**An Open-Label Phase 1 Compartmental Pharmacokinetic and Pharmacodynamic Assessment of Rilpivirine Long-Acting Pre-Exposure Prophylaxis for HIV Prevention**

**Authors:** Prof Ian McGowan MD1, Charlene S. Dezzutti PhD1,2, Aaron Siegel BS2, Jarret Engstrom MBA2, Alexiy Nikiforov BS2, Kathryn Duffill BS2, Cory Shetler BS2, Nicola Richardson-Harman PhD3, Kaleab Abebe PhD1, Prof David Back PhD4, Laura Else PhD4, Deidre Egan PhD4, Prof Saye Khoo MD4, James E. Egan PhD4, Prof Ronald Stall PhD5, Peter E. Williams MD6, Khaleel K. Rehman PhD2, Amy Adler RN7, Rhonda M. Brand PhD1, Beatrice Chen MD1, Sharon Achilles MD1, Ross D. Cranston MD1

**Affiliations:**

1University of Pittsburgh School of Medicine, Pittsburgh, PA, United States.

2Magee Women Research Institute, Pittsburgh, PA, United States.

3Alpha StatConsult LLC, Damascus, MD, United States.

4University of Liverpool, Liverpool, United Kingdom.

5University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, United States.

6Janssen Research and Development, Beerse, Belgium.

7The Translational Science Corp, Los Angeles, CA, United States.

**Correspondence**

\*Ian McGowan MD PhD FRCP

University of Pittsburgh School of Medicine

204 Craft Ave, Room B621

Pittsburgh, PA 15213

T: +1 412 641 4710

Email: [imcgowan@pitt.edu](mailto:imcgowan@pitt.edu)

**Summary**

**Background** Long-acting (LA) injectable antiretroviralagents are being developed for HIV prevention. The MWRI-01 study was undertaken to characterize the safety, acceptability, pharmacokinetic (PK), and pharmacodynamic (PD) profile of rilpivirine (RPV) LA.

**Methods** In the Phase 1 MWRI-01 trial, an open-label study conducted at the University of Pittsburgh, PA, USA, HIV-1 uninfected participants received a single intramuscular dose of either 1200 or 600 mg of RPV LA. Plasma, genital/rectal fluids, and tissue (rectal (RT), cervical (CT), and vaginal (VT)) were collected before and after exposure to RPV LA for assessment of PK and *ex vivo* biopsy challenge with HIV-1. The primary study objective was to characterize product safety and the analysis included all enrolled participants. This trial is registered with ClinicalTrials.gov, number NCT01656018.

**Findings** A total of 24 females and 12 males were enrolled in the study.Within the 36 participants enrolled in the study,there were a total of 204 adverse events reported of which 200 (98∙0%) were Grade 1/2. Geometric mean (GM; 90% CI) plasma RPV concentrations at Day 28 post-dose for the 1200 mg and 600 mg cohorts were 53 (38-67) ng/mL (female) / 43 (23-63) ng/mL (male) and 28 (19-37) ng/mL (female) / 17 (9-24) ng/mL (male) respectively. The RPV tissue: plasma ratio in RT was approximately two-fold higher than in VT or CT. Exposure to RPV LA was associated with significant suppression of viral replication in RT (p < 0∙0001) that persisted for up to four months. In contrast, no viral suppression was seen in CT or VT.

**Interpretation** Single dose administration of RPV LA was safe and well tolerated with dose dependent plasma PK exposure. This is the first study to demonstrate prolonged suppression of RT viral replication following systemic antiretroviral exposure.

The MWRI-01 study was funded by a grant from the Bill & Melinda Gates Foundation. **Introduction**

Long–acting (LA) injectable antiretrovirals are being developed for the prevention and treatment of HIV infection. (1, 2) TMC278 or Rilpivirine (RPV) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that is licensed for the treatment of chronic HIV infection. (3)NNRTIs are attractive pre-exposure prophylaxis (PrEP) agents because they act early in the viral replication cycle, are potent antiretroviral agents, and unlike TFV do not require cellular metabolism to be active against HIV. In a recent study conducted using BLT humanized mice, Kovarova et al. were able to show that mice treated with RPV LA were protected from HIV infection when challenged vaginally with HIV-1CH040. (4)

A series of Phase 1 clinical trials of RPV LA (alone or in combination with cabotegravir LA, an integrase inhibitor) have established the initial safety, acceptability, and PK profile of this formulation. In a recent study, participants received single doses of 300-1200 mg of RPV LA followed by comprehensive sampling of plasma, rectal fluid, cervical fluid, and more limited sampling of rectal and vaginal tissue. (5) All doses were well tolerated and provided prolonged plasma and genital-tract RPV exposure through 84 days. There was a significant correlation between the concentration of RPV in cervicovaginal lavage fluid and the degree of HIV inhibition.

The purpose of the MWRI-01 study was to further characterize the safety and acceptability of RPV LA, to provide compartmental PK data, and to determine whether exposure to RPV LA was associated with protection of rectal, cervical, and vaginal tissue biopsies from HIV-1 infection.

**Materials and Methods**

**Study Design**

This study was a Phase 1, single centre, open-label, exploratory dose ranging study of RPV LA conducted in healthy HIV seronegative men and women. The study was performed at the Magee-Womens Hospital Clinical and Translational Research Center, Pittsburgh, PA, USA. The final protocol was approved by the Institutional Review Board at the University of Pittsburgh and study participants provided written informed consent in English.

**Participants**

Participants were initially eligible for inclusion if they were between 18 and 45 years of age, had a body mass index between 18 and 35 kg/m2, were HIV-1 seronegative, were not pregnant or breastfeeding, had a regular menstrual cycle, were willing to use an acceptable form of contraception, had satisfactory cervical cytology and were willing to abstain from sperm donation for the duration of the study. The full list of inclusion criteria is provided in the study protocol (appendix p 14).

Major exclusion criteria were the use of antiretroviral PrEP or post exposure prophylaxis, unprotected insertive or receptive anal intercourse within six months of screening, non-therapeutic injection drug use, use of immunomodulatory medications, use of the following medications (heparin, warfarin, clopidogrel bisulfate, any drugs likely to increase the risk of post-biopsy bleeding, rectally or vaginally administered medications, rifamycin, anticonvulsants, dexamethasone, and St. John’s wort), history of or electrocardiogram (ECG) demonstrating a prolonged QT interval (QTc), abnormalities of the cervical, vaginal, or rectal mucosa which would be a contraindication to taking biopsies, at screening symptoms or laboratory test evidence of untreated rectal or reproductive tract infection, any of the following laboratory abnormalities (hemoglobin <10∙0 g/dL, platelet count <100,000 per L, white blood count <2,000 or >15,000 cells per L, serum creatinine >1∙3× the local upper limit of normal (ULN), alanine transaminase (ALT) and aspartate aminotransferase (AST) >2∙5× ULN, +1 glucose or +2 protein on urinalysis, hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) antibody positive. The full list of exclusion criteria is provided in the study protocol (appendix p 14).

**Product Allocation**

The participants were not randomized to the two doses. Instead the first female or male participant received the 1200 mg dose and thereafter participants were alternately assigned to the 600 mg or 1200 mg study arms. The RPV LA (300 mg/mL) was administered as one 2 mL (600 mg) or two 2 mL (1200 mg) intramuscular (IM) gluteal injections. The two 2 mL injections were given approximately 1 hour (hr) apart. There was no oral RPV lead-in in any subject.

**Study Product**

The investigational product (RPV LA) was supplied in vials as a sterile aqueous nanosuspension formulation (G001) at a concentration of 300 mg RPV/mL. Prior to use the RPV LA was stored in a temperature controlled environment (2-8oC).

**Procedures**

There were a total of twelve study visits. After obtaining informed consent all participants were screened with a medical history, a physical examination including rectal and vaginal examination, and laboratory tests to exclude hematological, renal, or hepatic abnormality (Visit (V) 1). In addition, samples were collected to exclude pregnancy or anorectal/cervicovaginal sexually transmitted infections (STIs). A 12 lead ECG was recorded in triplicate. Participants who met the inclusion/exclusion criteria during the Screening Visit were enrolled into the study. At the Enrollment Visit (V2), a web-based behavioral questionnaire was administered, and a focused physical examination was performed. Samples were collected to exclude pregnancy and STIs. Flexible sigmoidoscopy was performed with collection of 10 rectal biopsies acquired at approximately 15 cm from the anal verge as previously described (6-8). Female participants had two ectocervical and three vaginal biopsies. Biopsies were used for PK and PD (*ex vivo* biopsy challenge). Participants then received an IM injection of RPV LA. Blood and rectal/genital tract fluids were collected 24 hr (V3), 1 week (V4), and 2 weeks (V5) after the injection. Additional blood and fluids were collected every 2 weeks thereafter until V12 (16 weeks after the injection of RPV LA). Rectal (RT), cervical (CT), and vaginal (VT) tissue was collected 4 weeks after the injection (V6) and every 4 weeks thereafter (V8, V10, and V12). Study participants could elect to have two additional visits (Visits 12A and 12B) where additional PK and PD samples were collected at 20 and 24 weeks after the injection of RPV LA.

**Clinical Safety and Laboratory Assessments**

Emergent adverse events (AEs) were graded using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1∙0, December 2004 as well as Addenda 1-3 (<http://rsc.tech-res.com/safetyandpharmacovigilance/>).

**Behavioral Evaluations and Procedures**

Computer-assisted self-interview (CASI) was completed by the participant at V2. In addition to demographics, this questionnaire also assessed the participants’ history of HIV sexual risk behaviors and experience with safer sex practices. Participants completed product acceptability CASIs at V3, V4, and V12. Participants were asked to rate acceptability on a scale from one to ten, with one representing less acceptable/unlikely to use in the future. In this paper, we will only report product acceptability and factors that might influence product use in the future as a second behavioral manuscript is in preparation.

**Pharmacokinetic Analysis**

Merocel sponges were used to collect RF while Dacron swabs were used to collect CVF. Merocel® sponges are a type of PVA-based sponge. The bioanalytical method (used for PK analysis) as described by Else et al was fully validated for PVA-based sponges, in accordance with FDA bioanalytical guidelines. (9) The analytical method used a mixture of hexane/ethyl acetate to extract RPV from the Merocel® sponges. Extraction (or percentage recovery) of RPV was acceptable (as per FDA guidelines). An organic solvent could not be used to extract RPV from the Dacron polyester swabs for the TZM-bl assay as this would have lysed the cells. The Dacron swab extraction process was non-validated.

RPV concentrations in all matrices (plasma, mucosal fluids (rectal, endocervical, and vaginal), and tissue (rectal, ectocervical, and vaginal)) were quantified by validated high-pressure liquid chromatography-mass spectrometry. Full methodological validation of this process is provided in a separate publication. (9) RPV concentrations in all matrices were expressed as ng/mL. Tissue homogenate and rectal/vaginal/endocervical fluid samples were initially quantified using an ng/sample calibration curve and then converted to ng/mL by adjusting for the recorded tissue and fluid volumes. Concentrations that were below the assay limit of quantification (LLOQ) were expressed as ½ LLOQ values.

**Pharmacodynamic analysis**

At Baseline (V2) and post-product exposure timepoints (V6, V8, V10, and V12), rectal, cervical, and vaginal biopsies were collected in 20 mL RPMI (with 1∙125 µg/mL of Fungizone and 0.5 mg/mL of Zosyn) and transported to the laboratory for *ex vivo* infection within 30 minutes using a common viral stock of HIV-1BaL (105 TCID50 for rectal and 5×104 TCID50 for cervicovaginal tissue), as previously described. (10-13) Results were adjusted for initial biopsy weight, and reported as Day 14 cumulative p24 (p24 HIV antigen ELISA; Alliance; Perkin-Elmer Life Sciences Inc., Boston, MA) where the assay’s LLOQ was 10 pg/mL. Non-detectable cumulative p24 measures at Day 14 were converted to 1/2 LLOQ prior to log transformation. TZM-bl cells (NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH) were used to determine pharmacodynamic activity of the mucosal fluid. (14) Inhibition was determined based on deviations from the HIV-1 only control and presented as the percentage inhibition.

**Statistical Analyses**

Given the small sample size in this Phase 1 study, there was insufficient power to observe any adverse events other than those occurring at high frequency. We assessed between-arm differences in baseline characteristics by using two-sample t tests, chi-square tests, or their non-parametric counterparts. The proportion of participants experiencing a Grade 2 or higher adverse event was the primary outcome and was compared between arms using Fisher’s Exact test. Acceptability ratings related to study drug were compared between study arms and within study arms over the duration of participation. The proportion of product acceptability was calculated for each study arm along with the 95% confidence intervals. Fisher’s exact test was utilized to compare arms with regard to this endpoint.

Plasma and compartmental PK endpoints included the area under the concentration-time curve over 112 days (AUC112), maximum concentration (Cmax), time to maximum concentration (Tmax), half-life (t½) and concentrations measured at 4-weekly intervals (C28, C56, C84, and C112). For male participants this included plasma and rectal secretions and tissue. For female participants this included values from plasma, endocervical and vaginal secretions, and CT and VT. Rectal secretion and RT PK data were also available from those female participants who elected rectal samples collected. PK parameters were calculated using non-compartmental analysis (WinNonlin Phoenix, version 6∙1; Pharsight, Mountain View, CA). Data were expressed as geometric means (GM) with 90% confidence intervals (CI). Measures of drug exposure and ratios of compartmental-to-systemic drug ([RPV]COMP/[RPV]Plasma) over 112 days were compared between arms and concentration differences were analyzed.

Changes in culture supernatant HIV-1 p24 antigen were the primary outcome in the biopsy studies, which were designed to assess potential pharmacodynamic activity as realized by a negative slope between HIV-1 p24 release and drug concentrations. Multiple tissue sites (cervical, vaginal, and rectal) were infected, *ex vivo*, with HIV-1BaL. Using a two-sided paired t-test with α = 0∙05, the study had 80% power to detect effect sizes as small as 0∙89 in the female arms and 1∙43 in the male arms for RT. Comparisons between study arms for each gender with respect to p24 antigen across visits were made using linear mixed models with fixed effects for visit, arm, and their interaction plus a random effect for subject. Detectable PK drug levels and cumulative p24 antigen levels for measures taken from the same subjects/visits were correlated using a linear mixed effect model to test for a significant slope estimate for each PK/PD pair. Statistically significant linear PK/PD relationships were further tested using a non-linear Emax model. The non-linear Emax model was chosen using the Akaiki Information Criteria. The upper and lower asymptotes were constrained to 100 and 0% virus control, respectively.

**Role of the funding source**

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

**Results**

*Participants*

Enrollment was conducted from the 3rd January, 2013 through 17th February, 2014. Study follow up was completed on the 4th August, 2014. A total of 62 participants were screened and 36 were enrolled in the study (Figure 1 and Table 1). Eighteen of the participants (12 females and 6 males) received a single 1200 mg dose of RPV LA and 18 participants (12 females and 6 males) received a single 600 mg dose. All 36 participants completed the original final study visit (Visit 12; 112 days post dose). Seven of the 24 female participants completed two additional visits; Visits 12A and 12B (28 and 56 days following Visit 12 respectively) and two female participants completed Visit 12A but not Visit 12B. Although the study participants were not randomized there were no significant demographic differences between the two groups (1200 mg versus 600 mg RPV LA).

*Safety*

For the primary safety endpoint, there were a total of 204 adverse events (AEs) reported in 36 participants: 170 (83∙3%) Grade 1, 30 (14∙7%) Grade 2, 2 (1∙0%) Grade 3, and 2 (1∙0%) Grade 4 (Table 2). All Grade 3 and 4 AEs were deemed Not Related to study product. There were no statistically significant differences in Grade 2 or higher AE reporting at a participant level within sex cohorts between the 1200mg and 600mg dose (Table 2). Injection site reaction (ISR) was the most frequent AE, and was reported in 32 of 36 participants. In both male and female cohorts, the ratio of participants with Grade 1/2 ISR AEs was approximately 2:1 (appendix p 5). The average length of Grade 1 and 2 ISR AEs was 2-3 days and 1-2 days, respectively in all participants.

Electrocardiograms were performed throughout the study. There were 28 participants with a prolonged QT interval (QTc) (26 participants with Grade 1 and 2 participants with Grade 2). Both Grade 2 AES were in the 1200mg cohort (appendix p 5). There was one skin rash reported during the study that was deemed possibly related to product. The rash was noted at Visit 4, was Grade 1, and resolved spontaneously after 5 days.

**Behavioral Evaluation and Product Acceptability**

Overall, participants reported acceptable levels of anxiety related to injections (Mean score (M) = 6∙6, SD = 2∙4). On average, however, anxiety was significant lower among women than men (β = -1∙78, p = 0∙04). Similarly, mean scores suggested participants did not experience pain associated with the injection (M = 6∙6, SD = 1∙98). These data, based on self-reported survey responses, differ from findings on injection site pain collected in the safety data. The behavioral data did not capture this difference.

When asked about uptake of the product (assuming ‘very good’ protection), likelihood was high that participants reported they would use this product (M = 6∙83 SD = 2∙21). When asked if they might use the product given a specific price, the likelihood decreased as the price increased. At $25 the mean score was just over 6 (M = 6∙29, SD = 2∙31), at $50 the mean score dropped to about 5 (M = 4∙92, SD = 2∙16), and finally, at $100 the mean score dropped to less than 4 (M = 3∙76, SD = 2∙20). Of the suggested barriers to uptake, participants were most concerned with cost (M = 7∙60, SD = 2∙02), possible medication side effects (M = 7∙19, SD = 1∙98), and fear that medications could be harmful to their health (M = 6∙30, SD = 2∙32). Least likely to impact future uptake was: the ability to protect oneself without their partner knowing (M = 4∙06, SD = 1∙97), stigma associated with using an HIV medication (M = 3∙18, SD = 2∙06), and fear of needles (M = 3∙05, SD = 2∙32).

**Pharmacokinetic Analysis**

PK profiles (Geometric Mean [GM]; 90% Confidence Interval [CI]) for RPV in the plasma, genital tract (vaginal fluid [VF], endocervical fluid [EF] and VT, and CT) and rectum (rectal fluid [RF] and RT) of females over 168 days after dosing (Figure 2, and appendix p 7-8). RPV PK profiles for the male subjects are presented in the appendix p 2 and 12-13. The target concentration for HIV prevention is unknown, but for comparison we utilized the *in vitro* EC90 for wild-type HIV, corrected for protein binding to yield a putative target concentration of 12∙2 ng/mL. (15)

*Plasma*

RPV persisted in the plasma for up to 112 days in both males and females, and was also detectable at 168 days post dose in females that undertook additional visits in the 2 arms (GM = 5∙8 and 13∙3 ng/mL). Time to peak plasma concentrations was variable, and in individual cases could be delayed beyond the Tmax observed in the population profiles which are based on mean concentrations at each time point (Figure 2 and appendix p 2). RPV exposures (AUC112) in plasma were not significantly different between males and females receiving an equivalent 600mg and 1200 mg dose. Plasma concentrations >EC90 were reached for all subjects, irrespective of dose or gender, at the time of the first PK sampling at 1-day post dose (Visit 3). Following a 1200 mg dose, all female subjects had RPV concentrations above the protein adjusted EC90 for up to 98 days, and 92% remained above the EC90 at 112 days post dose. However, in the 600 mg cohort, the percentage of female subjects with plasma concentrations >EC90 fell below 100% by day 42 (92%), and all subjects were below this target by 112 days post dose. In males, plasma concentrations were above the EC90 for up to 14 days (600 mg dose) and 84 days (1200 mg dose), and the percentage above target fell to 17% and 33% at 112 days post dose.

*Female genital tract*

VF Cmax was achieved slightly later than systemic levels (Figure 2b), suggesting a delay in drug absorption into the female genital tract. RPV exposures (AUC112) in VF were consistently higher (significant for the 600 mg dose; p=0∙02) than corresponding plasma levels: VF-to-plasma drug ratios were ≥1 (appendix p 7). Concentrations in EF were lower: EF-to-plasma ratios = 0∙69-0∙77 for 600 and 1200mg doses (p>0∙08). Both matrices were associated with plasma (VF *r*2 = 0∙423; EF *r*2 = 0∙471); VF and EF were also highly correlated (r2 = 0∙760).

*Rectum*

RPV concentrations in both female and male RF were highly variable and substantially lower than levels observed in plasma: GM RF-to-plasmaratios ranged between 0∙28-0∙66, and no gender-related differences were observed in overall RF AUC112 (p>0∙23). Furthermore, unlike the cervical/vaginal fluids, RF was only weakly correlated with corresponding plasma drug concentrations (*r*2 = 0∙09).

*Tissue*

Tissue biopsies were taken at 4-weekly intervals between 28 and 112 days (and at 168 days in women undertaking additional visits). For females, VT and CT concentrations, when expressed as ng/mL, were significantly lower than paired plasma (p<0∙0001): GM Tissue-to-plasma ratios were 0∙44-0∙72 for VT and 0∙42-0∙77 for CT over the course of 112 days. Plasma and VT (r2=0∙721) and CT (r2=0∙666) concentrations were also highly correlated.

Ten of 12 females in each dosing arm also opted to undergo RF and RT sampling. RT concentrations in females, when expressed as ng/ml, exceeded systemic levels (GM RT-to-plasma ratios ranged between 1∙10-1∙22; p=0∙001) and were approximately 1∙5 to 2∙5-fold higher (p<0∙003) than drug exposures in the female genital tract (in VT or CT). RT concentrations were comparable in males and females (both doses; p>0∙566) and were strongly associated with paired plasma concentrations (r2=0∙795, females; r2=0∙812, males).

**Pharmacodynamics**

*Ex vivo* HIV-1 infection (log10 p24 pg/mg) in RT, CT, and VT was compared between doses (600 versus 1200 mg) and across Days 0-168 post IM injection of RPV LA (Figure 3) by mixed model ANOVA. There was an effect of dose for RT (p = 0∙04; Figure 3a) but no significant effects of dose were found for either CT (p = 0∙55; Figure 3b) or VT (p = 0∙07; Figure 3c). There was also an effect of visit for RT (p < 0∙0001) but, again, no effects of visit were found for either CT or VT.

Pairwise comparisons were performed for rectal infection results between Baseline (Visit 2) and Days 28 (Visit 6), 56 (Visit 8), 84 (Visit 10), 112 (Visit 12), 140 (Visit 12A), and 168 (Visit 12B) for each dose group. Suppressed p24 was found at Visits 6-12 compared to Visit 2 for both the 600mg and 1200mg doses (p < 0∙05). At Visits 12A and 12B the p24 levels had reverted back to Visit 2 levels (p > 0∙05) for both doses. Pairwise comparisons were performed to compare rectal infection between doses for each visit; the only visits where a significant difference in p24 infection was found between the 1200 mg and 600 mg arms were Visits 10 and 12 where p24 infection levels were lower for the 1200 mg dose group. RF, EF, and VF were unable to demonstrate drug-related inhibition of HIV-1 in the TZM-bl assay (appendix p 3). This is likely due to the poor recovery of the hydrophobic RPV from the collection matrix.

**Pharmacokinetic / Pharmacodynamic Relationship**

*Ex vivo* infection results in RT, CT, and VT were correlated with paired PK drug levels in the respective tissue and fluids or plasma across all visits (Figure 4). A linear mixed effect model was performed to correlate the PK levels (i.e. concentration in tissue, fluid and plasma) with the PD response (i.e. *ex vivo* HIV-1 p24 in the explant supernatant fluid). A significant negative slope value supports a finding of drug mediated virus suppression and was found for rectal PK: PD models (p < 0∙001; Figure 4a, b, and c). There were no significant PK: PD relationships found for cervical (Figure 4d, e, and f) or vaginal (Figure 4g, h and i) linear models. The significant linear mixed PK: PD effects found for rectal tissues were further explored with an Emax model.

The EC50 (i.e. the concentration of RPV predicted to suppress 50% of *ex vivo* infection) for rectal tissue (1.01 log10 ng/mL) and plasma (0.92 log10 ng/mL) were similar in both compartments. Drug levels at or above 1∙1 log10 ng/mL were mostly found for the 1200 mg dose (appendix p 4). The rectal tissue EC90 = 1.96 (1.83-2.09; 95% CI) log10 ng/mL and the plasma EC90 = 1.87 (1.74-2.00) log10 ng/mL values were higher than the in vitro EC90 for wild-type HIV, corrected for protein binding to yield a putative target concentration of 1.09 log10 ng/mL. Only 6% (4/67) of the rectal explant PK-PD pairs from the 1200 mg dose group and none of the 600 mg dose group were found to have ≥ 1.96 log10 ng/mL EC90.

**Discussion**

Single dose administration of RPV LA was safe and well tolerated. The majority of AEs were Grade 1 and there were no related Grade 3 or Grade 4 events. There was a non-significant trend towards increased Grade 2 events in the 1200 mg arm of the study. The most common AE was injection site discomfort which was mild or moderate, transient, and did not appear to diminish participants’ willingness to consider this form of HIV prevention in the future. Although Grade 1 prolongation of the QTc interval was fairly common, none of the values in the study exceeded the FDA definition of QTc prolongation (450 and 470 msec for males and females respectively).

The behavioral data suggest that study participants were not dissuaded by fear of, or experienced pain with, injections and were highly likely to use the product. It remains to be seen whether multiple injections will be equally acceptable to participants and this question is currently being addressed the HPTN-076 study (NCT02165202) where participants will receive six injections at eight week intervals.

After a single injection, maximum plasma concentrations of RPV were achieved by 9 days and RPV was still detected in samples collected at Visit 12B (Day 168 post-dosing). Tissue levels were variable but in general, RT/plasma ratios were > 1∙0 and cervicovaginal tissue/plasma ratios were < 1∙0. RT RPV concentrations were 1∙5 to 2∙5-fold higher than CT or VT concentrations. These data confirm RPV as an LA formulation but raise questions concerning the compartmental PK profile and whether there might be differential levels of protection associated with rectal and vaginal transmission of HIV infection. The presence of subtherapeutic levels of RPV during an extended pharmacological tail, after the end of an RPV LA dosing regimen, might lead to the acquisition of NNRTI class resistance if the individual becomes infected with HIV.

*Ex vivo* infection rates were lower in RT after both 600 mg and 1200 mg single doses showing evidence of drug mediated virus suppression and this was sustained out to 112 days post RPV LA administration. There were no significant reductions in virus infection in either CT or VT even though drug levels were detectable in both compartments. The *ex vivo* TZM-bl assays did not provide any evidence of antiviral activity in either compartment due to the high levels of endogenous antiviral activity in RF and probably the failure to elute RPV from the sponges. The divergent results from the rectal and cervicovaginal biopsy models are intriguing and may reflect differences in the models and/or differential PK requirements to suppress tissue infection in either compartment (16). A key consideration is whether these data might predict differential outcomes in populations at risk of HIV infection through vaginal or rectal intercourse.

Clear evidence of drug mediated virus suppression was found when drug PK levels in RT, plasma and RF were correlated with infectivity in RT. When HIV infectivity was compared to baseline (Visit 2), a non-linear model determined that approximately 12∙6 ng/mL of RPV in both RT and plasma compartments predicted 50% suppression in virus growth, which predominately occurred following the 1200 mg dose. It is uncertain what the *in vivo* target range for HIV prevention will be but extrapolating from these biopsy data suggests that protective levels could be achieved in the rectal compartment with 1200 mg injections of RPV LA every two months.

One limitation of both cabotegravir LA and RPV LA is that once the products have been injected they cannot be removed and so any idiosyncratic AEs associated with their use will be difficult to manage. Some of the current trials evaluating these products are using an oral run-in period to try and identify individuals who might be vulnerable to product-related AEs. Data from our study would suggest that product associated adverse events are generally mild and raises the question as to whether oral run is necessary. Alternatively, there is now increasing interest in developing implantable devices that could be easily removed should a significant adverse event occur. Ideally, these products would gradually release the antiretroviral over a period of months if not years. (17)

A strength of this study was our ability to undertake longitudinal multi-compartmental sample collection to characterize the PK: PD profile of LA RPV over an extended period of time. Despite the intensity and duration of sample collection, all study participants completed the study. Limitations of the study included the fact that this was an open label randomized trial and so we were unable to determine whether adverse events such as injection site pain were related to the method of product administration or to the actual product. In addition, *in vitro* susceptibility targets may not accurately reflect the situation *in vivo*, especially in anatomical sites of transmission, such as genital tract and rectum, where the protein content and drug binding are uncharacterized. The explant challenge model is a stringent test of product pharmacodynamics and protective concentrations in efficacy trials may be less than those required in the explant model.

In this Phase 1 study we have demonstrated that single dose administration of RPV LA is safe and acceptable. Moreover, exposure to single dose RPV LA is associated with prolonged PK and associated viral suppression in colorectal but not cervicovaginal tissue. Ongoing studies, including a Phase 2 study in US, South African, and Zimbabwean women (HPTN-076), will characterize longer term safety and acceptable of multiple injections and help determine whether this product should advance to assessment of efficacy in preventing HIV infection.

**Contributors**

I.M. designed the study protocol with assistance from R.D.C., A.A., K.K., and K.A. B.C. and S.A. collected female genital tract samples. Laboratory assays were run by A.S., J.E., A.N., K.D., and C.S. and overseen by C.S.D., R.B.; D.B., L.E., D.E., and S.K were responsible for the analysis of all pharmacokinetic samples; R.S. and J.E.E designed, conducted, and analyzed the behavioral component of the study; K.A. and N.R.H analyzed and interpreted the data; P.W. provided access and regulatory support for the provision of rilpivirine LA; I.M. wrote the manuscript. All authors have read and commented on the final manuscript.

**Declaration of Interests**

I.M. has has served on advisory boards for Novicol Life Sciences and ABIVAX and received research grants from ViiV Healthcare and Janssen. S.K. has received research grants and speaker’s honoraria from Merck, Gilead Sciences, and ViiV Healthcare. The Liverpool HIV Drug Interaction website has received support from ViiV Healthcare, Gilead Sciences, Janssen, Bristol-Myers Squibb and Merck. C.S.D. has served on an advisory board for Pfizer.  D.J.B. has served on speaker bureaus or advisory boards for Gilead Sciences, Janssen, Viiv, Merck, Bristol-Myers Squibb and AbbVie and has also received educational grants from Gilead Sciences, Janssen, ViiV Healthcare, Merck, Bristol-Myers Squibb and AbbVie. N.R.H. is an employee of Alpha StatConsult. P.W. is a full-time employee of Janssen.

**Acknowledgments:**

The MWRI-01 study was funded by a grant from the Bill & Melinda Gates Foundation (OPP1045325). The authors would like to thank the participants for their commitment to the MWRI-01 study.

**Competing interests**:

None

**References**

[1] Spreen WR, Margolis DA, Pottage JC, Jr. Long-acting injectable antiretrovirals for HIV treatment and prevention. *Curr Opin HIV AIDS* 2013; **8**:565-71.

[2] Jackson A, McGowan I. Long-acting rilpivirine for HIV prevention. *Curr Opin HIV AIDS* 2015; **10**:253-7.

[3] Ripamonti D, Bombana E, Rizzi M. Rilpivirine: drug profile of a second-generation non-nucleoside reverse transcriptase HIV-inhibitor. *Expert Rev Anti Infect Ther* 2014; **12**:13-29.

[4] Kovarova M, Council OD, Date AA, Long JM, Nochii T, Belshan M, et al. Nanoformulations of Rilpivirine for Topical Pericoital and Systemic Coitus-Independent Administration Efficiently Prevent HIV Transmission. *PLoS Pathog* 2015; **11**:e1005075.

[5] Jackson AG, Else LJ, Mesquita PM, Egan D, Back DJ, Karolia Z, et al. A compartmental pharmacokinetic evaluation of long-acting rilpivirine in HIV-negative volunteers for pre-exposure prophylaxis. *Clin Pharmacol Ther* 2014; **96**:314-23.

[6] Anton PA, Elliott J, Poles MA, McGowan IM, Matud J, Hultin LE, et al. Enhanced levels of functional HIV-1 co-receptors on human mucosal T cells demonstrated using intestinal biopsy tissue. *AIDS* 2000; **14**:1761-5.

[7] Anton PA, Poles MA, Elliott J, Mao SH, McGowan I, Lenz HJ, et al. Sensitive and reproducible quantitation of mucosal HIV-1 RNA and DNA viral burden in patients with detectable and undetectable plasma viral HIV-1 RNA using endoscopic biopsies. J Virol Methods 2001; **95**:65-79.

[8] Shacklett BL, Yang O, Hausner MA, Elliott J, Hultin L, Price C, et al. Optimization of methods to assess human mucosal T-cell responses to HIV infection. *J Immunol Methods* 2003; **279**:17-31.

[9] Else LJ, Tjia J, Jackson A, Penchala SD, Egan D, Boffito M, et al. Quantification of rilpivirine in human plasma, cervicovaginal fluid, rectal fluid and genital/rectal mucosal tissues using liquid chromatography-tandem mass spectrometry. *Bioanalysis* 2014; **6**:1907-21.

[10] Anton PA, Cranston RD, Kashuba A, Hendrix C, Bumpus NN, Richardson-Harman N, et al. RMP-02/MTN-006: A Phase 1 rectal safety, acceptability, pharmacokinetic and pharmacodynamic study of tenofovir 1% gel compared to oral tenofovir disoproxil fumarate. *AIDS Res Hum Retroviruses* 2012; **11**:1412-21.

[11] Richardson-Harman N, Hendrix CW, Bumpus NN, Mauck C, Cranston RD, Yang K, et al. Correlation between compartmental tenofovir concentrations and an ex vivo rectal biopsy model of tissue Infectibility in the RMP-02/MTN-006 Phase 1 study. *PLoS ONE* 2014; **9**:e111507.

[12] Anton PA, Saunders T, Elliott J, Khanukhova E, Dennis R, Adler A, et al. First Phase 1 double-blind, placebo-controlled, randomized rectal microbicide trial using UC781 gel with a novel index of ex vivo efficacy. *PLoS ONE* 2011; **6**:e23243.

[13] Dezzutti CS, Uranker K, Bunge KE, Richardson-Harman N, Macio I, Hillier SL. HIV-1 infection of female genital tract tissue for use in prevention studies. *J Acquir Immune Defic Syndr* 2013; **63**:548-54.

[14] Wei X, Decker JM, Liu H, Zhang Z, Arani RB, Kilby JM, et al. Emergence of resistant human immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy. *Antimicrob Agents Chemother* 2002; **46**:1896-905.

[15] Azijn H, Tirry I, Vingerhoets J, De Bethune MP, Kraus G, Boven K, et al. TMC278, a next-generation nonnucleoside reverse transcriptase inhibitor (NNRTI), active against wild-type and NNRTI-resistant HIV-1. *Antimicrob Agents Chemother* 2010; 54:718-27.

[16] Dezzutti CS, Else LJ, Yandura SE, Shetler C, Russo J, Back DJ, et al. Distinct pharmacodynamic activity of rilpivirine in ectocervical and colonic explant tissue. *Antimicrob Agents Chemother* 2016; **60**:2765-70.

[17] Gunawardana M, Remedios-Chan M, Miller CS, Fanter R, Yang F, Marzinke MA, et al. Pharmacokinetics of Long-acting Tenofovir Alafenamide (GS-7340) Subdermal Implant for HIV Prophylaxis. *Antimicrob Agents Chemother* 2015; **59**:3913-9.

**Figure Legends**

**Figure 1 Consort Flow Diagram.**

**Figure 2** **Female Pharmacokinetic Data.** RPV concentrations in female study participants **(a)** plasma, **(b)** plasma and fluids (vaginal, endocervical, and rectal), **(c)** vaginal, ectocervical, and rectal tissue. The solid line represents the geometric mean with error bars representing the 90% CI

**Figure 3** ***Ex vivo* / *in vitro* explant infection.** Tissue explants **(a)** rectal, **(b)** ectocervical, and **(c)** vaginal in rectal, cervical, and vaginal tissue were exposed to HIV-1BaL (105 TCID50 for rectal and 5 x 104 TCID50 for cervicovaginal tissue) and incubated for 14 days. A Day 14 cumulative HIV-1 p24 (weight adjusted) was calculated for each visit.

**Figure 4** **Pharmacokinetic/pharmacodynamic correlations between explant infection and RPV concentration**. Data are presented as Day 14 weight adjusted HIV-1 p24 in the explant supernatant (**A-C** rectal; **D-F** cervical, and **G-I** vaginal tissue).

**Supplemental Figure 1** **Male Pharmacokinetic Data.** RPV concentrations in male study participants **(a)** plasma, **(b)** plasma and fluids (vaginal, endocervical, and rectal), **(c)** vaginal, ectocervical, and rectal tissue. The solid line represents the geometric mean with error bars representing the 90% CI

**Supplemental Figure 2** **TZM-bl Infection Model.** Inhibition of TZM-bl infection following exposure to **(a)** rectal, **(b)** endocervical, or **(c)** vaginal fluid

**Supplemental Figure 3** **Concentration of RPV predicted to suppress 50% of *ex vivo* infection (EC50).** The degree to which explant infection is suppressed compared to baseline explant infection is plotted as a percentage (y axis) against the Log10 transformed concentration of RPV in rectal tissue or plasma (x axis).