Long-acting rilpivirine as potential pre-exposure prophylaxis for HIV-1 prevention (the MWRI-01 study): an open-label, phase 1, compartmental, pharmacokinetic and pharmacodynamic assessment

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Summary

Background Long-acting injectable antiretroviral agents are being developed for HIV-1 prevention. The MWRI-01 study was done to characterise the safety, acceptability, and pharmacokinetic and pharmacodynamic profile of long-acting rilpivirine.

Methods We did a phase 1 open-label study at the University of Pittsburgh. We enrolled healthy individuals (aged 18–45 years) who were seronegative for HIV-1. Participants were assigned alternately one intramuscular dose of either 1200 mg or 600 mg long-acting rilpivirine, beginning with the 1200 mg dose. We obtained plasma specimens, genital and rectal fluids, and tissue samples (rectal, cervical, and vaginal) before and after exposure to long-acting rilpivirine for assessment of pharmacokinetics and ex vivo biopsy challenge with HIV-1. Our primary objective was to characterise product safety, and the analysis included all enrolled participants. This trial is registered with ClinicalTrials.gov, number NCT01656018.

Findings 36 participants were enrolled into the study, of whom 24 were women and 12 men. 12 women and six men received each dose. 204 adverse events were reported among the 36 participants, of which 200 (98%) were grade 1–2. The most common adverse event was injection site reaction. All grade 3 and 4 adverse events were deemed not related to rilpivirine. Geometric mean (90% CI) concentrations in plasma of rilpivirine at day 28 post dose were 53 ng/mL (38–67) in women and 43 ng/mL (23–63) in men for the 1200 mg dose and 28 ng/mL (19–37) in women and 17 ng/mL (9–24) in men for the 600 mg dose. The tissue-to-plasma ratio for rilpivirine in rectal tissue was about two-fold higher than in vaginal and cervical tissue (1·10–1·53 vs 0·61–0·72 and 0·50–0·71, respectively). Exposure to long-acting rilpivirine suppressed viral replication significantly in rectal tissue (p<0·0001), and this suppression persisted for up to 4 months. By contrast, no viral suppression was seen in cervical or vaginal tissue.

Interpretation Ongoing research will characterise longer term safety and acceptability of multiple injections and help ascertain whether long-acting rilpivirine should advance to assessment of efficacy in preventing HIV-1 infection.

Funding Bill & Melinda Gates Foundation.

Introduction Long-acting injectable antiretroviral agents are being developed for the prevention and treatment of HIV-1 infection.12 Rilpivirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that is licensed worldwide for the treatment of chronic HIV-1 infection.1 NNRTIs are attractive agents for pre-exposure prophylaxis because they act early in the viral replication cycle, are potent antiretroviral agents, and unlike tenofovir do not need cellular metabolism to be active. In a study by Kovarova and colleagues,4 BLT humanised mice treated with long-acting rilpivirine were protected from infection when challenged vaginally with HIV-1_LAI.

In a series of phase 1 clinical trials of long-acting rilpivirine (alone or in combination with long-acting cabotegravir, an integrase inhibitor), the initial safety, acceptability, and pharmacokinetic profile of this formulation was established.3 Participants in one study (n=66) received single doses of 300–1200 mg long-acting rilpivirine followed by comprehensive sampling of plasma, rectal fluid, and cervical fluid and limited sampling of rectal and vaginal tissue.4 All doses were well tolerated and provided prolonged exposure to rilpivirine in plasma and the genital tract for 84 days. A significant correlation was recorded between the concentration of long-acting rilpivirine in cervicovaginal lavage fluid and the degree of HIV-1 inhibition.

The aim of the MWRI-01 study was to further characterise the safety and acceptability of long-acting rilpivirine, to provide compartmental pharmacokinetic data, and to ascertain whether exposure to long-acting rilpivirine protected biopsy specimens of rectal, cervical, and vaginal tissue from HIV-1 infection.

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Methods

Study design and participants
We did a phase 1, single-centre, open-label, exploratory, dose-ranging study of long-acting rilpivirine in men and women at the Magee-Womens Hospital Clinical and Translational Research Center, Pittsburgh, PA, USA. Participants were initially eligible for inclusion if they were aged 18–45 years, had a body-mass index of 18–35 kg/m², were HIV-1 seronegative, were not pregnant or breastfeeding, had a regular menstrual cycle, were aged 18–45 years, had a body-mass index of <10 g/dL, platelet count <100 000 platelets per μL, white-blood-cell count <2000 cells per μL or >15 000 cells per μL, serum creatinine >1·3 × the upper limit of normal [ULN], alanine aminotransferase and aspartate aminotransferase >2·5 × ULN, +1 glucose or +2 protein on urinalysis, or hepatitis B surface antigen [HBsAg] or hepatitis C virus [HCV] antibody positive). Full exclusion criteria are described in the study protocol (appendix pp 14–115).

Major exclusion criteria were: use of antiretroviral pre-exposure or post-exposure prophylaxis; unprotected insertive or receptive anal intercourse within 6 months of screening; non-therapeutic injection drug use; use of immunomodulatory drugs; use of heparin, warfarin, clopidogrel, any drugs likely to increase the risk of post-biopsy bleeding, rectally or vaginally administered drugs, rifampycin, anticonvulsants, dexamethasone, or St John’s Wort; history of or ECG showing a prolonged QT interval (QTc); abnormalities of the cervical, vaginal, or rectal mucosa that would be a contraindication to taking a biopsy specimen; symptoms at screening or laboratory test evidence of untreated rectal or reproductive tract infection; or a laboratory abnormality (ie, haemoglobin <10 g/dL, platelet count <100 000 platelets per μL, white-blood-cell count <2000 cells per μL or >15 000 cells per μL, serum creatinine >1·3 × the upper limit of normal [ULN], alanine aminotransferase and aspartate aminotransferase >2·5 × ULN, +1 glucose or +2 protein on urinalysis, or hepatitis B surface antigen [HBsAg] or hepatitis C virus [HCV] antibody positive). Full exclusion criteria are described in the study protocol (appendix pp 14–115).

The final protocol was approved by the institutional review board at the University of Pittsburgh. All study participants provided written informed consent in English.

Procedures
Long-acting rilpivirine was supplied in vials as a sterile aqueous nanosuspension formulation at a concentration of 300 mg/mL. Before use, the formulation was stored in a temperature-controlled environment (2–8°C). We allocated participants alternately to either 1200 mg or 600 mg of long-acting rilpivirine; the first female or male participant received the 1200 mg dose and thereafter we assigned doses alternately. We administered the dose as either one 2 mL (600 mg) or two 2 mL (1200 mg) intramuscular gluteal injections. The two 2 mL injections were given approximately 1 h apart. We did not use an oral lead-in for any participant.

After obtaining informed consent, we screened all participants for medical history and did a physical examination, including the rectum and vagina, and
laboratory tests to exclude haematological, renal, or hepatic abnormality (visit 1). At this screening visit, we also collected urine samples to exclude pregnancy and anorectal or cervicovaginal sexually transmitted infections and did a 12-lead ECG in triplicate. Participants who met inclusion criteria during visit 1 were enrolled into the study. At the enrolment visit (visit 2), we asked participants to complete a web-based behavioural questionnaire (computer-assisted self-interview), to gather demographic information and assess their history of HIV-1 sexual risk behaviours and experience with safer sex practices. We also did a symptom-directed physical examination and sample collection (urine, blood, and rectal and genital fluid) to exclude sexually transmitted infection, and a urine pregnancy test if indicated. We did flexible sigmoidoscopy to obtain ten rectal biopsy specimens at approximately 15 cm from the anal verge, as previously described.7–9 We also obtained two ectocervical and three vaginal biopsy samples from female participants. We used these biopsy specimens for pharmacokinetic and pharmacodynamic analyses (ex-vivo biopsy challenge). We then gave participants their assigned dose of long-acting rilpivirine. We collected blood samples and rectal and genital tract fluids 24 h (visit 3), 1 week (visit 4), and 2 weeks (visit 5) after administration of long-acting rilpivirine, and every 2 weeks thereafter until 16 weeks post dose (visits 6–12). We gathered rectal, cervical, and vaginal tissue samples by biopsy 4 weeks after administration of long-acting rilpivirine (visit 6) and every 4 weeks thereafter until 16 weeks post dose (visits 8, 10, and 12). We did another ECG 4 weeks after administration of long-acting rilpivirine (visit 6) and every 4 weeks thereafter until week 16 post dose (visits 8, 10, and 12). Study participants could elect to have two further visits (visits 12A and 12B), at which we collected additional blood samples and rectal and genital fluids, and rectal, cervical, and vaginal tissue, for pharmacokinetic and pharmacodynamic analyses, at 20 weeks and 24 weeks after administration of long-acting rilpivirine.

We graded emergent adverse events using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, version 1.0 (December, 2004) and addenda 1–3.10 The investigator of record at the trial site (visit 1) had to judge if adverse events were related to study drug. The investigator of record at the trial site (visit 1) had to judge if adverse events were related to study drug and anorectal or cervicovaginal sexually transmitted infection and to administer a physical examination and sample collection (urine, blood, and rectal and genital fluid) to exclude sexually transmitted infection, and a urine pregnancy test if indicated. We did flexible sigmoidoscopy to obtain ten rectal biopsy specimens at approximately 15 cm from the anal verge, as previously described.7–9 We also obtained two ectocervical and three vaginal biopsy samples from female participants. We used these biopsy specimens for pharmacokinetic and pharmacodynamic analyses (ex-vivo biopsy challenge). We then gave participants their assigned dose of long-acting rilpivirine. We collected blood samples and rectal and genital tract fluids 24 h (visit 3), 1 week (visit 4), and 2 weeks (visit 5) after administration of long-acting rilpivirine, and every 2 weeks thereafter until 16 weeks post dose (visits 6–12). We gathered rectal, cervical, and vaginal tissue samples by biopsy 4 weeks after administration of long-acting rilpivirine (visit 6) and every 4 weeks thereafter until 16 weeks post dose (visits 8, 10, and 12). We did another ECG 4 weeks after administration of long-acting rilpivirine (visit 6) and every 4 weeks thereafter until week 16 post dose (visits 8, 10, and 12). Study participants could elect to have two further visits (visits 12A and 12B), at which we collected additional blood samples and rectal and genital fluids, and rectal, cervical, and vaginal tissue, for pharmacokinetic and pharmacodynamic analyses, at 20 weeks and 24 weeks after administration of long-acting rilpivirine.

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Participants completed computer-assisted self-interviews about product acceptability at visits 3, 4, and 12 and rated acceptability on a scale from 1 to 10, with 1 representing less acceptable or unlikely to use in the future. Here, we report only product acceptability and factors that might affect product use in the future; a behavioural report is in preparation.

We used polyvinyl alcohol (PVA)-based sponges (Merocel; Medtronic, Mystic, CT, USA) to collect rectal fluid and polyester swabs (Dacron) to collect cervicovaginal fluid. The bioanalytical method used for pharmacokinetic analyses10 was fully validated for PVA-based sponges, in accordance with US Food and Drug Administration (FDA) bioanalytical guidelines. The analytical method used a mixture of hexane and ethyl acetate to extract rilpivirine from the PVA sponges. Extraction (or percentage recovery) of rilpivirine was acceptable (as per FDA guidelines). An organic solvent could not be used to extract rilpivirine from the polyester swabs for the TZM-bl assay because it would have lysed the cells; the polyester swab extraction process was non-validated. To each swab we added 400 μL phosphate buffered saline (PBS), which we left at room temperature for 5 min. We transferred the swab and fluid to a SpinX column (Oneonta, NY, USA) and centrifuged it at 10 000 rpm for 10 min. We made aliquots of the eluate and stored them at –80°C until testing.

We quantified concentrations of rilpivirine in plasma, fluids (rectal, endocervical, and vaginal), and tissue (rectal, ectocervical, and vaginal) by validated high-pressure liquid chromatography-mass spectrometry. Full methodological validation of this process has been described elsewhere.11 We initially quantified tissue homogenate and rectal, vaginal, and endocervical fluid samples using a ng per sample calibration curve, then converted to ng/mL by adjusting for the recorded tissue and fluid volumes. Concentrations that were below the assay lower limit of quantification (LLOQ) were expressed as half LLOQ values.

The target concentration for HIV-1 prevention is unknown, but for comparison we used the in-vitro EC50 (effective concentration predicted to suppress 90% infection) for wild-type HIV-1, corrected for protein binding, to yield a putative target concentration of 12–2 ng/mL.12

We stored biopsy samples of rectal, cervical, and vaginal tissue in 20 mL RPMI (with 1–125 μg/mL of amphotericin B and 0·5 mg/mL of piperacillin–tazobactam) and transported them within 30 min to the laboratory at the University of Pittsburgh for ex-vivo infection with a common viral stock of HIV-1BaL (105 TCID50 [median tissue culture infective dose] for rectal and 5×104 TCID50 for cervicovaginal tissue), as described previously.13–16 We adjusted results for initial biopsy weight and reported data as day 14 cumulative p24 (Alliance HIV-1 p24 antigen ELISA; Perkin-Elmer Life Sciences, Boston, MA, USA); the LLOQ for the assay was 10 pg/mL. Non-detectable cumulative p24 measures at day 14 were converted to half LLOQ before log transformation. We used TZM-bl cells (National Institute of Health AIDS Research and Reference Reagent Program, Germantown, MD, USA) to ascertain pharmacodynamic activity of mucosal fluid.17 Inhibition was ascertained on the basis of deviations from the HIV-1 only control and presented as the percentage inhibition.
Statistical analysis

In view of the small sample size, our phase 1 study had insufficient power to report any adverse events other than those occurring at high frequency. We assessed differences in baseline characteristics between participants receiving the 1200 mg and 600 mg doses with two-sample $t$ tests, $\chi^2$ tests, or their non-parametric counterparts. The proportion of participants having a grade 2 or worse adverse event was the primary safety endpoint and was compared between 1200 mg and 600 mg doses with Fisher's exact test. Acceptability ratings related to study drug were compared between 1200 mg and 600 mg dose groups and by sex over the duration of participation. We calculated the proportion (95% CI) of product acceptability for each dose group. We used Fisher's exact test to compare 1200 mg and 600 mg doses with respect to product acceptability.

Plasma and compartmental pharmacokinetic endpoints included the area under the concentration–time curve over 112 days (AUC$_{112}$), maximum concentration ($C_{\text{max}}$), time to maximum concentration ($T_{\text{max}}$), half-life ($t_{1/2}$), and concentrations measured at intervals of 4 weeks ($C_{28}$, $C_{56}$, $C_{84}$, and $C_{112}$). For male participants, we analysed pharmacokinetic data for plasma, rectal secretions, and rectal tissue; for female participants, we assessed values from plasma, endocervical and vaginal secretions, and cervical and vaginal tissue. Pharmacokinetic data for rectal secretions and rectal tissue were also available for some women who elected to have rectal samples collected. We calculated pharmacokinetic variables with non-compartmental analysis (WinNonlin Phoenix, version 6.1; Pharsight, Mountain View, CA, USA) and expressed data as geometric means with 90% CIs. We compared measures of drug exposure and ratios of compartment-to-systemic rilpivirine over 112 days between dose groups and analysed concentration differences.

Change in amount of HIV-1 p24 antigen in culture supernatant was the primary outcome in the biopsy studies, which were designed to assess potential pharmacodynamic activity as realised 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-1$_{BaL}$. Using a two-sided paired $t$ test with an $\alpha$ of 0·05, the study had 80% power to detect effect sizes as small as 0·89 in women in both dose groups and 1·43 in men for rectal tissue. We compared amount of p24 antigen between dose groups for each sex across visits by use of linear mixed models, with fixed effects for visit, dose, and their interaction, plus a random effect for participant. We correlated detectable pharmacokinetic drug concentrations and cumulative p24 antigen levels for measures taken from the same participants and at the same visits by use of a linear mixed effect model to test for a significant slope estimate for each pharmacokinetic/pharmacodynamic (PK/PD) pair. We further tested significant linear PK/PD relations using a non-linear E_max model, which was chosen by use of the Akaike information criterion. The upper and lower asymptotes were constrained to 100% and 0% virus control, respectively.

Role of the funding source

The funder 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 data in the study and had final responsibility for the decision to submit for publication.

Results

Between Jan 3, 2013, and Feb 17, 2014, 62 individuals were screened for the study and 36 were enrolled, 12 men and 24 women (figure 1). Six men and 12 women received a single 1200 mg dose of long-acting rilpivirine and the same numbers of participants received a single 600 mg dose. All 36 participants completed the final study visit (visit 12, 112 days post dose). Seven women completed two additional optional visits (visit 12A and 12B, 28 days and 56 days after the final visit, respectively) and two women completed visit 12A but not visit 12B. Although study participants were not randomly allocated a dose of rilpivirine, no significant demographic differences were noted between the two dose groups (table 1). Follow-up was completed on Aug 4, 2014.

Figure 1: Participant recruitment and follow-up

STI=sexually transmitted infection. UTI=urinary tract infection.

62 screened (18 men, 44 women)
26 screen failures
  6 withdrew before enrolment
  6 STI or UTI at screening
  4 excluded by the investigator
  3 ECG abnormality
  7 other
36 enrolled (12 men, 24 women)
18 received 1200 mg rilpivirine
  (6 men, 12 women)
18 received 600 mg rilpivirine
  (6 men, 12 women)
18 completed visit 12
  (6 men, 12 women)
18 completed visit 12
  (6 men, 12 women)
3 completed visit 12A
  (3 women)
6 completed visit 12A
  (6 women)
3 completed visit 12B
  (3 women)
4 completed visit 12B
  (4 women)
204 adverse events were reported in 36 participants (table 2). Adverse events of grade 2 or worse did not differ within sex cohorts between the 1200 mg and 600 mg dose (men, p=0·21; women, p=0·55; overall, p=0·086). Injection site reactions were the most frequent adverse event, which was reported in 32 of 36 participants (appendix pp 5, 6). The average duration of grade 1 and grade 2 injection site reactions was 2–3 days and 1–2 days, respectively, in all participants. 28 participants had a prolonged QT interval (appendix pp 5, 6). Two participants with grade 2 prolonged QT interval received the 1200 mg dose. One participant had a grade 1 skin rash that was deemed possibly related to product. The rash was noted at visit 4 and resolved spontaneously after 5 days. One woman who received 1200 mg long-acting rilpivirine had an exercise-related grade 3 and grade 4 increase in aspartate aminotransferase and a grade 4 increase in creatine kinase. A second woman receiving the 1200 mg dose had a grade 3 bleeding event after vaginal biopsy. No grade 3 or 4 adverse events were deemed related to rilpivirine.

On the behavioural questionnaires, participants reported acceptable levels of anxiety related to injections (mean score 6·6, SD 2·4). However, anxiety was significantly lower among women than men (p=0·04). Scores similarly suggested participants did not feel pain associated with the injection (mean 6·6, SD 1·98). These data, based on self-reported survey responses, differ from findings on injection site pain reported in the safety data, based on self-reported survey responses, differ from findings on injection site pain reported in the safety analysis. Behavioural data did not capture this difference.

When asked about uptake of the product (assuming very good protection), participants reported they would be likely to use this product (mean score 6·83, SD 2·21). When asked if they might use the product given a specific price, the likelihood of use decreased as the price increased. At US$25, the mean score was 6·29 (SD 2·31), at $50, the mean score fell to 3·76 (2·20). Of the suggested barriers to uptake were the ability to protect oneself without their partner knowing (mean score 4·06 [SD 1·97]), stigma associated with using an anti-HIV drug (3·18 [2·06]), and fear that the drug could be harmful to their health (6·30 [2·32]). Factors least likely to affect future use were the ability to protect oneself with a condom (7·19 [2·32]), and fear of needles (3·05 [2·32]). Product acceptability did not differ by dose or sex (data not shown).

Geometric mean (90% CI) concentrations in plasma of rilpivirine at day 28 after dose were 53 ng/mL (38–67) in women and 43 ng/mL (23–63) in men for the 1200 mg dose and 28 ng/mL (19–37) in women and 17 ng/mL (9–24) in men for the 600 mg dose. Rilpivirine persisted in plasma for up to 112 days in both men and women, and was detectable at 168 days after dose in the women who undertook additional visits, in both dose groups (geometric mean 5·8 ng/mL for the 600 mg dose and 13·3 ng/mL for the 1200 mg dose). Time to peak plasma concentration (Tmax) was variable and, in individual cases, could be delayed beyond the Tmax observed in population profiles, which are based on mean concentrations at each timepoint (figure 2A; appendix p 2). Rilpivirine exposures (AUCinf) in plasma did not differ significantly between men and women receiving an equivalent 600 mg and 1200 mg dose. Concentrations of rilpivirine in plasma greater than the EC90 (12·2 ng/mL) were reached for all participants, irrespective of dose or sex, at the time of the first pharmacokinetic sampling, 1 day after the dose (visit 3). After the 1200 mg dose, all women had concentrations of rilpivirine 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 proportion of women with a concentration in plasma greater than the EC90 fell below 100% by day 42 (92%), and all participants were below this target by 112 days post dose. In men, concentrations of rilpivirine in plasma were above the EC90 for up to 14 days (600 mg dose) and 84 days (1200 mg dose), and the proportion of male participants above target fell to 17% and 33% at 112 days after the dose.
In vaginal fluid, \( C_{\text{max}} \) was achieved slightly later than in plasma (figure 2B), suggesting a delay in drug distribution in the female genital tract. Rilpivirine exposures (AUC\(_{112}\)) in vaginal fluid were higher (600 mg dose, \( p=0.02 \); 1200 mg dose, \( p=0.18 \)) than corresponding plasma concentrations: vaginal fluid-to-plasma drug
ratios were 1·18–1·37 (appendix p 7). Concentrations in endocervical fluid were lower, with ratios of 0·69 for the 600 mg dose (p=0·08) and 0·77 for the 1200 mg dose (p=0·12). Both types of fluid were associated with plasma (vaginal fluid, $r^2=0·423$; endocervical fluid, $r^2=0·471$); vaginal and endocervical fluid were also highly correlated with each other ($r^2=0·760$). Concentrations of rilpivirine in rectal fluid from men and women were highly variable and substantially lower than those recorded in plasma (figure 2B; appendix pp 2, 7): rectal fluid-to-plasma ratios were 0·28–0·66, and no sex differences were observed in overall rectal fluid AUC (600 mg dose, p=0·23; 1200 mg dose, p=0·44). Unlike cervical and vaginal fluids, rectal fluid correlated weakly with corresponding concentrations of rilpivirine in plasma ($r^2=0·09$).

Concentrations of rilpivirine in vaginal and ectocervical tissue were significantly lower than in paired plasma (all comparisons p<0·0001): tissue-to-plasma ratios were 0·44–0·72 for vaginal tissue and 0·42–0·77 for ectocervical tissue for 112 days post dose. Concentrations of rilpivirine were highly correlated in plasma and vaginal tissue ($r^2=0·721$) and ectocervical tissue ($r^2=0·666$). Ten of 12 women in each dosing group opted to undergo sampling for rectal tissue. Concentrations of rilpivirine in rectal tissue from women exceeded amounts in plasma, with tissue-to-plasma ratios of 1·10–1·22 (600 mg dose, p=0·006; 1200 mg dose, p=0·03) and were approximately 1·5–2·5-fold higher than drug exposures in vaginal or cervical tissue (all comparisons p<0·0001). Concentrations of rilpivirine in rectal tissue were comparable in men and women (600 mg dose, p=0·57; 1200 mg dose, p=0·77) and were associated strongly with paired plasma concentrations (women, $r^2=0·795$; men, $r^2=0·812$).

A dose effect for change in amount of HIV-1 p24 antigen in rectal, vaginal, and cervical tissue after ex-vivo infection with HIV-1BaL and incubation for 14 days was noted for rectal tissue (p=0·04; figure 3A) but no significant effect of dose was recorded for either cervical tissue (p=0·55; figure 3B) or vaginal tissue (p=0·07; figure 3C). The amount of p24 antigen in rectal tissue increased significantly over time (p<0·0001), but no time effect was noted for ectocervical tissue (p=0·70) or vaginal tissue (p=0·12). Pairwise comparisons were done 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 noted at days 28–112 compared with baseline for both the 600 mg and 1200 mg doses; however, by days 140 and 168, amounts of p24 had reverted back to baseline levels for both doses (600 mg dose: visit 2 vs visit 6, p=0·0001; visit 2 vs visit 8, p=0·0023; visit 2 vs visit 10, p=0·05; visit 2 vs visit 12, p=0·39; 1200 mg dose: visit 2 vs visit 6, p=0·0001; visit 2 vs visit 8, p=0·0009; visit 2 vs visit 10, p=0·0001; visit 2 vs visit 12, p=0·12). Pairwise comparisons were done to compare rectal infection between doses for each visit; the only visits at which a significant difference in p24 infection was noted were between 1200 mg and 600 mg doses were visits 10 and 12 (days 140–168), when p24 infection levels were lower for the 1200 mg dose group (600 mg vs 1200 mg: visit 2, p=0·11; visit 6, p=0·066; visit 8, p=0·19; visit 10, p=0·004; visit 12, p=0·0025). Drug-related inhibition of HIV-1 was not shown for rectal, cervical, and vaginal fluid in the TZM-bl assay (appendix p 3), probably because of poor recovery of rilpivirine (which is hydrophobic) from the collection matrix.
For rectal PK/PD models, a significant negative slope value was recorded, supporting a finding of drug-mediated virus suppression (figure 4). No significant PK/PD relations were noted for cervical or vaginal models. The significant PK/PD linear effects seen for rectal tissues were investigated further with an Emax model. The EC50 for rectal tissue (1.1 log10 ng/mL) and plasma (0.92 log10 ng/mL) were similar in both compartments. Drug concentrations at or above 1.1 log10 ng/mL were mostly seen with the 1200 mg dose (appendix p 4). The rectal tissue EC90 of 1.96 log10 ng/mL (95% CI 1.83–2.09) and the plasma EC90 of 1.87 log 10 ng/mL (1.74–2.00) were higher than the in-vitro EC90 for wild-type HIV-1 (0.67 ng/mL), corrected for protein binding, to yield a putative target concentration of 1.09 log10 ng/mL. Only four (6%) of 67 rectal explant PK/PD pairs from the 1200 mg dose group, and none of those from the 600 mg dose group, had an EC90 of 1.96 log10 ng/mL or higher.

Discussion
Single-dose administration of long-acting rilpivirine was safe and well tolerated. Most adverse events in our study were grade 1, and no treatment-related grade 3 or 4 events were reported. The most frequent adverse event was injection site discomfort, which was mild or moderate, transient, and did not seem to diminish participants’ willingness to consider this form of HIV-1 prevention in the future. Although grade 1 prolongation of QT interval was fairly common, none of the values in the study exceeded the FDA definition of QT interval prolongation (450 ms for men and 470 ms for women).

The behavioural data we obtained suggested that participants were not dissuaded by fear of painful injections, or did not experience pain, and were highly likely to use the product. However, whether multiple injections will be equally acceptable to participants remains to be seen, and this question is being addressed.

Figure 4: Pharmacokinetic/pharmacodynamic correlations between explant infection and rilpivirine concentration
Plots show correlations of day 14 weight-adjusted HIV-1 p24 antigen in explant supernatant of (A–C) rectal, (D–F) cervical, and (G–I) vaginal tissue with the respective rilpivirine concentrations in these compartments.
in the phase 2 HPTN-076 study (NCT02165202), in which participants will receive 1200 mg doses every 8 weeks at six consecutive dosing visits.

After one injection of long-acting rilpivirine, maximum concentrations in plasma were achieved by 9 days, and the drug was still detected in samples collected at visit 12B (day 168 post dose). Amounts of rilpivirine in tissue were variable but, in general, rectal tissue-to-plasma ratios were greater than 1·0 and cervicovaginal tissue-to-plasma ratios were less than 1·0. Concentrations of rilpivirine in rectal tissue were 1·5–2·5-fold higher than those in cervical or vaginal tissue. These data confirm rilpivirine as a long-acting formulation but raise questions about the compartmental pharmacokinetic profile and whether there might be differential levels of protection associated with rectal and vaginal transmission of HIV-1 infection. The presence of subtherapeutic amounts of rilpivirine during an extended pharmacological tail, at the end of a dosing regimen, might lead to selection of NNRTI class resistance if the individual becomes infected with HIV-1.

Ex-vivo infection rates were lower in rectal tissue than in vaginal and cervical tissue after both 600 mg and 1200 mg single doses, showing evidence of drug-mediated virus suppression, and this level was sustained to 112 days post dose. No significant reductions in virus infection were noted in either cervical or vaginal tissue, even though drug concentrations were detectable in both compartments. The ex-vivo TZM-bl assays did not provide any evidence of antiviral activity in either compartment because of high levels of endogenous antiviral activity in rectal fluid and, probably, failure to elute rilpivirine from the PVA sponges. The divergent results from the rectal and cervicovaginal biopsy models are intriguing and might reflect differences in the models or differential pharmacokinetic requirements to suppress tissue infection in either compartment. A key consideration is whether these data might predict differential outcomes in populations at risk of HIV-1 infection through vaginal or rectal intercourse.

Evidence of drug-mediated virus suppression was recorded when concentrations of rilpivirine in rectal tissue, plasma, and rectal fluid were correlated with infectivity in rectal tissue. When HIV-1 infectivity was compared with baseline (visit 2), a non-linear model established that approximately 12·6 ng/mL (1·1 log₃ ng/mL) of rilpivirine in both rectal tissue and plasma compartments predicted 50% suppression in virus growth, which predominately occurred after the 1200 mg dose. The in-vivo target range for HIV-1 prevention is uncertain, but extrapolating from these biopsy data suggests that protective concentrations could be achieved in the rectal compartment with 1200 mg injections of long-acting rilpivirine every 2 months.

One limitation of both long-acting cabotegravir and long-acting rilpivirine is that once the products have been injected they cannot be removed and, therefore, any idiosyncratic adverse events associated with their use will be difficult to manage. Some trials currently assessing these products are using an oral run-in period to identify individuals who might be vulnerable to product-related adverse events. Data from our study suggest that product-associated adverse events are generally mild and question whether an oral run-in is necessary. Alternatively, interest is now increasing in developing implantable devices that could be removed should an important adverse event occur. Ideally, these products would gradually release the antiretroviral drug over a period of months, if not years.

A strength of our study was the longitudinal multi-compartmental sample collection to characterise the PK/PD profile of long-acting rilpivirine over an extended period. Despite the intensity and duration of sample collection, all participants completed the study. Limitations of our study include that it was an open-label trial and, thus, we were unable to establish whether adverse events such as injection site pain were related to the method of product administration or to the actual product. Moreover, in-vitro susceptibility targets might not reflect accurately the situation in vivo, particularly in anatomical sites of transmission, such as the genital tract and rectum, in which the protein content and drug binding are uncharacterised. The explant challenge model is a stringent test of product pharmacodynamics, and protective concentrations in efficacy trials might be less than those needed in the explant model.

The findings of our phase 1 study indicate that single-dose administration of long-acting rilpivirine is safe and acceptable. Moreover, exposure to single-dose rilpivirine is associated with prolonged pharmacokinetics and viral suppression in colorectal, but not cervicovaginal, tissue. Ongoing research, including the phase 2 HPTN-076 study in women from the USA, South Africa, and Zimbabwe, will characterise longer term safety and acceptability of multiple injections and help ascertain whether long-acting rilpivirine should advance to assessment of efficacy in preventing HIV-1 infection.

Contributors
IM designed the study protocol with assistance from RDC, AA, KKR, and KA. BC and SA collected female genital tract samples. Laboratory assays were run by AS, JE, AN, KD, and CS and were overseen by CSD and RMB. DB, LE, DE, and SK were responsible for analysis of all pharmacokinetic samples. RS and JEE designed, conducted, and analysed the behavioural component of the study. KA and NR-H analysed and interpreted the data. PEW provided access and regulatory support for the provision of long-acting rilpivirine. IM wrote the report. All authors have read and commented on the final report.

Declaration of interests
IM has served on advisory boards for Novocil Life Sciences and ABIVAX and has received research grants from ViIV Healthcare and Janssen. SK has received research grants and speaker’s honoraria from Merck, Gilead Sciences, and ViIV Healthcare. CSD has served on an advisory board for Pfizer. DB has served on speaker bureaus or advisory boards and has received educational grants from Gilead Sciences, Janssen, ViIV Healthcare, Merck, Bristol-Myers Squibb, and AbbVie. NR-H is an employee of Alpha StatConsult. PEW is a full-time employee of Janssen. All other authors declare no competing interests.
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