar and hypertonic solutions injures the colonic mucosa resulting in cell degeneration and sloughing of epithelia, net intraluminal fluid and mucus secretion, and altered permeability (Billich and Levitan 1969; Kameda et al. 1968; Rubsamen and Hornicke 1982). Rectally administration of hypertonic solutions (Fleet Enema<sup>TM</sup>) and tap water also cause sloughing of the rectal epithelia in a temporal relationship between epithelial injury and exfoliation (Meisel et al. 1977; Schmelzer et al. 2004). This relationship with time was also seen when N9 was rectally administrated in MSM (Phillips et al. 2000). Sheets of epithelial cells were found in the rectal lavage fluid within 15 minutes of dosing and the mucosal layer was absent from colorectal tissue sections histologically. By 8 hours after dosing, these mucosal changes had been restored to normal. In another study evaluating rectal gel toxicity in MSM who practice RAI, hyperosmolar and iso-osmolar gels were compared (Fuchs et al. 2007). After one and a half hour of gel dosing, biopsies taken by endoscopy showed a higher grade of epithelial integrity loss when hyperosmolar gel was used (Figure 3) (Fuchs et al. 2007).

## Vehicle Selection

In this study two approaches were considered in formulating an efficient rectal microbicide. The first approach was to use a formulation that spreads and coats all tissue surfaces of the rectum and distal colon rapidly prior to RAI; the second was to create a deformable, erodible barrier that would stay at the site of administration (rectal ampulla) until re-distributed by



RAI. It was possible to evaluate these two approaches using either an aqueous or lipid vehicle. These considerations lead to the previous development of four candidate vehicles with various viscosity, lipophilicity, and mucoadhesive characteristics to meet the characteristics of an ideal rectal microbicide (Wang et al. 2011). In this study the four vehicles were referred as:

1. Aqueous Fluid (AF) – easily spreadable; fluid with viscosity consistent with rapid rectal/colonic distribution.
2. Aqueous Gel (AG) – erodible; semisolid with viscosity parameters consistent with erosion and distribution instigated by rectal intercourse.
3. Lipid Fluid (LF) – easily spreadable; fluid with viscosity consistent with rapid rectal/colonic distribution.
4. Lipid Gel (LG) – erodible; semisolid with viscosity parameters consistent with erosion and distribution instigated by rectal intercourse.

Their complete formulas and testing information are shown in Table 1 and 2 respectively. Condom compatibility testing was performed as follows: lubricated and non-lubricated latex condoms were exposed to the test lipid mixtures for 5, 15, and 30 minutes, washed with water and dried with a paper towel. After treated, condoms were fixed on the gel/film fixture and force applied with a ball probe (TA8A, 1/8 inch) until the breaking point was reached. Water and baby oil were used as a negative and positive control, respectively. The four candidate vehicles were also tested commercially for condom compatibility by the Family Health International, Product Quality and Compliance – Testing Laboratory with 3 lots each of non-lubricated latex, silicone lubricated, aqueous lubricated and polyurethane condoms. The aqueous candidate vehicles were compatible with all the condoms tested,

however, the lipid candidate vehicles were compatible with the polyurethane condoms, but not with the latex condoms.

The objective of the present clinical study was to evaluate toxicity, luminal distribution, and acceptability of the four previously described formulations that could potentially be used as vehicles for rectal microbicides. A cross-over study design with randomized product sequence was used in which research participants served as their own controls.

## MATERIALS AND METHODS

### Study Participants

This was a randomized, partially-blinded, comparative study of four different enema formulations. After obtaining Institutional review board approval eight HIV-seronegative healthy male research participants with a mean age of 39.8 years (SD = 8.5) were recruited in the study between November 2009 and July 2010. All research participants had history of RAI at least twice per month in the prior 3 months. Four formulations were evaluated in all participants. Each of the four formulations was evaluated in an intensive inpatient and outpatient phases. The primary purpose of the inpatient phase evaluation was to assess the toxicity, infectibility and pharmacokinetics (PK) variables. For these purposes all participants during each inpatient phase underwent flexible sigmoidoscopy including a baseline at least two weeks before administering the first formulation. A washout period of two weeks between colonic biopsies was used to prevent carry-over effects associated with intraluminal and tissue sampling. The outpatient phase period was designed primarily to address acceptability of the formulations in the context of use prior to RAI and, consequently, did not absolutely need to follow the inpatient period immediately. Research participants received separately all four formulations thereby serving as their own controls to reduce the effects of inter-individual variability on outcome variables. The sequence of study product evaluation was randomized.

## Microbicide Vehicle Formulations and Administration

In this study, four formulations previously described were used (Table 1) (Wang et al. 2011). In brief, microbicide vehicle formulations consisted of two iso-osmolar aqueous-based and two lipid-based formulations. Within each of these categories, one formulation has a viscosity consistent with liquid, and the other formulation had a viscosity similar to a semi-solid gel. The concentration of polymer or gelling agent has been adjusted in each case to provide a viscosity range of 20 to 100 cps (centipoises) for the fluid vehicles allowing them to spread easily and coat all tissue surfaces of the rectum and distal colon rapidly prior to RAI, and up to 50,000 cps for the gel vehicles allowing them to create a deformable, erodible barrier that would remain relatively localized at the administration site until distributed by RAI. For this reason the gel vehicles exhibit a rheological yield stress (stress that must be exceeded for flow to occur). These differences in each formulation allowed comparison of products with a wide range of hydrophilic characteristics (aqueous to lipid) and rheological properties (Newtonian, shear thinning, thermal sensitive and thixotropy).

Formulations for the inpatient phase were mixed with  $^{99m}\text{Tc}$ -DTPA from Cardinal Health (Dublin, OH) and a previously determined total volume of approximately 10 ml was intrarectally administered at time zero (Fuchs et al. 2007). One hour after dosing, flexible sigmoidoscopy was performed. Colonic mucosal biopsies and luminal brushes were obtained at 5, 10, and 20 cm from the anus.

## Histology

Colonic biopsies were blinded and examined by a pathologist using a categorical scales for surface denudation and lamina propria hemorrhage from 0, 1, 2, and 3 as follows: none, <33%, >33% and <66%, and >66% of mucosal involvement respectively. In addition number of apoptotic cells per biopsy was recorded to determine cellular damage.

## Mucosal Interferon-Gamma

Levels of IFN-gamma (IFN- $\gamma$ ) mRNA were measured using qPCR as previously described (McGowan et al. 2004). In brief, total RNA was extracted from two colonic biopsies obtained at 20 cm from the anus using a TRIzol isolation technique from Invitrogen (Carlsbad, CA). Reverse transcription of total RNA was accomplished using a First Strand ProSTAR RT-PCR kit from Stratagene (Cedar Creek, TX) using random primers. IFN- $\gamma$  primers optimized for real-time PCR were designed using the cDNA sequences from the GenBank database in conjunction with Primer Express software (Applied Biosystems, Foster City, CA). PCR products were designed to be approximately 150 base pairs and verified by agarose gel electrophoresis. Relative quantitation of cytokine gene expression was performed with the Applied Biosystems Geneamp 5700 sequence detection system (Applied Biosystems) using the standard curve method. The primers used were 5'-ATG TCC AAC GCA AAG CAA TA-3', 5'-CGA CCT TGA AAC AGC ATC TG-3'. Detection of PCR products was monitored by measuring the increase in fluorescence caused by the binding of SYBR Green to double-stranded DNA. Standardization across samples was achieved by

expressing cytokine copy number per  $10^6$  beta-actin copies per sample. All assays were performed in triplicate, and a mean calculated from the 3 measurements was obtained.

#### HIV Exposure and HIV-1 p24 Protein Measurement

A method using tissue explant has been explored to determine conditions that either increased or decreased susceptibility to HIV infection (unpublished data). It has been demonstrated that a  $TCID_{50}$  (50% tissue culture infective dose) dose dependent amplification with  $10^3$  and  $10^4$   $TCID_{50}$  per mL, result in infection of all challenged biopsies. HIV-1 p24 antigen concentration detected was a mean (SD) of 27.2 (8.4) and 56.8 (26.7) with  $10^3$  and  $10^4$   $TCID_{50}$  per mL, respectively. These findings were based on 6 biopsies placed in individual wells in a non-polar explant method described as follows. Six colonic biopsies were rinsed with RPMI 1640 medium containing L-Glutamine, 25mM HEPES (Mediatech, Herndon, VA) pen/strep antibiotic and 10% FBS (Invitrogen, Carlsbad, CA). Biopsies were exposed to  $10^3$   $TCID_{50}$  per ml of HIV-1, strain Ba-L from Advanced Biotechnologies, Inc (Columbia, MD) in a 24-well plate. After 2-hours in a cell culture incubator with 5%  $CO_2$ /95% air at 37 °C, biopsies were rinsed three times and placed on six separate wells containing Surgifoam (Cardinal Health) and culture media. Plates were incubated for 15 days. On days 3, 6, 9, and 12 culture media was harvest a replaced with fresh media. Harvested media was stored at -20 °C. HIV-1 p24 capsid protein concentration was measured using the HIV-1 p24<sup>CA</sup> antigen capture assay kit from NCI (Frederick, MD) according to manufacturer's instructions. In brief, samples were thawed at room temperature and transferred in a pre-coated 96-well plate. Rabbit anti-HIV (MN) p24 and Goat anti Rabbit IgG (H+L)-HRP were used as primary and secondary antibody respectively. Plates

containing substrate were read at 450 nm wavelength with a reference of 650 nm. Total cumulative amount of p24 produced per biopsy during 15 days was used in the analysis.

### Permeability Studies

Blood samples were taken at times 0 (before dosing), 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.33, 2.66, 3, 3.5, 4, 8, 16, and 24 hours after dosing and blood cells were separated by centrifugation. Serum, colonic biopsies and luminal brushes  $^{99m}\text{Tc}$  activity was measured using a 2480 Wizard<sup>2</sup> automatic gamma counter from PerkinElmer Inc. (Waltham, MA). Concentration values were normalized as a fraction of the dose administered for each subject (dose ratio). Serum values were analyzed calculating AUC ( $\text{AUC}_{0-24}$ ),  $C_{\text{max}}$ , and  $T_{\text{max}}$  using a noncompartmental model. Results were expressed as radiation dose-adjusted  $\mu\text{Ci/mL}$  as done in a previous study (Fuchs et al. 2007).

### Imaging Distribution

a) SPEC-CT. Soon after sigmoidoscopy, SPEC-CT imaging was performed to assess colonic distribution of the radiolabeled formulation. Participants were imaged using a dual-head VG SPECT-CT series system (GE Medical Systems, Waukesha, WI) equipped with a low-dose CT (computed tomography) unit (Hawkeye) as previously described (Caffo et al. 2008; Hendrix et al. 2008; Louissaint, Nimmagadda, et al. 2012) at 2, 4 and 24 hours after dosing. In brief, CT images were acquired before SPECT acquisition for anatomical references and imaging reconstruction. CT images were reconstructed with filtered back projection onto a  $256 \times 256$ -matrix size. After SPECT acquisition, images were reconstructed using the EM

algorithm (Hudson and Larkin 1994) and fused using the software General Electric eNTEGRA workstation, version 1.04 (GE Medical Systems, Waukesha, WI) into a 128 x 128 x 128 matrix size with each voxel representing 3.45 mm<sup>3</sup>.

b) Bladder and external contamination signal subtraction. In images showing activity in the bladder or skin contamination (e.g. gluteal region), the signal was subtracted from the original image by setting the voxel values of a spherical volume of interest (VOI) placed centrally over the bladder to a value of zero ("0"). Once the center and radius of the spherical VOI was identified, we used in-house developed software to perform the actual voxel removal.

c) Curve fitting algorithm. Attenuation corrected image data was exported as dicom files and converted to the Mayo Analyze format as using the software MRIcro version 1.40 (McCausland Center - University of South Carolina, Columbia, SC). Curve-fitting and concentration-by-distance calculations were done using R version 2.13.1 (The R Foundation for Statistical Computing, Vienna, Austria) using previously described algorithms (Caffo et al. 2008; Goldsmith et al. 2011). In short, a flexible principal curve algorithm is used to construct a three-dimensional curve through the colon images. This method allows user-specified endpoints and constrained interior points for the curve to ensure accuracy in the final fit. Additionally, the curve is gradually “molded” to the data to allow adaptation to complex structures. Image points that are more than 5 cm from any point on the fitted curve are omitted from further analysis; retained image points are sorted based on their orthogonal projections onto the curve. After the centerline is constructed, a concentration-by-distance curve is estimated using a kernel-based moving average that weighs intensity values within voxels according to distance measured along the centerline using the orthogonal projections.



The origin of the centerline was normalized to the coccygeal plain defined as the transverse plain crossing the coccyx in the SPEC-CT as previously described (Cao et al. 2012). The distance between the origin of the centerline and the coccygeal plain was recorded as  $D_{\min}$  (distance associated with the most distal signal). Previously defined imaging pharmacokinetic-distance parameters  $D_{\max}$  (furthest point where radiosignal was detected),  $DC_{\max}$  (distance at concentration maximum), and  $D_{\text{ave}}$  (mean residence distance) were calculated for further analysis (Cao et al. 2012).

## Acceptability

The response variables from the acceptability assessment included:

- a) Inpatient acceptability ratings (1-completely unacceptable; 2-somewhat unacceptable; 3-somewhat acceptable; and 4 highly acceptable) after one-time use in the hospital.
- b) Outpatient acceptability ratings after use:
  - How much they liked each product (overall), the consistency of each product, how each product felt inside their rectum immediately and 30 minutes after inserting it.
  - How easy it was to administer the product.
  - How much they liked anal sex after using the product including how sexually satisfied they felt and how much their partners liked each product.
  - How likely they would be to use each of the products: 1. every time they have RAI, 2. on occasions when they don't use condoms, and 3. if they had to wait 30 minutes after application before having intercourse.

At the end of the study, research participants were asked to make comparative assessments of the 4 formulations. An intensive structured interview was also performed.

### Statistical Analysis

Histological data was analyzed using multilevel analysis since multiple samples per participant and product were obtained. Results were adjusted when required by distance from anus where biopsies were taken. Dependent variables were analyzed as ordinal and compared to baseline. The logarithm of the interferon-gamma values was calculated and analyzed using paired t-test. All formulations were compared to baseline. HIV-1 p24 data was analyzed using multilevel analysis. Dependent variable was defined as the logarithm of the cumulative p24 protein produced per biopsy and analyzed as a continuous variable and compared with baseline. Permeability studies and pharmacokinetic-distance parameters dependent variables were analyzed as categorical using Friedman's test and Wilcoxon signed-rank test for multiple comparisons among products. Imaging data was analyzed using multilevel analysis since multiple samples per participant and product were obtained at different times.

Product acceptability assessment was analyzed using a Latin square design to control for variations in acceptability ratings due to differences between participants and due to the order in which study products were used (sequence). Acceptability ratings were examined as a function of product. The ANOVA model was completely additive with no interaction terms. The two extraneous factors –sequence and subject– were important to include in order to provide a more precise evaluation of the difference in acceptability ratings due to

the products; they were used to determine whether or not accounting for them was efficient in reducing the variability in the experimental units (Ott and Longnecker 2010). Also, due to violations of the normality assumption in the acceptability data, non-parametric tests were done. However, because the Kruskal Wallis test involves one independent variable and given the need to include all three variables (product plus the two extraneous variables), ranks of the ratings were obtained (using the mean for ties) and ANOVAs were performed with Tukey's HSD for post-hoc multiple comparisons. It was also calculated the KW critical value as another way to look at post-hoc pairwise comparisons (differences in the mean rankings).

In all cases when logarithm conversions were performed, zero values were transformed by adding one tenth of the minimum rounded-down numerical value to all the data. Pharmacokinetics parameters were calculated using the WinNonlin® 5.0.1 software package (Pharsight, Sunnyvale, CA). Data was analyzed using the statistical package STATA/IC 11.2 for Windows software (StataCorp LP, College Station, TX). Differences showing a p-value less than 0.05 or a  $\beta$  coefficient 95% confidence interval that does not include zero or 1 if exponential were considered statistically significant.

## RESULTS

### Histology

The degrees of colonic mucosal surface denudation induced by the products were analyzed adjusting by distance. Table 3 shows that all the products apparently increased the grade of surface denudation ranging from a 4% to 69% higher than baseline. However, statistical analysis showed that this change was not statistically significant. Analysis of the degree of lamina propria hemorrhage is shown in Table 3. AG and LG showed a statistically significant 3.2 and 2.4 times increase respectively in comparison with baseline after adjusting for distance where biopsies were taken. Another statistically significant increase in lamina propria hemorrhage was seen when comparing biopsies obtained at 10 and 20 cm with 5 cm ones. The analyses showed a statistically significant 2.0 and 3.6 times increase at 10 cm and 20 cm respectively in comparison with 5cm and adjusted by formulation. Number of apoptotic cells was not different among products when compared with baseline (data not shown).

### Mucosal Cytokine Profile

The induction of IFN- $\gamma$  mRNA expression in colonic mucosa after exposure to each product was measured. After paired analysis, none of the products showed changes in the levels of IFN- $\gamma$  mRNA expression in comparison with baseline (data not shown).

## HIV infectibility in colonic mucosal explants

Changes in the cumulative levels of p24 protein expression after 15 days in comparison with baseline are shown in Table 3. LF product showed a statistically significant 5.35 times increase ( $\beta$  coefficient of 5.35, 95% CI, 1.42 - 20.13) in p24 expression. The other three products did not show any difference compared with baseline.

## Permeability

Concentration-time curves by product and by product per participant are shown in Figures 4 and 5 respectively. Figure 4 shows that lipid products reach higher concentrations than aqueous products. The pharmacokinetic parameters  $AUC_{0-24}$ ,  $C_{max}$ , and  $T_{max}$  calculated using a noncompartmental model, were used to determine differences in permeability among products (Table 4). Paired analysis showed statistically significant higher  $C_{max}$  and  $AUC_{0-24}$ s in lipid products when compared with aqueous ones.  $T_{max}$  of LF formulation was statistically significant lower when compare with the other three products. AF, AG, and LG showed similar median  $T_{max}$ .

## Luminal Concentrations

Luminal brushes containing product were obtained by flexible sigmoidoscopy one hour after dosing. Multilevel analysis of the ratios of luminal concentration per dose administered comparing with AF product are shown in Table 3. AG and LF products showed statistically significant decrease in luminal content, 1.5 and 7.1 times lower respectively ( $\beta$  coefficients of

0.63 and 0.14 respectively) in comparison with AF after adjusting by distance where brushes were taken, which were at 5, 10 and 20 cm from the anal sphincter

When distances were analyzed, brushes taken at 20 cm showed a statistically significant 3.7 times decrease in product content compared with brushes taken at 5 cm adjusted by product used. Brushes taken at 10 cm did not show statistically significant difference.

### Tissue Concentrations

Colonic biopsies were also taken at 5, 10, and 20 cm from the anus. Results of the multilevel analysis comparing with AF formulation biopsies are shown in Table 3. LF tissue concentrations showed a statistically significant 2.2 times decrease ( $\beta$  coefficient of 0.44) in comparison with AF after adjusting by the distance from the anal sphincter. Analyzing by tissue distance and adjusting by formulation, 10 cm biopsies showed a statistically significant 2.2 times decrease and 20 cm biopsies a statistically significant 4 time decrease in tissue concentration in comparison with biopsies taken 5 cm from the anal sphincter (data not shown).

### Imaging Distribution

Tube fitting and concentration by distance results were calculated in each participant at 2, 4 and 24 hours after intra-rectal administration of each formulation. Figure 6 shows imaging results corresponding to SPEC/CT,  $^{99m}\text{Tc}$  DTPA radionuclide activity imaging, tube fitting reconstruction using the previously described algorithm, and the calculated concentration by

distance curves at 2, 4 and 24 hour following intra-rectal administration of AG formulation in one participant. Figure 7 shows imaging results corresponding to SPEC/CT,  $^{99m}\text{Tc}$  DTPA radionuclide activity imaging, tube fitting reconstruction, and the calculated concentration by distance curves 2 hours following separately intra-rectal administration of each product AF, AG, LF, and LG formulation in the one participant. Results of the imaging pharmacokinetic-distance parameters by product and by time after dosing and multilevel analysis results are shown in Table 5.  $D_{\max}$  at 4 hours showed a statistically significantly lower value than at 2 hours after dosing (mean difference = -4.12 cm, 95 % CI = -7.4 -0.8,  $p=0.014$ ) after adjusting for all formulations. In addition, LF formulation had a statistically significant lower  $D_{\max}$  when compared with AF ( $p=0.009$ ) after adjusting by time from dose. No differences were found among products or time after dosing when analyzing  $DC_{\max}$ ,  $D_{\text{ave}}$ , and  $D_{\min}$ .

### Acceptability

### Inpatient Ratings

The main effect for product was statistically significant ( $F(3, 18) = 3.967$ ,  $p = .025$ ). The gels (both aqueous and lipid) were ranked higher than the AF ( $M_{\text{rank}} = 20.75$  and  $19.75$ , respectively vs.  $11.38$ ). Comparisons using Tukey's HSD indicated that the AG was ranked statistically significantly higher than the AF ( $p = .04$ ). The comparison between the LG and AF was only marginally statistically significant. However, none of the comparisons were statistically significant when using the Kruskal Wallis critical value of  $12.039$ ,  $p = .05$ . The actual ratings (on a scale of 1-4) are presented in Table 6.

## Outpatient Ratings

Overall, participants rated the four products similarly. The LG was ranked higher than the other products, but this was not statistically significant ( $\eta^2 = .036$ ). On a scale from 1 to 10, the ratings for AF and AG were similar (mean = 6.38,  $SE = 1.034$  (95%CI = 3.93, 8.82), and mean = 6.75,  $SE = .84$  (95%CI = 4.76, 8.74), respectively) indicating that participants felt neutral about them and liked the aqueous products equally. With regard to consistency, the AG was ranked lowest relative to the other products, but this was not statistically significant ( $\eta^2 = .132$ ). The gels were rated more favorably in terms of how each product felt inside their rectum immediately after (AG was ranked higher) and 30 minutes after inserting it (lipid gel was ranked higher); however, no statistically significant differences were found (the effect sizes were small - .075 and .106, respectively). Difficulties of formulation administration results are shown in Table 7. Mean ranks indicate that the fluids were easier to administer ( $M_{rank} = 17.19$  for both aqueous and lipid fluids); the AG was ranked the lowest ( $M_{rank} = 13.38$ ). The differences were non- statistically significant ( $\eta^2 = 0.067$ ). Participant ratings indicate that all of the products were fairly easy to administer. Only a few problems were reported by participants, including leakage and soiling of underwear and linens. These were reported with the LF and both of the aqueous products. No problems were reported with the LG. The LG was ranked highest with respect to how much they liked anal sex after using each of the products, followed by the AG ( $\eta^2 = .108$ ). In terms of how sexually satisfied they felt after using the products, the lipid products were ranked higher, followed by the AG ( $\eta^2 = .192$ ). The AF was ranked the lowest. We examined the difference between how sexually satisfied they felt after using each of the products and how sexually satisfied they feel in general with this partner when they have RAI not using this study product. The differences



were greater for the aqueous products (-1.71 for AF, -1.50 for the AG, -0.50 LF, and -0.43 LG). When the differences were ranked, the F test was not statistically significant; however, power was only .324 (eta-square = .221 - indicating a small effect size). Participants indicated that they would want to use the study products without the knowledge of a steady sexual partner for added protection (e.g. “extra backup”, “insurance protection”, “added insurance”) and when not in a monogamous relationship or if unsure about infidelity in the relationship. All said that it would be possible to use the products without a steady partner or casual partners noticing them with the exception of the lipid products (both gel and fluid) – two participants said that it would not be possible after they used them. Also, one participant thought that steady or casual partners would notice the AG.

Participants were asked to indicate how likely they would be to use each of the formulations in the future and results are shown in Table 8. The AF was ranked the lowest; however, the ratings were in the middle of the 10-point scale, indicating that participants were neutral about future use. When asked if they would use the product every time they have RAI, the AF was ranked the lowest. Similarly, it was ranked the lowest if they had to wait 30 minutes after application before having intercourse. The four products were rated similarly when asked about likelihood of use when condoms are not used.

## DISCUSSION

This study evaluated in eight participants the toxicity, luminal distribution, and acceptability of four formulations that could potentially be used as vehicles for rectal microbicides. The cross-over study design allowed each participant to serve as their own controls. Despite the small sample size, it was possible to discriminate between products in several important features.

Histological examination of the colonic biopsies taken at 5, 10, and 20 cm one hour after dosing, showed no statistically significant difference in surface denudation when each product was compared with biopsies taken before the administration of any formulation (baseline). This study also found an increase in lamina propria hemorrhage when both gel formulations were used. It has been described that lamina propria hemorrhage without changes in the epithelium or edema could be as a result of trauma due to bowel preparation or biopsy artifact (Chlumska et al. 2010). So it is possible that the risk of trauma due to bowel preparation or endoscopy be increased with the use of gel formulation. About the high rate of lamina propria hemorrhage at 20cm, as part of the study more biopsies were taken at 20 cm, which increased the exposure to endoscope manipulation independently of the product. However, there is not enough evidence to conclude that the gel formulations produce relevant changes that could increase the transmission of HIV since none of the gel formulations produced surface denudation or epithelial damage during the first hour.

Results of HIV infection of colonic explants previously exposed *in vivo* to LF formulation showed a 5-time increase in the infection rate when compared with baseline. This result although unexpected is not far from reality since multiples studies have shown an increase in the risk of HIV transmission due to douches, lubricants, or microbicides (Coates et al. 1988;

Moss et al. 1987; Myer et al. 2006; Phillips et al. 2004). These findings also confirm the ability of our technique to determine differences in HIV infection rate between formulations. The mechanism that increases the risk of HIV infection in colonic explants needs to be determined, since increased epithelial denudation was not observed in colonic biopsies exposed to LF formulation.

Product permeability using pharmacokinetic parameters was calculated for each formulation. Lipid products had higher AUC and  $C_{\max}$  than aqueous formulations. These findings indirectly favor the idea that aqueous formulations stay longer in colon since they have lower permeability. In addition, permeability of LF shows a very short  $T_{\max}$ .

Luminal and tissue concentrations for each formulation were calculated. LF showed statistically significant lower concentration in lumen and tissue. The analysis also showed that LF concentrations are the lowest at 5, 10 and 20 cm in lumen and in tissue when compared with the other three formulations. Further analysis by distance found that all the formulations have less product at 20 cm than at 5 cm, suggesting that more formulation was accumulated at distal levels (e.g. 5 cm) compared to more proximal, but this was not only for LF. So probably a portion of the LF formulation was eliminated with the stools and that will explain its lower values at all the levels. However, the clinical relevance of this low concentration still has to be determined. Most importantly, the low tissue concentration of LF compromises its future use as a vehicle in the development of a RM. AG formulation concentration was lower in the lumen but not in tissue when compared to other formulations. However, it is difficult to establish the clinical relevance of this finding since the upper 95% CI was 0.99. These findings about LF together with the previous about very

short  $T_{\max}$  support the idea that this formulation may be crossing faster than the other formulations from the colon into the bloodstream.

Analysis of the imaging pharmacokinetic-distance parameter  $D_{\max}$  showed that between 2 and 4 hours after dosing, there was a decrease in colon distribution with all the formulations. In addition, multilevel analysis of the  $D_{\max}$  comparing all products with AF, showed statistically significant lower values with LF formulation. This finding in LF product agrees with our previous results above of luminal and tissue concentration. Although it does not allow proposing a mechanism that could explain how LF could be enhancing HIV infection in explants.

Analysis of the inpatient assessment ratings showed that aqueous and lipid gels receive higher ratings than the liquid formulation. Although the AG formulation appears to be preferred over the AF formulation, the interpretation of whether the differences can be considered statistically significant is restricted by assumptions about the normality of the distribution of the population. Nevertheless, in comments written in by participants in response to questions on what was liked the most and the least, there are fewer negative comments about the gel formulations than about the fluid ones.

Analysis of the outpatient data is restricted due to lack of power to detect significant statistical differences. Yet, trends were observed in the data analysis that indicate that the gels, (both aqueous and lipid) were rated more favorably than the fluid formulations on how they felt in the rectum after insertion, how participants liked anal intercourse after using them, and participants' likelihood of using them in the future. Ratings were similar among all four products on overall liking, consistency, and ease of administration.

About product acceptability, there is a trend for gel products to be favored over fluid products, more clearly in the aqueous formulations. However, the small size of the sample and lack of statistical power should caution about this overall interpretation. It should be noted that participants used all products in small amounts, mainly as lubricants; they did not have enough product available to use the fluid formulations as an enema either before or after sexual intercourse. The fact that most ratings for the fluid formulations were in the liked or neutral ranges of the scales is encouraging, from a behavioral perspective, about the possibility of using the liquid formulation as an enema.

In summary, this study has shown that AF, AG, and LG formulations do not damage the colonic mucosa, and distribute similarly into the colon. Aqueous products turned out to have the most consistently favorable characteristics. Lipid formulations have higher permeability and reach higher concentrations in blood. Gel formulations seem to be more acceptable for MSM. LF was the formulation that behaves very differently than the other three and with the most consistently negative in term of PK, permeability and HIV explant infectivity, so it is in the opinion of the author that this formulation should not be considered a good candidate in the development of a RM. However it seems that LF has the characteristics of a vehicle to be used for the delivery of a topical drug.

This clinical study has demonstrated the ability to assess many highly relevant domains in a small first in human study of these four candidate vehicle formulations for the development of a microbicide to be used for PrEP in MSM.

On 2012, FDA approved the oral use of the combination of the reverse transcriptase inhibitors tenofovir and emtricitabine for PrEP (FDA 2012). This decision has been criticized since oral PrEP effectiveness is highly dependent of treatment adherence and its

use could increase the risk of HIV infection due to behavioral changes (Cohen 2012). Moreover previous studies highlighted the poor compliance of oral PrEP in MSM (McGowan 2011). However MSM are very interested in the development of a topical microbicide that can be used as lubricant before RAI. This last observation increases the acceptability and adherence of microbicide use as PrEP (Sullivan et al. 2012) and highlights the contribution of this study in the microbicide development pipeline.

Table 1. Candidate vehicles composition

Aqueous Fluid	Percent (w/w)	Function
- Poloxamer 407	15.00	Thickening Agent
- Glycerin	2.00	Cosolvent
- Methylparaben	0.18	Preservative
- Propylparaben	0.02	Preservative
- Sodium Citrate Dihydrate	0.30	Buffer
- Purified Water	82.58	Solvent
- 1N HCl or 1N NaOH qs	pH 7.0±0.20	pH Adjustment
Aqueous Gel		
- Carbomer 974P	0.50	Thickening Agent
- Glycerin	2.50	Cosolvent
- Methylparaben	0.18	Preservative
- Propylparaben	0.02	Preservative
- Disodium EDTA	0.01	Chelating Agent
- Sodium Hydroxide Solution 18%	1.15	pH Adjustment
- Purified Water	95.64	Solvent
- 1N HCl or 1N NaOH qs	pH 7.0±0.20	pH Adjustment
Lipid Fluid		
- Myristyl Myristate (Crodamol MM – Croda)	5	Gelling Agent
- Isopropyl Myristate (Crodamol IPM – Croda)	95	Solvent
Lipid Gel		
- Glyceryl Stearate and PEG-75 Stearate (Gelot 64 – Gattefosse)	25.0	Gelling Agent
- Caprylic/Capric Triglycerides (Crodamol GTCC – Croda)	74.9	Solvent
- Vitamin E Acetate	0.1	Antioxidant

qs: quantity sufficient; MM: Myristyl myristate; IPM: Isopropyl myristate; GTCC:

Caprylic/capric triglyceride; PEG: polyethylene glycol

Table 2. Candidate vehicles test parameters

	Aqueous Fluid	Aqueous Gel	Lipid Fluid	Lipid Gel
Phase Separation	None	None	None	None
Viscosity	Newtonian <sup>a</sup>	Shear Thinning Yield Stress <sup>b</sup>	Newtonian <sup>a</sup>	Shear Thinning Yield Stress <sup>b</sup>
Gel Strength/Adhesion	Low	High	Low	High
Microbial Limits USP <61>	Pass	Pass	Pass	Pass
Osmolarity	Iso-osmolar	Iso-osmolar	NA	NA
pH	7.0	7.0	NA	NA
Stability	> 6 months	> 6 months	> 6 months	> 6 months
Condom Compatibility	Compatible	Compatible	Limited to polyurethane	Limited to polyurethane
Preservative Effectiveness USP <51>	Pass	Pass	Pass	Pass
Explant Toxicity	Pass	Pass	Pass	Pass
Rectal Toxicity (Animal Model)	No adverse effects	No adverse effects	No adverse effects	No adverse effects

NA: not applicable

<sup>a</sup> fluid with constant viscosity

<sup>b</sup> viscosity decreases with the rate of shear



Table 3. Histology, HIV infectivity, and luminal and tissue concentration among products, Geometric Mean Ratios and 95% CI

Product	Surface denudation	Lamina propria hemorrhage	Total p24 pg per biopsy	Colonic brushes <sup>a</sup>	Colonic biopsies <sup>a</sup>
Baseline	1	1	1	-	-
Aqueous fluid	1.69 (0.7–3.9)	1.65 (0.7–3.8)	0.94 (0.2–3.4)	1	1
Aqueous gel	1.37 (0.6–3.1)	3.20* (1.3–7.5)	0.33 (0.09–1.2)	0.63* (0.4–0.9)	0.90 (0.5–1.4)
Lipid fluid	1.04 (0.4–2.4)	2.29 (0.9–5.3)	5.35* (1.4–20.1)	0.14* (0.09–0.2)	0.44* (0.2–0.7)
Lipid gel	1.63 (0.7–3.7)	2.40* (1.03–5.5)	1.76 (0.4–6.5)	0.77 (0.4–1.1)	1.51 (0.9–2.3)

\*p<0.05

<sup>a</sup> In comparison with Aqueous fluid product,

Table 4. Permeability parameters by product. Median (Q25, Q75) x 10<sup>6</sup>

	Aqueous Fluid	Aqueous Gel	Lipid Fluid	Lipid Gel
AUC( $\mu$ Ci.hr/ml)*	8.04 (3.6, 9.5)	4.37 (3.3, 7.6)	108 (36.6, 148)	95.3 (91.5, 123.5)
C <sub>max</sub> ( $\mu$ Ci/ml)*	0.83 (0.45, 1.34)	0.73 (0.56, 1.11)	10.55 (4.13, 15)	9.08 (7.53, 13.2)
T <sub>max</sub> (hr)	1.7 (1.7, 1.9)	2.2 (1.6, 3.0)	0.6** (0.3, 1.4)	1.8 (1.4, 2.9)

\* Wilcoxon Paired analysis showed statistically significant difference (p<0.05) when comparing aqueous products with lipid products

\*\* Wilcoxon Paired analysis showed statistically significant difference (p<0.05) when comparing lipid fluid with the other three products

Table 5. Imaging pharmacokinetic-distance parameters by product.at 2 and 4 hours after dosing. Median (Q25, Q75)

	Aqueous Fluid	Aqueous Gel	Lipid Fluid	Lipid Gel
$D_{\max}$ (cm)				
2 h	23.2 (20.7, 28.9)	21.1 (19.1, 25.3)	19.9 (0, 24.9)*	19.8 (15.4, 24.6)
4 h**	23.1 (14.9, 25.1)	12.9 (11.6, 20)	17.3 (6, 24.1)*	21.3 (3.9, 24.9)
$DC_{\max}$ (cm)				
2 h	5.8 (3.6, 9.9)	7.2 (5.4, 12.1)	7.1 (0, 12.5)	8.5 (5.8, 11.1)
4 h	5.3 (3.3, 7.2)	5.1 (2.0, 8.3)	7.3 (2.5, 12.2)	8.7 (0.8, 10.5)
$D_{\text{ave}}$ (cm)				
2 h	8.5 (5.4, 11.7)	7.3 (6.3, 9.6)	7.8 (0, 11.4)	8.3 (6.1, 12.0)
4 h	6.8 (4.7, 10.2)	6.4 (4.7, 7.5)	9.0 (2.4, 12.2)	9 (1.0, 12.2)
$D_{\min}$ (cm)				
2 h	-3.9 (-5.8, -2.2)	-3.6 (-5, -1.2)	-3.5 (-5.2, -1.7)	-3.4 (-4.3, -1.2)
4 h	-3.8 (-3.8, -3.5)	-2.6 (-3.5, 0.8)	-3.1 (-4.5, -1.0)	-3.4 (-4.8, -2.1)

$D_{\max}$ : furthest point where radiosignal was detected

$DC_{\max}$ : distance at concentration maximum

$D_{\text{ave}}$ : mean residence distance

$D_{\min}$ : distance associated with the most distal signal

\*  $p < 0.05$ , multilevel analysis showed in Lipid fluid formulation a statistically significant lower

$D_{\max}$  when compare with aqueous fluid after adjusting by time from dose

\*\*  $p < 0.05$ , multilevel analysis showed a statistically significant lower  $D_{\max}$  at 4 hours in

comparison with 2 hours after dosing for each of the four formulations

Table 6. Participants rating of the four formulations

Question: Please indicate your opinion. Overall, this product felt... (1-completely unacceptable; 2-somewhat unacceptable; 3-somewhat acceptable; and 4 highly acceptable)				
			95% Confidence Interval	
	Mean	SE	Lower Bound	Upper Bound
Aqueous Fluid	3.13	.227	2.59	3.66
Aqueous Gel	3.75	.250	3.16	4.34
Lipid Fluid	3.38	.183	2.94	3.81
Lipid Gel	3.75	.164	3.36	4.14

Table 7. Difficulties for each formulation administration.

Question: How easy was it to administer the product? (1-extremely difficult to 10-extremely easy)				
			95% Confidence Interval	
	Mean	SE	Lower Bound	Upper Bound
Aqueous Fluid	8.8	.61	7.4	10.3
Aqueous Gel	8.2	.79	6.3	10.1
Lipid Fluid	8.8	.61	7.4	10.3
Lipid Gel	8.8	.55	7.5	10.2

Table 8. Likelihood to use the formulations in the future

Question: If a gel were available that contributed to provide some protection against HIV, and it looked like the one you have used in this study, how likely would you be to use it every time you have receptive anal intercourse?				
			95% Confidence Interval	
Product	Mean	SE	Lower Bound	Upper Bound
Aqueous Fluid	6.3	1.20	3.5	9.2
Aqueous Gel	8.6	.73	6.9	10.3
Lipid Fluid	9.2	.25	8.6	9.8
Lipid Gel	7.7	1.39	4.3	11.1
Question: How likely would you be to use a gel that contributed to provide some protection against HIV on the occasions when you don't use condoms				
Aqueous Fluid	8.5	1.00	6.1	10.8
Aqueous Gel	9.7	.18	9.2	10.1
Lipid Fluid	9.5	.18	9.0	9.9
Lipid Gel	8.8	.85	6.7	10.9
Question: How likely would you be to use a gel if you had to wait				
Aqueous Fluid	5.6	1.30	2.5	8.7
Aqueous Gel	7.3	1.05	4.8	9.8
Lipid Fluid	8.1	.58	6.7	9.5
Lipid Gel	7.5	.84	5.5	9.6

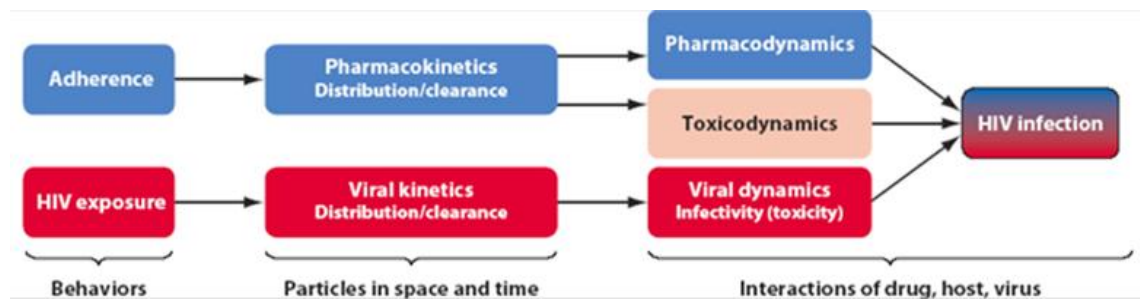


Figure 1. Linkage of human (*blue, upper row*) and viral (*red, lower row*) processes, moving from left to right, influencing HIV infection outcome with the use of a microbicide (Hendrix, Cao, and Fuchs 2009). This figure is reproduced with permission of the publisher.

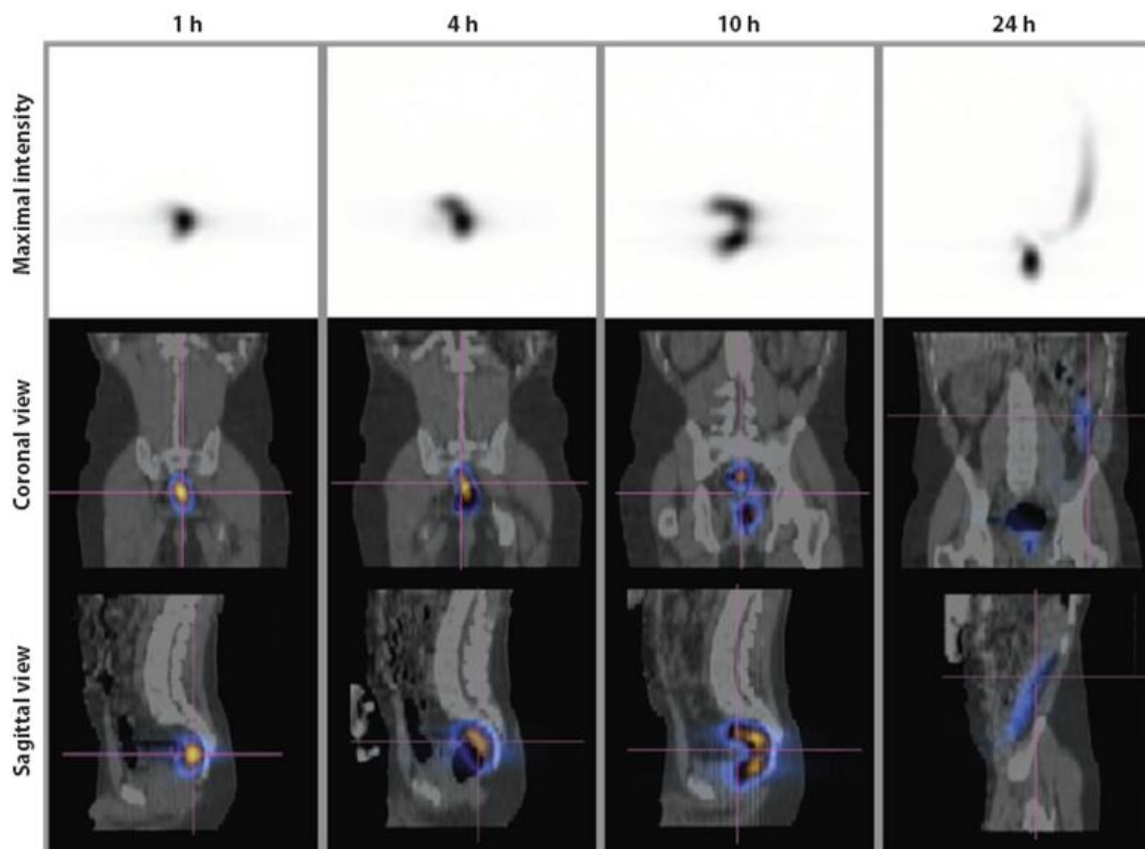


Figure 2. Fusion of single photon-emitted computed tomography (SPECT) and computed tomography (CT) images showing microbicide surrogate gel temporal migration retrograde to the splenic flexure after rectal dosing. Microbicide surrogate is an iso-osmolar mixture of over-the-counter sexual lubricants with  $^{111}\text{In}$ -DTPA added for imaging to simulate a small-molecule (492 Da) microbicide active ingredient. Temporal sequence (*left to right*) includes 1st hour to 24th hour after dosing. Top row of images panel is grayscale maximal intensity projection of  $^{111}\text{In}$  signal. Lower two rows of images are coronal and sagittal views of CT images (grayscale: *light gray* is bone density; *darker gray* are soft tissues; *black* is air density) with superimposed SPECT images of  $^{111}\text{In}$  signal (*blue through yellow*) (Hendrix, Cao, and Fuchs 2009). This figure is reproduced with permission of the publisher.



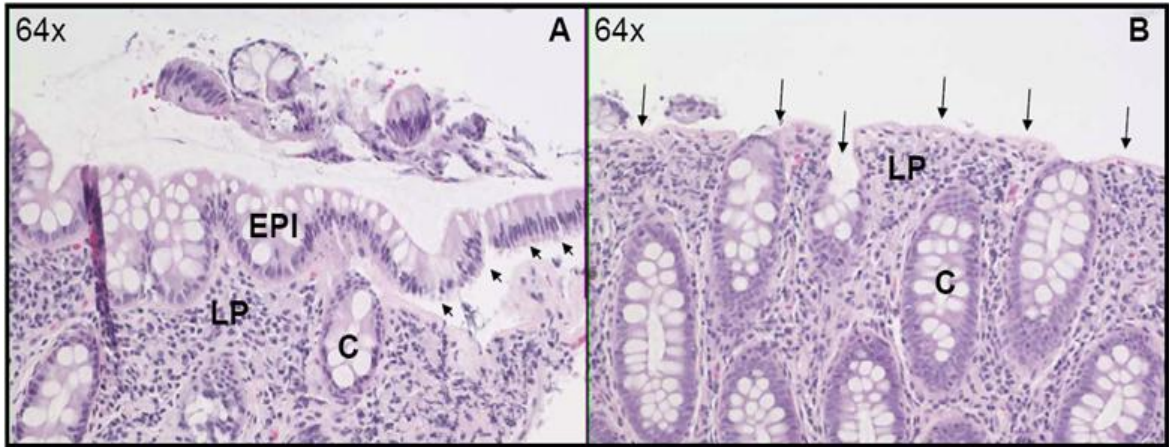


Figure 3. H&E stained sections from one research participant's rectal mucosa (10 cm beyond anus) 1 hour after rectal application of iso-osmolar gel (Panel A) and hyper-osmolar gel (Panel B). Grade 1 mucosal change in Panel A shows epithelial cell layer mostly intact with a section of mucin-depleted epithelial cells lifting off the basement membrane and separating from lamina propria (short arrows); the 'lift off' seen here could be handling artifact as well. Grade 3 changes in Panel B show complete absence of epithelium and an exposed lamina propria (long arrows) beneath the basement membrane. Grade 0 (Normal) looks like the left side of Panel A. EPI - epithelial cells. LP - lamina propria. C – goblet cells (Fuchs et al. 2007). This figure is reproduced with permission of the publisher.

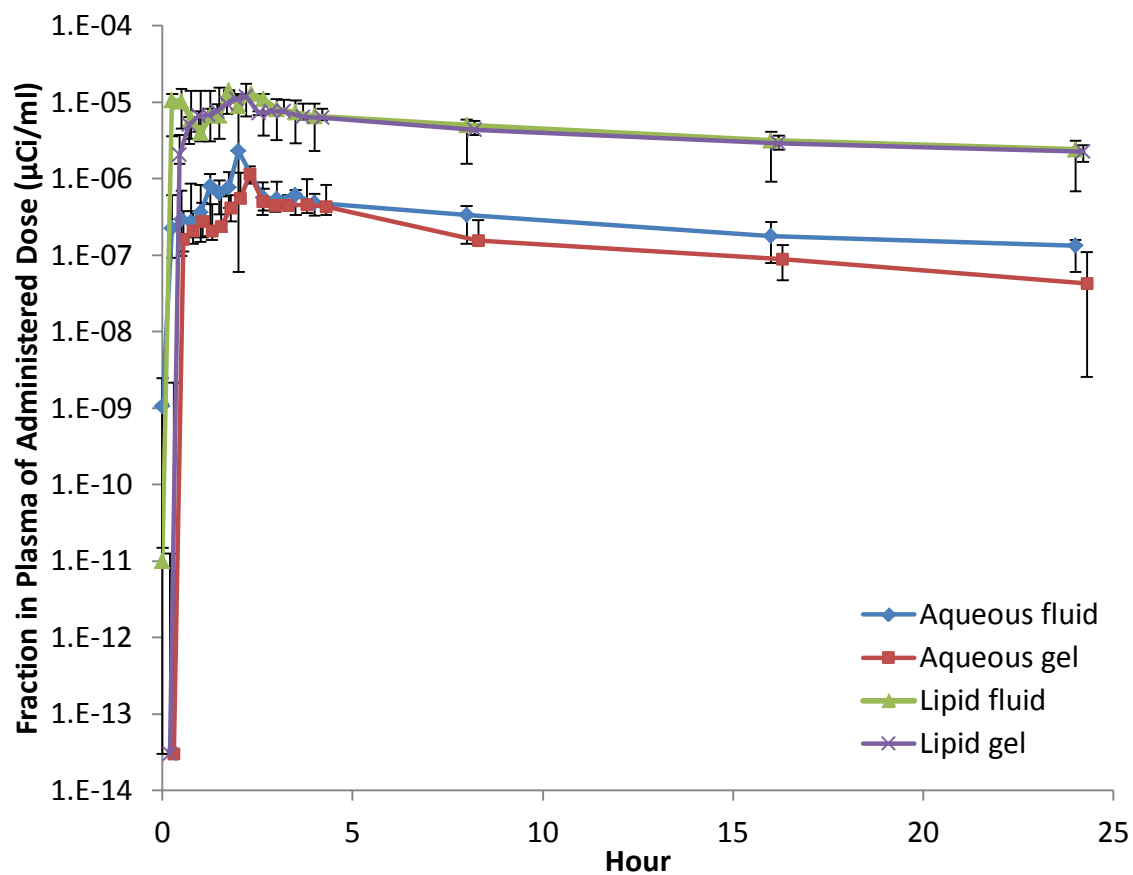
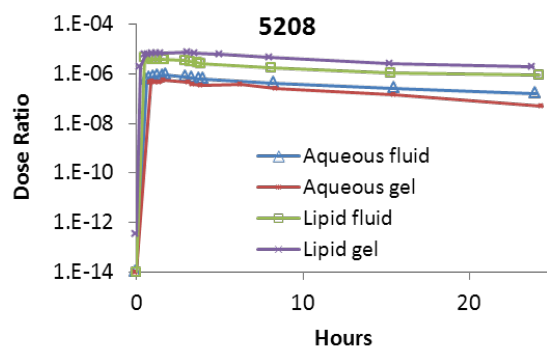
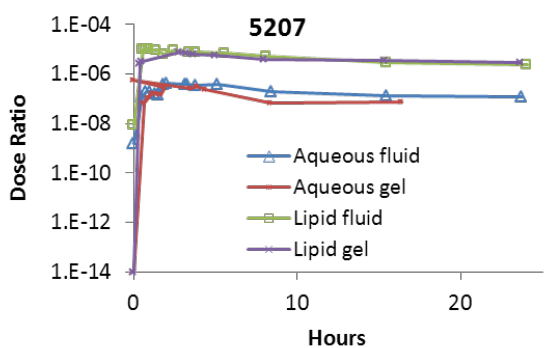
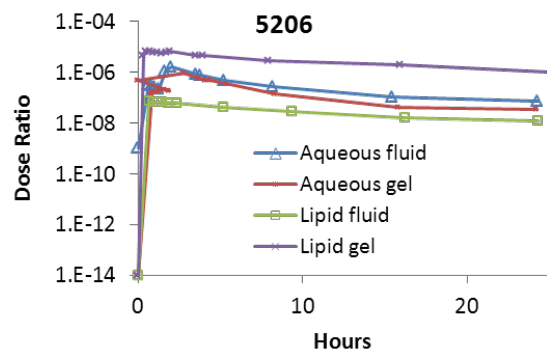
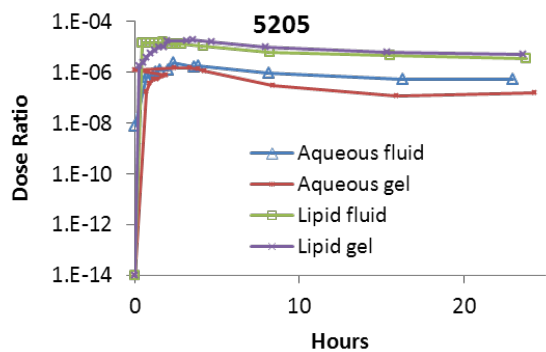
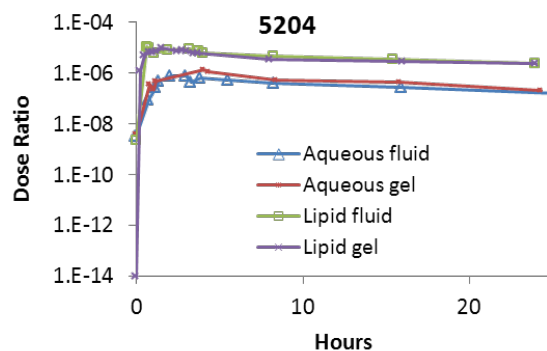
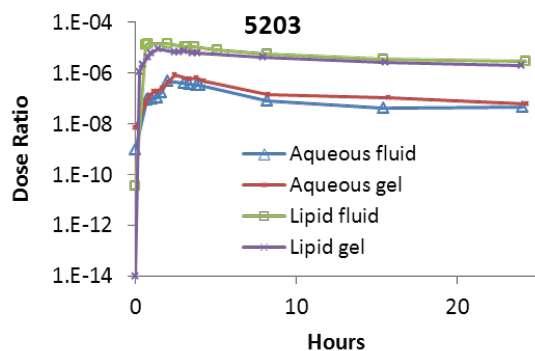
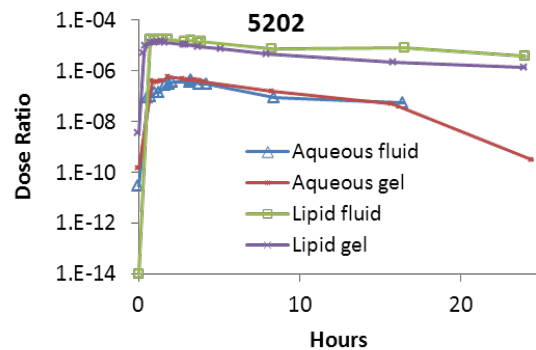
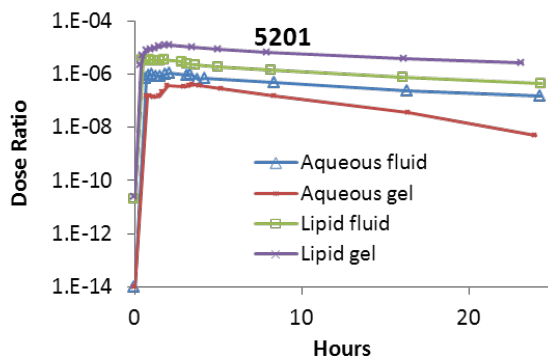


Figure 4. Fraction of  $^{99m}\text{Tc}$  DTPA labeled formulation rectally administered found in blood obtained at different times in eight participants per formulation. Median and Q25-Q75

Figure 5. Dose Ratio or fraction of  $^{99m}\text{Tc}$  DTPA labeled formulation rectally administered found in blood at different times in each participant. Concentration values were normalized as a fraction of the dose administered for each subject (dose ratio).



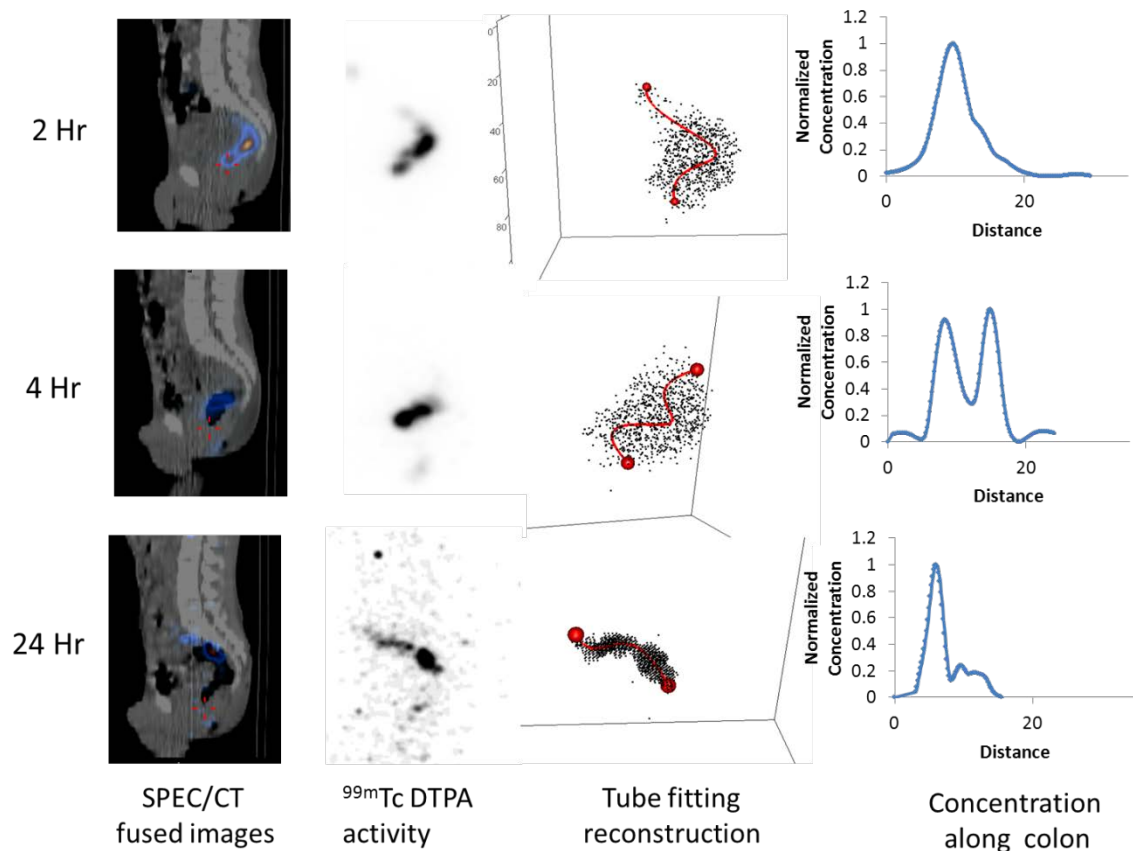
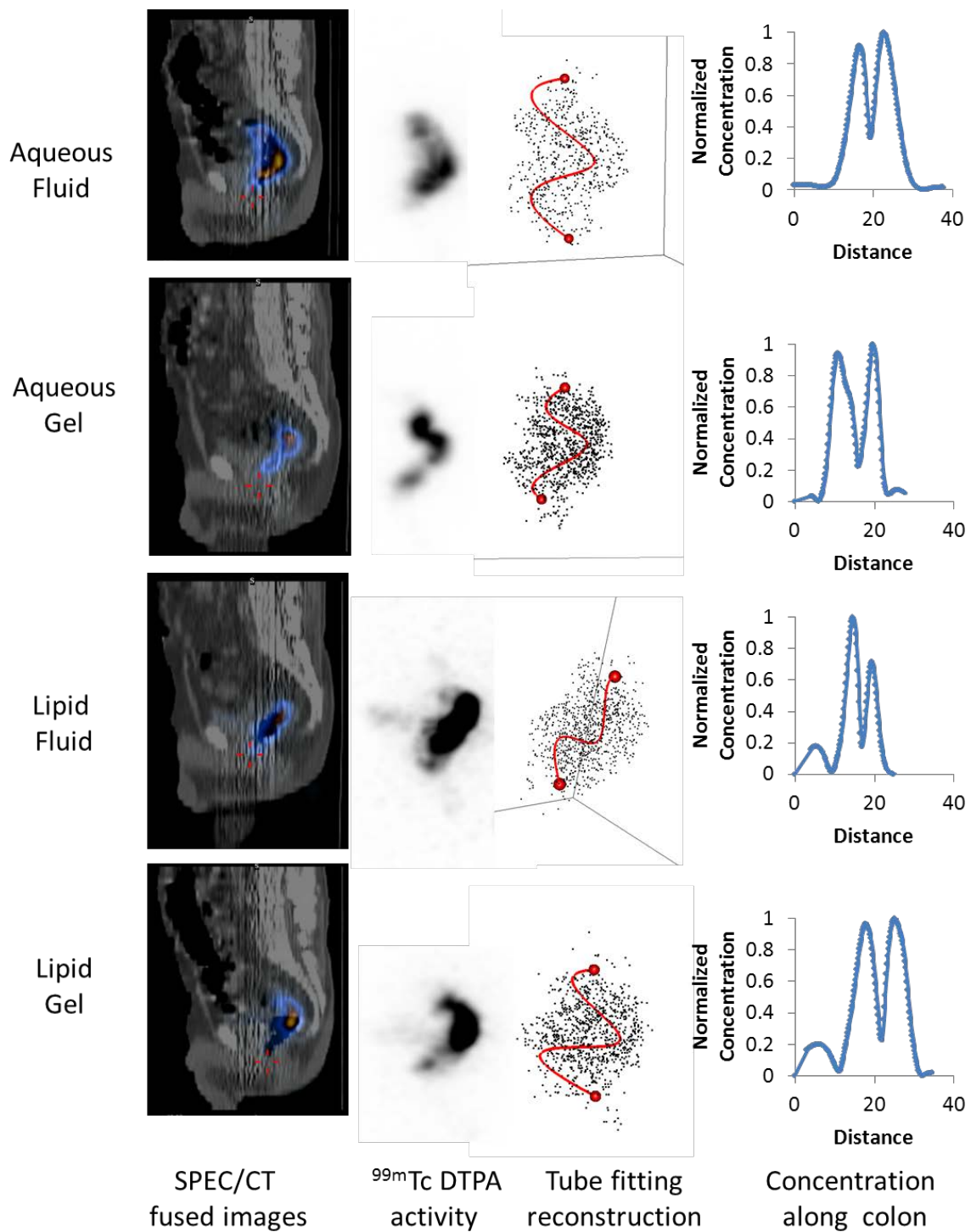


Figure 6. Imaging of one participant at 2, 4, and 24 hours following intra-rectal dosing with aqueous gel formulation. First column to the left shows sagittal view of SPECT and computed tomography (CT) images. Second column shows  $^{99m}\text{Tc}$  DTPA activity of the corresponding left sagittal view image. Third column shows the reconstructed radioisotope activity image using the previously described algorithm and in red the curve corresponding to the central axis of the tube fitting modeling. Fourth column shows concentration-distance curves calculated after tube fitting modeling.

Figure 7. Imaging of one participant at 2 hours following intra-rectal dosing with each formulation: aqueous fluid, aqueous gel, lipid fluid, and lipid gel. First column to the left shows sagittal view of SPECT and computed tomography (CT) images. Second column shows  $^{99m}\text{Tc}$  DTPA activity of the corresponding left sagittal view image. Third column shows the reconstructed radioisotope activity image using the previously described algorithm and in red the curve corresponding to the central axis of the tube fitting modeling. Fourth column shows concentration-distance curves calculated after tube fitting modeling.



APPENDIX 1  
RESEARCH PROTOCOL

IPCP U-19 Microbicide Development Project

Project 5 – Aim 2

A Randomized, Partially-Blinded, Comparative Study of the Safety,  
Mucosal Toxicity, Mucosal Infectibility, Colorectal Distribution, and Participant  
Acceptability of Four Different Rectal Microbicide Vehicle Candidates

Sponsored by:

Division of AIDS, US National Institute of Allergy and Infectious Diseases  
US National Institutes of Health

Principal Investigator:  
Craig W. Hendrix, MD  
Johns Hopkins University School of Medicine  
Baltimore, MD USA

Version 1.0  
2 July 2009



## MDP Project 5 – Aim 2

### A Randomized, Partially-Blinded, Comparative Study of the Safety, Mucosal Toxicity, Mucosal Infectibility, Colorectal Distribution, and Participant Acceptability of Four Different Rectal Microbicide Vehicle Candidates

#### SCHEMA

**Purpose:** To study safety, mucosal immunological toxicity, infectibility, colorectal distribution, and participant acceptability of the 4 different rectal microbicide candidate vehicle formulations

#### Design:

Study Phase	Screen	Randomization	Base-line	Stage 1		Stage 2		Stage 3		Stage 4		Exit Interview
Visit Number	1		2	3	4	5	6	7	8	9	10	11
Location	OUTPT		OUTPT	INPT	OUTPT	INPT	OUTPT	INPT	OUTPT	INPT	OUTPT	OUTPT
Visit Focus												
Safety	x		x	X	x	X	x	x	x	x	x	
Distribution				X		X		x		x		
Acceptability			x	X	x	X	x	x	x	x	x	x

**Consent, Screening, Randomization.** At Visit 1, following education of prospective research participants regarding study eligibility, procedures, risks, and benefits, and resolution of all research participant questions, participants will be asked to sign the informed consent document. Following this, eligibility and safety screening will be completed on an outpatient basis in the research clinic. Research participants who are deemed eligible after this screening period will be randomized into one of four product sequence-specific study groups (see table below). The four candidate vehicles (A, B, C, and D) will differ from one another in their viscosity, lipophilicity, and mucoadhesive characteristics.

Group	Vehicle Sequence			
	Stage 1	Stage 2	Stage 3	Stage 4
1	A	B	C	D
2	B	D	A	C
3	D	C	B	A
4	C	A	D	B

After a baseline safety and acceptability assessment (Visit 2), each group will begin dosing with their assigned test vehicles in 4 stages (Visits 3 – 10). Each stage is identical, with the exception of the test vehicle used. Each stage (one vehicle type tested per stage) includes a single inpatient visit and a single outpatient evaluation. The

inpatient visit includes one dose of the test vehicle (according to the randomization sequence) followed by safety and PK assessments prior to discharge. The outpatient evaluation involves a single self-administered test vehicle dose (according to the randomization sequence) followed by an outpatient research clinic visit to evaluate for any adverse effects related to outpatient product use.

The inpatient test vehicle evaluation must precede the related outpatient evaluation of the same vehicle. However, another vehicle may be evaluated on an inpatient basis before the prior vehicle's outpatient evaluation is completed to allow maximum flexibility in scheduling inpatient stays. The inpatient visits must occur in order defined by the randomization – visit 3, visit 5, visit 7, visit 9. However, a research participant may progress through each of 4 inpatient stages independently of completing any of the 4 outpatient stages (visit 4, 6, 8, and 10). Similarly, the outpatient test vehicle evaluations may occur out of the strict alternating sequence shown in the study schema, but must occur in the randomization order as specified for the research participant group following the designated order - visit 4, visit 6, visit 8, visit 10 – regardless of the timing of the inpatient visits.

The time period between inpatient visits (Visits 3, 5, 7, 9) will be at least 2 weeks to allow a sufficient period of time for the mucosal biopsies to heal. A 72-hour period of abstinence from RAI is required following any inpatient biopsy and preceding any inpatient dose.

**Baseline Visit. Baseline Behavioral Questionnaire (BBQ).** Following randomization, but prior to receipt of the first inpatient dose, the BBQ will be administered by Computer Assisted Self-Interview (CASI). Research participants may complete the web-based CASI in the privacy of their own home. If a research participant does not have access to the Internet, prefers to complete the BBQ at the study site, or requires assistance in using the CASI, he will be accommodated at the study site. A baseline outpatient endoscopy with 24 biopsies will also be performed prior to research participants receiving any test vehicle.

**Candidate Vehicle Evaluation (Visit 3 through Visit 10).** The participant is admitted to the inpatient research unit (for ~26 hours), and has an interval medical history and directed physical exam. Then, the radiolabeled candidate vehicle is administered to the participant. Over a 24 hour period, the participant will undergo pharmacokinetic and permeability studies (periodic sampling of blood and urine for 24 hours following dosing), endoscopic evaluation with 24 biopsies and 9 luminal brushings (1 hour after dosing), and imaging by SPECT/CT (1.5, 4, and 24 hours after dosing). During the inpatient visit, at some time following administration of the test vehicle, the research participant will complete a Brief Acceptability Questionnaire (BAQ). The participant will then be discharged home with a single dose of test vehicle (not radio-labeled). Participants will be instructed to allow for an approximately 72 hour recovery period (to ensure healing of the biopsy sites) prior to initiating use of the test vehicle. Research participants will be encouraged to use the test vehicle in the context of RAI. Consistent with standard HIV counseling, participants will be strenuously encouraged to use condoms.

As soon as possible following the outpatient use of the test vehicle, the research participant will complete an online Product Acceptability Questionnaire (PAQ) either from any accessible computer with web access or at the research clinic where a terminal will be made available. Also, upon notification of study personnel that the outpatient test

vehicle use has been completed, an outpatient research clinic visit will be scheduled, preferably within one week. At this visit, the research participant will undergo a targeted history and physical exam to assess the safety of the outpatient test vehicle used in the setting of RAI. No additional testing similar to those conducted during the inpatient exposure will occur.

**Study Exit Interview (Visit 10).** After completion of all 4 stages, or upon early termination after initiating product use, the participant will complete an online Overall Product Preference Questionnaire (OPPQ) followed by an in-depth interview.

**PRN visits:** Research participants may be asked to return to the research clinic for evaluation of any complications that arise.

**Study Population:** HIV negative men with a history of recent RAI (at least twice per month in the prior 3 months)\*

*\*Refers to coitus only, does not include manual stimulation or the use of sex toys or purgatives.*

**Study Size:** A total of 8 participants

**Study Duration:** Participant accrual will take approximately 4 months and each participant will be on study for approximately 12-24 weeks. The duration of the clinical phase of the study will be approximately 7 to 10 months. Specimen and data analysis will require an additional 3 months.

**Primary Objective:** To evaluate the safety of four candidate test vehicles of varying viscosity, lipophilicity, and mucoadhesive characteristics using adverse event grading, endoscopic inspection, and histopathology.

**Secondary Objectives:** To evaluate the mucosal toxicity, infectibility, luminal distribution, and acceptability of candidate vehicles of varying viscosity, lipophilicity, and mucoadhesive characteristics using the following parameters:

Toxicity:	Mucosal mononuclear cell phenotype Mucosal cytokine profile Permeability
Infectibility:	Colonic explant HIV challenge
Pharmacokinetics:	Distribution/Migration studies Luminal Concentration
Acceptability:	Acceptability assessments

## APPENDIX 2

### INFORMED CONSENT DOCUMENT

#### RESEARCH PARTICIPANT INFORMED CONSENT AND PRIVACY AUTHORIZATION FORM

**Protocol Title:** A Randomized, Partially-Blinded Comparative Study of the Safety, Mucosal Toxicity, Mucosal Infectibility, Colorectal Distribution, and Participant Acceptability of Four Different Rectal Microbicide Vehicle Candidates

**Version:** 0.6

**Sponsor:** National Institutes of Health (NIH),  
The Johns Hopkins Institute for Clinical and Translational Research/Clinical Research Unit (CRU)

**Principal Investigator:** Craig W. Hendrix, M.D.

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#### 1. What you should know about this study:

- You are being asked to join a research study.
- This consent form explains the research study and your part in the study.
- Please read it carefully and take as much time as you need.
- Please ask questions at any time about anything you do not understand.
- You are a volunteer. If you join the study, you can change your mind later. You can decide not to take part or you can quit at any time. There will be no penalty or loss of benefits if you decide to quit the study.
- During the study, we will tell you if we learn any new information that might affect whether you wish to continue to be in the study.
- Ask your study doctor or the study team to explain any words or information in this informed consent that you do not understand.
- You may not be in this study if:
  - You are a woman
  - You have HIV
  - You have problems with your anus or rectum, like warts or hemorrhoids, that could make this study painful for you
  - You have inflammatory bowel disease
  - You are taking aspirin or other types of pain medications on a regular basis, because these could increase your risk of bleeding.
  - You are taking medicines that suppress your immune system (your body's natural defense system against infections).
  - You are unwilling to have your specimens (data, tissue, blood and samples) stored for future use.

## **2. Why is this research being done?**

One of the main purposes of this research is to help develop a rectal microbicide that can be used before anal sex to help prevent HIV transmission. This study is being done to look at the differences between 4 different products (called “vehicles” in this study) that are being considered for use with a rectal microbicide. We are calling these “vehicles”, because they will be used to “carry” the active ingredient of the rectal microbicide so that it can be applied to the rectum in either a gel or liquid form. We will be studying what changes each of the vehicles may cause in the rectum including if they can be safely used. We will also study how far the vehicles travel in the colon and how people like to use each of the vehicles. These vehicles have never been used in humans.

Two of the vehicles will have a thickness like an enema. The two other vehicles will have a thickness like sexual lubricants sold in stores. The Food and Drug Administration has not approved any of these study vehicles.

We are asking men to join this study who:

- have had receptive (“bottom”) anal sex
- have used enemas before sex during the past year
- have used a sexual lubricant before or during sex at least some of the time
- and are HIV negative and agree to use condoms during the study

The study will be done at the Clinical Research Unit (CRU) of The Johns Hopkins Hospital. Between 8 and 30 men will join the study.

## **3. What will happen if you join this study?**

The amount of time you are in the study will be about 12-24 weeks depending on the amount of time between the visits. Below is a description of what will happen while you are in the study.

### **Screening (Visit 1):**

If you join this study, we will ask you to come to the clinic for a physical exam, a rectal swab, and some blood tests. This visit will last about 2 hours.

The screening will help make sure that you do not have any health problems that might make some parts of the study more uncomfortable or dangerous for you. It will be done by a physician or physician assistant (PA). At the visit, we will also ask you some questions about your medical history, medications you take and your sexual practices.

For the blood tests, we will take blood (a little more than 1 tablespoon) from one of your veins. The blood will be used to make sure that your blood count is good and that your blood clots normally. As part of being in this study, you will have a test for HIV virus (the virus that causes AIDS). You will be given the State of Maryland HIV consent form as part of that process. If this test is positive, you will be referred for proper medical care and counseling. The law requires us to report positive tests to the health department.

The rectal swab is done by putting a q-tip like device into your anus. These swabs will be tested for the sexually transmitted infections, chlamydia and gonorrhea. If you have one of these infections you can get treatment from your primary care doctor or another clinic and then be re-screened for the study. We will not provide treatment or pay for treatment of these infections. The law requires us to report positive tests to the health department.

If the screening tests show that it is safe for you to take part in the study, we will ask you to come back to start the study.

If you join the study, you must not have anal sex for 72 hours before each of four inpatient visits and for 72 hours after these visits. You must not use condoms with Nonoxynol-9 (a spermicide) or any products with Nonoxynol-9 at any time during the study. You must not take any aspirin or NSAIDS (like ibuprofen, Motrin®, naproxen, Naprosyn®) for 72 hours before your visits and for 72 hours after your visit, because these may increase your risk of bleeding. You may take Tylenol (acetaminophen) for discomfort.

During the study you will receive calls or e-mails from the study staff reminding you of your visits. We ask that you keep track of all medications you use during the study and bring this to every visit.

### **Baseline (Visit 2):**

At this visit we will ask you to not have anything to eat until after the study visit. We will also ask that you not have receptive (bottom) anal sex for three days before the visit. At Visit 2 you will again be examined by a study investigator and have blood tests and a rectal swab repeated.

You will be given instructions on how to complete a computerized questionnaire. The questionnaire will ask many questions about your sexual behaviors and previous experiences with similar products. You should know that the web site used to collect the questionnaire information does not use encryption to send data between the web browser and web server. If you feel uncomfortable as a result of any of the questions, you may refuse to answer. This information is confidential and WILL NOT be placed in your medical record. The questionnaire will only be labeled with your study ID number. The local research staff will not be able to view the answers to your questions. A separate member of the research team located in New York will review the data.

After this you will be taken to the endoscopy area where a doctor will perform a flexible sigmoidoscopy (also known as an endoscopy). To do this, the doctor will use an endoscope to look inside your rectum. An endoscope is a long, thin tube with a camera in it. The doctor will use a long wire with a tiny pincher to take up to 24 small biopsies of your intestinal lining. Each biopsy is a small piece of tissue measuring smaller than the surface of a pencil eraser and about the thickness of a dime. This visit will last about 4 hours.

### **Inpatient Visits (Visits 3, 5, 7 and 9):**

There are 4 inpatient visits during the study. Each visit will be the same as described below, but will involve a different vehicle type each time.

The order in which you get each of the four vehicle types will be random. The process by which the order is decided is like flipping a coin. You will not know the order in which you are getting the vehicles.

**Day 0:** You will be admitted to the General Clinical Research Center inpatient unit before 6PM. You will have an examination by the study investigator and rectal swab (for STD testing) will be collected. This exam and rectal swab may also be done on Day 1.

**Day 1:** Every time you urinate, you will urinate in a bottle, so we can collect the urine. There will also be specific times that we will ask you to urinate for the next 24 hours. We will tell you when these times are and encourage you to drink a lot for the first 4 hours.

Blood samples (about 1 ½ tablespoons) will be collected for an HIV test and to look for certain types of cells in your blood.

You will be given an enema to help clean out your rectum. We will ask that you hold the enema for 5 minutes and then let it out into the toilet.

You will be given one of the study vehicles, which will have a radioactive tracer (technetium) in it. We will ask you to sit or lie on your back from this point in the study until after the SPECT/CT study described below, about 2 to 3 hours.

About an hour after the enema you will have the same endoscopy procedure as described earlier in this form (Visit 2). During the procedure the doctor will also use a small brush inserted through the endoscope to take samples of fluid and cells along the rectal surface.

After the endoscopy procedure you will have 2 SPECT/CT scans about 2 hours apart. A third SPECT/CT scan will be done on Day 2. This procedure takes pictures like an x-ray (CT scan) and measures the radioactive tracer in your colon (SPECT). For the CT scan, you lay quietly on your back on a padded table, which moves into a large donut shaped device. For the SPECT part of the study, the machine revolves slowly around you. The total amount of time for each SPECT/CT scan is about one hour.

You will not be allowed to eat breakfast this morning, but may eat after the endoscopy procedure and the first SPECT/CT scan are finished.

Blood samples (about 1-2 teaspoons each) will be collected to see how much of the radioactive part of the vehicle is in your blood before the vehicle and at specific times after the vehicle is given.

At some point during your stay in the CRU, you will be asked to complete another short computerized questionnaire.

**Day 2:** You will finish collecting your urine and have the final blood sample taken.

You will repeat the SPECT/CT scan as described above and then you may go home. You should not have anal sex for 48 hours after discharge. You will be given the study vehicle to take home and use during the outpatient period as explained in the next section.

### **Outpatient Periods (Vehicle Take-Home Use):**

During the outpatient period we will ask you to use the study vehicle within one hour before anal sex one time. We will give you 2 doses of a study vehicle to take home in case you use one and don't end up having sex after using it. You will also be given 2 Fleet enemas and a specific type of condom (polyurethane) for your partner to wear when you use the vehicles. These condoms have been tested and proven not to break when exposed to the study vehicles. This may not be the case for other types of condoms. These condoms are commercially available, but not always easy to find so we will give them to you. You will be given a form on which to record when you used the product.

Once you have used the vehicle followed by anal sex you will complete one computer questionnaire. It is called the "Product Acceptability Questionnaire" (PAQ) and is a little longer than the one you completed as an inpatient. The PAQ is taken on the internet. The website is password protected, so you will have to logon to answer the questions. Your answers are confidential. Your name will never be recorded on any of the questionnaires. If you do not have access to the Internet at home or you need help with this, you can come into the study site to complete this in a private setting during your outpatient clinic visits.

Since we are studying 4 products, there will be 4 outpatient periods similar to this.

### **Outpatient Clinic Visits (Visits 4, 6, 8, and 10)**

Once you have completed the outpatient assessments, you will come to the clinic for a brief visit to make sure it is still safe for you to be in the study. You will then begin the next portion of the study. We expect that it will take about 2 weeks for you to use the study vehicle. Since there are 4 vehicle types we are studying in this project, there will be 4 visits similar to this.

### **In-Depth Exit Interview (Visit 11):**

Once you have finished all the evaluations for each of the 4 types of vehicles, you will complete a final computer questionnaire called the "Overall Product Preference Questionnaire" (OPPQ) and have an in depth telephone interview about the study. The interview will take about 45-60 minutes and will be audiotaped. Your name will not be on this recording and you do not have to give your name to the person interviewing you. The interviewers will ask you questions based on your answers to the computerized questionnaires.

### **Additional Visits:**

During the study it may be necessary to repeat some blood work or tests. We will ask you if this is ok with you before we repeat them. The results from the clinical tests and the flexible sigmoidoscopy exam done as part of this study are for research purposes, but will be made available to you.



We will notify you if we notice anything abnormal or unexpected in your blood work or during your exams. However, you must see your primary care doctor to receive any treatment you may need.

If you decide to leave the study or we decide you need to be withdrawn from the study, or when you have finished the study, there may be a reason we would like to schedule you for a follow up appointment, possibly including lab tests for your safety. Whether or not you need to have a follow up appointment will be decided on a case-by-case basis.

#### **Samples Remaining at the End of the Study:**

Samples obtained from you in this study may be used for future clinical pharmacology (drug) research or used to develop new laboratory tests. These samples will NOT be identified as coming from you. However, before any future research begins, the IRB (Ethics Committee) will review the research and the study procedures to protect your privacy. The research will not begin without IRB approval.

#### **4. What are the risks or discomforts of the study?**

**Risks of blood draws:** Blood drawing may cause discomfort. You may feel dizzy or faint, or develop a bruise, swelling or infection where the needle is inserted. The risks of the blood draws include pain, bruising, fainting, lightheadedness, and on rare occasions, infection. The total amount of blood collected during the entire study is about 1½ cups.

**Risks of rectal swab collection:** There is no risk from the rectal swab in itself. You may have minor discomfort from the insertion of the swab.

**Risks from flexible sigmoidoscopy with biopsies:** Flexible sigmoidoscopy is a common medical procedure and the endoscopic procedures done in this trial will not involve any unusual risks or discomforts. The risks associated with these procedures include mild discomfort and the feeling of having a “bloated stomach”. Small biopsies of your gut lining will be taken during the sigmoidoscopy. The biopsies are smaller than the surface of a pencil eraser and about the thickness of a dime. These biopsies are painless and heal quickly within 3-5 days, but you will probably notice the presence of minimal blood tinged mucus or stool from your rectum that may last for approximately 2-3 days. On very rare occasions, the endoscopic procedure or biopsies may lead to pain, infection (sepsis), bleeding or perforation (a hole or tear) in the lining of the gut. Perforation occurs approximately once out of every 10,000 procedures. If this very rare complication occurs, antibiotics and surgery to repair the tear may be necessary.

Things to look for after the procedure: After the procedure, you will probably notice some blood in your first few bowel movements. This is because the blood from the biopsy sites mixes with the stool and comes out when you go to the bathroom. This is expected and may last for up to 48 hours. If you are bleeding for more than 48 hours, blood is leaking in your underwear, have severe abdominal pain, are running a fever higher than 100.5°F, or are at all concerned, please call the emergency telephone number listed in section 12.c., on page 8 of the consent form. It is important that these symptoms are evaluated without delay.

**Risks from STD testing:** You may become embarrassed, worried, or anxious when completing the HIV-related interviews and/or receiving HIV/STD counseling. You also

may become worried or anxious while waiting for your test results or after receiving positive test results. Trained counselors will be available to help you deal with these feelings. Although the study site will make every effort to protect your privacy and confidentiality, it is possible that participant's involvement in the study could become known to others, and that social harms may result (i.e., because you could become known as "high risk" for HIV infection). For example, you could be treated unfairly or discriminated against, or could have problems being accepted by your family and/or community. In addition to referring you to treatment services, we can refer you to health or mental health services if you wish. However, Johns Hopkins and the NIH do not have funds to pay for treatment once you are referred.

If the HIV test result is negative, there is a small chance that the test result could be wrong. Most HIV test results are correct. However, "false-positive", "false-negative", or "unclear" (neither positive nor negative) results may occur. If your test results are unclear we will offer you repeat testing.

By law, positive results from some STD tests (including HIV) have to be reported to local health authorities. This reporting may result in a potential loss of confidentiality.

**Risks from enemas:** The main risk from having an enema is temporary discomfort. A hollow tube about the thickness of a pencil will be used to put approximately 125ml (one half cup) of the test article into the rectum and flush it out again, along with any stool that is there. This may cause a "bloated" or "crampy" feeling. Some air may be pumped into the rectum as well, causing flatulence (gas that comes out.) The tube is small, but it might cause some anal or rectal discomfort if you have any hemorrhoids or other painful conditions.

**Risks from the study vehicles:** You might find it uncomfortable that the study vehicle may leak from the rectum after you use it. You may use an absorbent pad under your clothing to protect your clothes. There is also a risk of allergic reaction. There may be other side effects of the study vehicles that are unknown since these have not been used in humans before.

**Risks associated with radiation exposure:**

The radiation exposure you will receive from the SPECT and CT scans in this study is equivalent to an exposure of approximately 1.9 rems to your whole body. Naturally occurring radiation (cosmic radiation, radon, etc.) produces whole body radiation exposure of about 0.3 rems per year. Occupationally exposed individuals are permitted to receive whole body exposures of 5 rems per year.

**Risks of Computer Assisted Questionnaire:** The web site used to collect the questionnaire information does not use encryption to send data between the web browser and web server. There may be discomfort or embarrassment related to questions dealing with sexual behaviors and personal habits. If some of the questions upset you or make you uncomfortable you may choose not to answer them.

**Risks of In-depth Phone Interview:** There may be discomfort or embarrassment related to questions dealing with sexual behaviors and personal habits. If some of the questions upset you or make you uncomfortable, you may choose not to answer them. While not anticipated, there is also the potential risk of a violation of privacy and confidentiality, if someone overhears the telephone conversation.

The procedures listed above may involve risks that are currently unforeseeable.

**5. Are there benefits to being in the study?**

You will get no direct benefit from being a part of this study. However, what is learned in this study may be used in later studies to study or test a rectal microbicide. If you take part in this study, you may help others in the future.

**6. What are your options if you do not want to be in the study?**

Your alternative is not to take part in the study. You do not have to join this study. If you do not join, your care at Johns Hopkins will not be affected.

**7. Will it cost you anything to be in this study?**

The meals, tests, and procedures in this study will not cost you anything. The only cost to you is the time you must spend and what it costs you to get to the CRU.

Your insurance company is unlikely to pay for medical care for any injury that you sustain as a result of being in this study. Emergency care will be provided, but you will be responsible for any costs not covered by your insurance. Treatment of a serious injury could be costly. If you have a bowel perforation from endoscopy, you will need major surgery. The cost for this is likely to be at least tens of thousands of dollars (probably \$20,000 or more). Check with your insurance company before you start this study to find out if your insurance company will pay.

**8. Will you be paid if you join this study?**

You will receive money for the time and inconvenience of being in the study. The amount you are paid will be based on the number of inpatient stays in the CRU and outpatient uses of the study vehicles.

You will receive \$2,120 total for your participation according to the schedule below.

- Baseline: \$335
- Vehicle sequence 1 inpatient: \$335
- Vehicle sequence 1 outpatient: \$112
- Vehicle sequence 2 inpatient: \$335
- Vehicle sequence 2 outpatient: \$112
- Vehicle sequence 3 inpatient: \$335
- Vehicle sequence 3 outpatient: \$112
- Vehicle sequence 4 inpatient: \$335
- Vehicle sequence 4 outpatient: \$112

All payments are made by check at the end of the study. If you withdraw before the study is completed, you will be paid for your participation up to that time.

NOTE: If you receive more than \$600 in total payment for your participation in this study, you are expected to report the dollar amount to the Internal Revenue Service

(IRS) as income. Johns Hopkins will not prepare or send any tax forms to you or to the IRS.

### **9. Can you leave the study early?**

- You can agree to be in the study now and change your mind later.
- If you wish to stop, please tell us right away.
- Leaving this study early will not stop you from getting regular medical care.

### **10. Why might we take you out of the study early?**

You may be taken out of the study if:

- Staying in the study would be harmful.
- You need treatment not allowed in this study.
- You fail to follow instructions.
- You test positive for HIV
- The study is cancelled (by the IRB, the study sponsor, the U.S. government, or other regulatory agencies).
- There may be other reasons that we don't know at this time to take you out of the study.

If you are taken out of the study before the study is completed, you will be paid for your participation up to that time.

### **11. How will your privacy be protected?**

Johns Hopkins has rules to protect information about you. Federal and state laws also protect your privacy. This part of the consent form tells you what information about you may be collected in this study and who might see or use it.

During the study, your study information will be stored at Johns Hopkins in locked file cabinets in an area only accessible by study staff. Generally, only people on the research team will know that you are in the research study and will see your information. However, there are a few exceptions that are listed later in this section of the consent form.

The people working on the study will collect information about you. This includes things learned from the procedures described in this consent form. They may collect other information including your name, address, date of birth, and other details.

The research team will need to see your information. Sometimes other people at Johns Hopkins may see or give out your information. These include people who review the research studies, their staff, lawyers, or other Johns Hopkins staff.

People outside of Johns Hopkins may need to see your information for this study. Examples include government groups, safety monitors, other hospitals in the study and companies that sponsor the study.

There is a possibility that the Food and Drug Administration may inspect your records.

We cannot do this study without your permission to use and give out your information. You do not have to give us this permission. If you do not, then you may not join this study.

We will use and disclose your information only as described in this form and in our Notice of Privacy Practices; however, people outside Hopkins who receive your information may not be covered by this promise. We try to make sure that everyone who needs to see your information keeps it confidential – but we cannot guarantee this.

The use of your information has no time limit. You can cancel your permission to use and disclose your information at any time by calling the Johns Hopkins Privacy Officer at 410-955-3008 or by sending a letter to:

Johns Hopkins Privacy Officer  
5801 Smith Avenue  
McAuley Hall, Suite 310  
Baltimore, MD 21209  
Fax: 410 735-6521

Please be sure to include the name of the principal investigator, the study number and your contact information.

If you do cancel your permission to use and disclose your information, your part in this study will end and no further information about you will be collected. Your cancellation would not affect information already collected in this study.

## **12. What treatment costs will be paid if you are injured in this study?**

Johns Hopkins and the federal government do not have programs to pay you if you are hurt or have other bad results from being in the study. However, medical care at Johns Hopkins is open to you as it is to all sick or injured people.

- If you have health insurance: The costs for any treatment or hospital care you receive as the result of a study-related injury will be billed to your health insurer. Any costs that are not paid for by your health insurer will be billed to you.
- If you do not have health insurance: You will be billed for the costs of any treatment or hospital care you receive as the result of a study-related injury.

## **13. What other things should you know about this research study?**

### **a. What is the Institutional Review Board (IRB) and how does it protect you?**

The Johns Hopkins Medicine IRB is made up of:

- Doctors
- Nurses
- Ethicists
- Non-scientists
- People from the local community

The IRB reviews human research studies. It protects the rights and welfare of the people taking part in those studies. You may contact the IRB if you have questions about your rights as a participant or if you think you have not been treated fairly. The IRB office number is 410-955-3008. You may also call this number for other questions, concerns or complaints about the research.

**b. What do you do if you have questions about the study?**

Call the principal investigator, Craig Hendrix at 410-955-3100. If you cannot reach the principal investigator or wish to talk to someone else, call the IRB office at 410-955-3008.

**c. What should you do if you are injured or ill as a result of being in this study?**

Call Edward Fuchs at 410-283-1075 if you have an urgent medical problem related to your taking part in this study. (This is a pager number. After the tone, enter the phone number where you can be called, press the # key, and hang up.)

Call the person in charge of this study Craig Hendrix M.D. at 410-955-3100, if you think you are injured or ill because of this study.

**d. What happens to Data, Tissue, Blood and Specimens that are collected in the study?**

Scientists at Johns Hopkins work to find the causes and cures of disease. The data, tissue, blood and specimens collected from you during this study are important to both this study and to future research.

If you join this study:

- You will not own the data, tissue, blood and specimens given by you to the investigators for this research
- Both Johns Hopkins and any sponsor of this research will study your data and the tissue, blood or other specimens collected from you
- Scientists may only use data, tissue, blood and specimens that identify you for future research with your consent or IRB approval
- You will not own any product or idea created by the investigators working on this study
- You will not receive any financial benefit from the creation, use or sale of that product or idea

**e. What are the organizations that are part of Johns Hopkins?**

Johns Hopkins includes the following:

- The Johns Hopkins University
- The Johns Hopkins Hospital
- Johns Hopkins Bayview Medical Center
- Howard County General Hospital
- Johns Hopkins Community Physicians.

**14. What does your signature on this consent form mean?**

Your signature on this form means that:

- you understand the information given to you in this form
- you accept the provisions in the form
- you agree to join the study

You will not give up any legal rights by signing this consent form.

**WE WILL GIVE YOU A COPY OF THIS SIGNED AND DATED CONSENT FORM**

---

<b>Signature of Participant</b>	<b>Date</b>
---------------------------------	-------------

---

<b>Signature of Person Obtaining Consent</b>	<b>Date</b>
--	-------------

**NOTE: A COPY OF THE SIGNED, DATED CONSENT FORM MUST BE KEPT BY THE PRINCIPAL INVESTIGATOR; A COPY MUST BE GIVEN TO THE PARTICIPANT; AND, IF APPROPRIATE A COPY OF THE CONSENT FORM MUST BE PLACED IN THE PARTICIPANT'S MEDICAL RECORD.**

APPENDIX 3  
DATA COLLECTION INSTRUMENTS

**INPATIENT VEHICLE**

**NON-CLINICAL PROCEDURES**

**Randomization, review and prep:**

Subject is randomized and Randomization ID assigned (Must be done prior to the visit)	<input type="checkbox"/> Yes	<input type="checkbox"/> No
<b>Randomization ID</b>	_____	
Locator information verified and updated, if necessary	<input type="checkbox"/> Yes	<input type="checkbox"/> No
Lab Results Reviewed	<input type="checkbox"/> Yes	<input type="checkbox"/> No
<i>Copy of results offered to subject</i>	<input type="checkbox"/> Yes	<input type="checkbox"/> No
*Referred for treatment, if applicable	<input type="checkbox"/> Yes	<input type="checkbox"/> N/A
*Verified appropriate treatment and adequate waiting period	<input type="checkbox"/> Yes	<input type="checkbox"/> N/A
Adverse Events Reported? (Record on AE log)	<input type="checkbox"/> Yes	<input type="checkbox"/> No
Medication Changes? (Record on Med log)	<input type="checkbox"/> Yes	<input type="checkbox"/> No
Following study precautions reviewed: <ul style="list-style-type: none"> <li>• No medication that may prolong bleeding, such as aspirin-containing products and/or NSAIDS for <u>72 hours prior to and following flex sig and biopsy</u>. (Acetaminophen, i.e. Tylenol, is allowed.)</li> <li>• No RAI for <u>48 hours before and after inpatient stay</u></li> </ul>	<input type="checkbox"/> Yes	<input type="checkbox"/> No

\* If gonorrhea or chlamydia result is positive, refer for treatment. Participant may proceed to the next visit one week after a CDC-approved treatment.

**CLINICAL PROCEDURES**

**First Vehicle – Inpatient Period**

**Day 0**  
\_\_\_\_/\_\_\_\_/\_\_\_\_

**Date**

Admitted to inpatient GCRC	<input type="checkbox"/> Yes	<input type="checkbox"/> No
----------------------------	------------------------------	-----------------------------



### Physical Exam

CV	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal	Explain:
Resp	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal	Explain:
GI, incl. rectal exam	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal	Explain:
Other:	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal	Explain:
Other:	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal	Explain:
<b>Performed By:</b> Print Name _____ Signature _____ Date _____		

### Samples Collected

Rectal swab for GC/CT testing	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Received <input type="checkbox"/> Entered into eCRF
Swab Collected By:		Date:
HIV-1 serology	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Received <input type="checkbox"/> Entered into eCRF

### Day 1

### Date

<u>Time</u>	<u>Procedure</u>		<u>Exact time</u>
Pre-dose	Enema administration	<input type="checkbox"/> Yes <input type="checkbox"/> No	
Dose	First Vehicle dose	<input type="checkbox"/> Yes <input type="checkbox"/> No	
1hr post dose	<u>Flexible sigmoidoscopy</u> Luminal brushes at 5 cm (x 3) Biopsies at 5 cm (x 2) Luminal brushes at 10 cm (x 3) Biopsies at 10 cm (x 2) Luminal brushes at 15 cm (x 3) Biopsies at 15-20 cm (x 16)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> No	

**Blood Collections**

<b>Collection Interval</b> (time post dose)	<b>Scheduled Draw Time</b>	<b>Actual Collection Time</b>
Pre-dose sample	N/A	
15 min		
30 min		
45 min		
1 hour		
1h 15min		
1h 30min		
1h 45min		
2 hours		
2h 20min		
2h 40min		
3 hours		
3h 30min		
4 hours		
8 hours		
16 hours		
24 hours		

**SPECT/CTs**

<b>Scan Interval</b>	<b>Actual scan start</b>	<b>Actual scan stop</b>
1.5 – 2 hours		
4 hours		
24 hours		

<b>Procedure</b>		<b>Time</b>
Inpatient BAQ complete	<input type="checkbox"/> Yes <input type="checkbox"/> No	
Discharged home	<input type="checkbox"/> Yes <input type="checkbox"/> No	
Reminder to refrain from RAI for 48 hrs after discharge	<input type="checkbox"/> Yes <input type="checkbox"/> No	N/A
Reminder to wait for phone call before using take-home dose	<input type="checkbox"/> Yes <input type="checkbox"/> No	N/A

**Inpatient/Outpatient Comments**

--

## APPENDIX 4

### PRODUCT ACCEPTABILITY QUESTIONNAIRE CONTENT

Section	Title:	Content
A	PRODUCT ACCEPTABILITY	Likes and dislikes of physical characteristics of the product and sensations
B	APPLICATION PROCESS	Ease of application, position, timing
C	APPLICATOR	Likes and dislikes about applicator, its tip, and its portability
D	CHANGES IN VOLUME USED	Any changes introduced by the user in the volume of product applied
E	EXPERIENCES USING THE PRODUCT	Leakage, soiling, fullness, or other product side effects, and how much the participant was bothered by them; feeling that the product was absorbed in the intestine
F	PARTNER'S REACTION	Disclosure of product use to partner, partner's reaction/product opinion
G	SEXUAL ENJOYMENT AFTER PRODUCT USE	Respondent and partner's enjoyment of sex after product use as compared to usual sexual experience
H	CONDOMS	Attitudes about condom use during the trial, if applicable
I	CHANGES IN SEXUAL PRACTICES DUE TO PRODUCT USE	Ease of penetration, interruption of sex, attitudes about it
J	INTENTION TO USE PRODUCT IN THE FUTURE	Willingness to use a product that contributed to provide some protection against HIV; willingness to use it when condoms are not used, if wait period were required, and according to its cost.
K	WILLINGNESS TO USE HIGHER VOLUME	Attitudes about different product volumes
L	POSSIBILITY OF COVERT USE	Possibility of non-disclosure of product use to different types of partners
M	RECOMMENDATIONS	Suggested changes to the product

## APPENDIX 5

### PERMISSION LETTERS TO REPRINT COPYRIGHTED MATERIAL

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**Publication:** Journal of Infectious Diseases

**Publisher:** Oxford University Press

**Date:** 03/01/2007

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## CURRICULUM VITAE

Francisco J. Leyva was born in Lima, Peru. Currently he is in his third year of the T32 Clinical Pharmacology fellowship training award at the Division of Clinical Pharmacology at Johns Hopkins University School of Medicine since August 2009 and enrolled in the Graduate Training Program in Clinical Investigation at Johns Hopkins Bloomberg School of Public Health since August 2010 pursuing a Master of Science degree in Clinical Investigation.

### Education and Training

- B.S., Medicine, 1995  
Universidad Peruana Cayetano Heredia, Lima, Peru
- M.D., Medicine, 1995  
Universidad Peruana Cayetano Heredia, Lima, Peru
- Clinical Research Postdoctoral Fellow Pulmonary Medicine(1998-2000)  
Pulmonary medicine, internal medicine specialties  
The University of Texas M.D. Anderson Cancer Center, Houston, TX
- Ph.D., Toxicology, 2007  
University of Montana, Missoula, MT
- IRTA Postdoctoral Fellow in Translational Medicine (05/2007- 05/2009)  
Translational Medicine Branch, National Heart, Lung, and Blood Institute  
National Institutes of Health, Bethesda, MD
- IRTA Postdoctoral fellow in Division of Lung Diseases (05/2009-08/2009)  
Division of Lung Diseases, Extramural Research Program, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD

### Educational Activities

- Research Methods and Biostatistics Lecturer (1997-1998)  
Scientific Research Office  
University of Peru Cayetano Heredia, Lima, Peru
- Instructor of Anatomy (2004-2006)  
College of Health Professions and Biomedical Sciences  
University of Montana, Missoula, MT

- Organizer and Primary Instructor of Toxicology (09/2008-12/2008)  
Foundation for Advanced Education in the Sciences (FAES) – National Institutes of Health, Bethesda, MD

#### Recognitions and Awards

- Diversity Advisory Council Student Achievement Award, The University of Montana, Missoula, MT, 2006.
- Nominee “Graduate Assistant Teaching Award”, The University of Montana, Missoula, MT, 2006.
- Intramural Research Training Award, NHLBI- National Institutes of Health, Bethesda, MD, 2007.
- T-32 Fellowship award in Clinical Pharmacology, The Johns Hopkins University School of Medicine, Baltimore, MD, 2009.

#### Presentations

1. Leyva FJ, Ige M. 1993. 1st International Meeting of Intensive Care Medicine / 2nd International Conference. Peruvian Society of Intensive Care Medicine. “Recurrent Pulmonary Tromboembolism: Case report and discussion”. Lima, Peru. Nov. 25-28th.
2. Leyva FJ, Huaranga AJ. 1999. Annual Meeting: Pulmonary/Critical Care Course Review, Challenge in Pulmonary Medicine. Texas Thorax Society. “Lung Permeability Index: An estimation of Pulmonary Capillary Permeability”. Austin, TX. Feb 26-27.
3. Leyva FJ, Huaranga AJ. 1999. Seventeenth Annual Houston Conference on Biomedical Engineering Research. The Houston Society for Engineering in Medicine and Biology. “Lung Permeability Index: An estimation of Pulmonary Capillary Permeability”. Houston, TX. Feb 11-12.
4. Leyva FJ. 2004. Pre-Med Association of The University of Montana. “Primum Non Nocere, First Do No Harm. Challenges in Clinical Investigation”. The University of Montana. Missoula, MT
5. Leyva FJ. 2006. Health Careers Opportunity Program (HCOP) Reach out. “Pulmonary Diseases”. The University of Montana. Missoula, MT
6. Leyva FJ. 2006. Victor High School, Montana. “Pulmonary Diseases”. Victor, MT
7. Leyva FJ. 2009. Division of Lung Diseases. “Analysis of Self-Reported Snoring Population, NHANES 2005-2006”. National Lung, Heart, and Blood Institute – NIH. Bethesda, MD

8. Leyva FJ. 2010. Division of Clinical Pharmacology. “ HIV transmission: are enemas helpful or harmful, study project”. Johns Hopkins University School of Medicine. Baltimore, MD
9. Leyva FJ. 2011. Division of Clinical Pharmacology. “HIV transmission: are enemas helpful or harmful, study results”. Johns Hopkins University School of Medicine. Baltimore, MD
10. Leyva FJ. 2011. Sleep Disorders Center. Analysis of Self-Reported Snoring Population, NHANES 2005-2006”. Johns Hopkins University School of Medicine. Baltimore, MD
11. Leyva FJ. 2012. Division of Clinical Pharmacology. “Clinical Evaluation of Rectal Microbicide Formulations, Why still in Phase 1?” Johns Hopkins University School of Medicine. Baltimore, MD

### Publications

#### Peer-Reviewed Original Research Articles:

1. Salvatierra EJ, Ige M, Leyva FJ. 1998. Clinical and laboratory characteristics in septic shock patients: cross sectional study in patients from HNAL-ICU. Boletín de la Sociedad Peruana de Medicina Interna. Aug 11(2):51-58. [[Spanish](#)]
2. Huaranga AJ, Leyva FJ, Giralt SA, Blanco J, Signes-Costa J, Velarde H, Champlin RE. 2000. Outcome of bone marrow transplantation patients requiring mechanical ventilation. Critical Care Medicine. Apr 28(4):1014-1017. PMID: 10809275
3. Huaranga AJ, Leyva FJ, Signes-Costa J, Morice RC, Raad I, Darwish AA, Champlin RE. 2000. Bronchoalveolar lavage in the diagnosis of pulmonary complications of bone marrow transplant patients. Bone Marrow Transplant. May 25(9):975-979. PMID: 10800066.
4. Verschraegen CF, Gilbert BE, Huaranga AJ, Newman R, Harris N, Leyva FJ, Keus L, Campbell K, Nelson-Taylor T, Knight V. 2000. Feasibility, phase I, and pharmacological study of aerosolized liposomal 9-nitro-20(S)-camptothecin in patients with advanced malignancies in the lungs. Annals of the New York Academy of Sciences. Jan 922(1):352-354. PMID: 11193921.
5. Llanos-Zavalaga F, Leyva FJ, Miranda JJ, Mayca J, Zeballos C. 2002. Auditoría médica concurrente de certificados de incapacidad temporal para el trabajo (Concurrent medical audit certificates of temporary incapacity for work). Revista Medica Herediana Jun 13(1):26-31. [[Spanish](#)]
6. Huaranga AJ, Barriga C, Leyva FJ, Lainez R, Darwish A, Delpasand A, Ro J. 2004. Gallium scan predicts activity of diffuse parenchymal lung disease. The Internet Journal of Pulmonary Medicine. (www.ispub.com). Jul 3(2):1-9



7. Pfau JC, Schneider JC, Archer AJ, Sentissi J, Leyva FJ, Cramton J. 2004. Environmental oxygen tension affects phenotype in cultured bone marrow-derived macrophages. *American Journal of Physiology. Lung Cellular and Molecular Physiology*. Feb 286(2):L354-L362. PMID: 14527932.
8. Leyva FJ, Pershouse MA. 2009. Quantitative and qualitative methods using fluorescence microscopy for the study of modified low density lipoproteins uptake. *Scanning*. Jul 31(4):167-73. PMID: 19768737.
9. Leyva FJ, Pershouse MA, Holian A. 2010. Modified low density lipoproteins binding requires a lysine cluster region in the murine macrophage scavenger receptor class A type II. *Molecular Biology Reports*. Jul 37(6):2847-52. PMID: 19774489, PMCID: PMC2941145.
10. Leyva FJ, Roberts K. 2010. Crocidolite induces PGI<sub>2</sub> release mediated by vitronectin receptor and cyclooxygenase-2 in lung cells. *Lung*. Apr 188(2):133-41. PMID: 20155273, PMCID: PMC2862624.
11. Ong DS, Anzinger JJ, Leyva FJ, Rubin N, Addadi L, Kruth HS. 2010. Extracellular cholesterol-rich microdomains generated by human macrophages and their potential role in reverse cholesterol transport. *Journal of Lipid Research*. Aug 51(8):2303-13. PMID: 20421591, PMCID: PMC2903806.
12. Anzinger JJ, Chang J, Xu Q, Buono C, Li Y, Leyva FJ, Park BC, Greene LE, Kruth HS. 2010. Native low-density lipoprotein uptake by M-CSF-differentiated macrophages is mediated by macropinocytosis and micropinocytosis. *Arteriosclerosis Thrombosis and Vascular Biology*. Oct 30(10):2022-31. PMID: 20634472, PMCID: PMC3170564
13. Huaranga AJ, Leyva FJ, Glassman AB, Haro MH, Arellano-Kruse A, Kim EE. 2011. The lung permeability index: a feasible measurement of pulmonary capillary permeability. *Respiratory Medicine*. Feb 105(2):230-5. PMID: 21094033
14. Leyva FJ, Anzinger JJ, McCoy JP, Kruth HS. 2011. Evaluation of transduction efficiency in macrophage colony-stimulating factor differentiated human macrophages using HIV-1 based lentiviral vectors. *BMC Biotechnology* Jan 31;11:13. PMID: 21281514, PMCID: PMC3045310
15. Leyva FJ, Lankapalli RH, and Twery MJ. 2012. Association between sleep disordered breathing and physiological changes in the U.S. population: National Health and Nutrition Examination Survey NHANES 2005-2008. In submission
16. Bakshi RP, Leyva FJ, Hendrix CW. 2012. A randomized, partially-blinded, comparative study of the safety, mucosal toxicity, mucosal infectibility, colorectal distribution, and participant acceptability of three different preparatory enemas (hypo- iso- and hyper-osmolar). In preparation.

17. Leyva FJ, Bakshi RP, Hendrix CW. 2012. A randomized, partially-blinded, comparative study of the safety, mucosal toxicity, mucosal infectibility, colorectal distribution, and participant acceptability of four different rectal microbicide vehicle candidates. In preparation.

#### Review Articles:

1. Ormea A, Ige M, Torres L, Loja D, Caceres L, Leyva FJ. 1996. Complicated tetanus management: parasympathetic dysautonomy. Boletín Sociedad Peruana de Medicina Interna. Jun 9(2):60-66. [[Spanish](#)]
2. Ormea A, Leyva FJ. 1998. Central venous catheterism and myocardial perforation. Revista Medica Herediana. Jan 9(1):119-122. [[Spanish](#)]
3. Huaranga AJ, Herrera NE, Leyva FJ, Sotelo CE. 2000. Boop-like reaction: an unusual presentation of pulmonary Histiocytosis X. The Internet Journal of Pulmonary Medicine ([www.ispub.com](http://www.ispub.com)). Jan 1(1):1-9.
4. Huaranga AJ, Leyva FJ. 2000. Diffuse parenchymal lung disease: a practical approach. The Internet Journal of Pulmonary Medicine ([www.ispub.com](http://www.ispub.com)). Jan 1(1):19-29.
5. Huaranga AJ, Zetola NM, Leyva FJ, Blanco JJ, Herrera NE. 2001. Necrotizing granulomatosis causing a malignant airway obstruction. The Internet Journal of Pulmonary Medicine. ([www.ispub.com](http://www.ispub.com)). Jan 1(2):22-27.
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#### Abstracts/Posters:

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2. Leyva FJ, Pershouse MA, Holian A, and Roberts K. 2008. Crocidolite asbestos induces prostacyclin release mediated by vitronectin receptor and cyclooxygenase 2 in lung cells. Annual Fellows Retreat. National Heart, Lung, and Blood Institute – National Institutes of Health. Annapolis, MD
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5. Kruth, HS, Anzinger JJ, Chang J, Leyva FJ, Park BC, and Greene LE. 2009. Native low-density lipoprotein uptake by macrophage colony-stimulating factor differentiated macrophages is mediated by macropinocytosis and micropinocytosis. Society of Leukocyte Biology Annual Meeting. Lisbon, Portugal. Cytokine. Volume 48, Issues 1-2, October-November 2009, Page 135.
6. Leyva FJ, Fuchs EJ, Ventuneac A, Carballo-Diequez A, Lee LA, Torbenson MS, Li L, and Hendrix CW. 2011. Hyper-osmolar (fleet) enema damages colorectal lining compared to iso-osmolar (normosol-r) enema in randomized, blinded, cross-over comparison of toxicity, distribution, and acceptability study of three rectal douches (enemas) identifying a safe, preferred delivery vehicle for rectal microbicides. 18<sup>th</sup> Conference on Retroviruses and Opportunistic Infections (CROI). Boston, MA.

#### DNA Sequences Published in NCBI GeneBank

1. Leyva FJ, Evans CM, Tuvim MJ, Dickey BF. 2002. *Mus musculus* signal transducer and activator of transcription 6 (Stat6) mRNA, complete cds. Accession: AF481809.
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