Original article

HIV-1 genital shedding in HIV-infected patients randomized to second-line lopinavir/ritonavir monotherapy versus tenofovir/lamivudine/lopinavir/ritonavir

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Background: HIV-1 shedding in genital secretions is associated with HIV transmission risk. Limited data exist on the effect of second-line lopinavir/ritonavir monotherapy (mLPV/r) on genital secretion of HIV RNA.
Methods: We measured HIV-1 in genital secretions of HIV-infected adults at time of failure from non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens and at 48 weeks after being randomized to second-line mLPV/r versus tenofovir/lamivudine/LPV/r (TDF/3TC/LPV/r). Plasma and genital secretion (semen, vaginal swab) HIV RNA was quantified by the CobasAmpliprep/TaqMan assay.
Results: Forty enrolled (15 on mLPV/r and 25 on TDF/3TC/LPV/r). Median age was 37.8 years and 35% were male. Median baseline CD4$^+$ T-cell count was 222 cells/mm^3, plasma HIV RNA was 4.1 $\log_{10}$ copies/ml and genital secretion HIV RNA was 2.3 $\log_{10}$ copies/ml. At week 48, the proportion of patients with plasma HIV RNA<50 copies/ml was 13/15 (87%) in mLPV/r and 21/25 (84%) in TDF/3TC/LPV/r arms. Median genital HIV RNA was significantly decreased from baseline in both arms (P=0.009 in mLPV/r and P=0.001 in TDF/3TC/LPV/r). In subjects with suppressed plasma HIV RNA, 12/34 (35%; 6/13 [46%] in the mLPV/r and 6/21 [29%] in the TDF/3TC/LPV/r arms) had detectable HIV RNA (range 74–957 copies/ml) in the genital secretions (P=0.41). By multivariate analysis, the only predictor of having genital HIV RNA>50 copies/ml at week 48 was baseline genital secretion HIV RNA>50 copies/ml (P=0.049).
Conclusions: LPV/r either given alone or in combination with TDF/3TC as second-line treatment achieved high genital secretion HIV RNA suppression rate. Genital secretion HIV RNA remained detectable at low levels in one-third of patients with suppressed plasma viraemia.

Introduction

HAART does not lead to total viral eradication, as HIV-1 remains in sanctuary sites such as the central nervous system and the genital compartments. Most nucleoside reverse transcriptase inhibitors (NRTIs) do achieve acceptable or even high drug levels in different tissue compartments, however, protease inhibitors (PI) achieve very low drug levels in the genital tract [1,2]. Possible consequences of limited drug penetration in the genital compartment are suboptimal viral suppression and development of drug-resistant variants [3]. In addition, the high levels of HIV-1 in genital secretions are likely to play an important role in HIV transmission risk [4–6].

In the past few years, several studies of boosted PI monotherapy (mono-bPI) had been reported [7,8]. Mono-bPI has the theoretical advantages of regimen simplification, improved adherence and avoidance of long-term toxicity associated from NRTIs. Lopinavir/ritonavir monotherapy (mLPV/r) has been the most investigated because of its co-formulation with ritonavir and its high genetic barrier to resistance [8]. One major concern about using mLPV/r is its limited penetration to the genital compartment [9].

Mono-bPI in adults has been shown to be effective and safe [8,10]. However, most of mono-bPI studies enrolled virologically suppressed patients and have demonstrated efficacy for maintaining viral suppression in the blood [7,8]. In contrast, our team had conducted a randomized study of mLPV/r versus LPV/r-based HAART in patients who were failing first-line non-nucleoside reverse transcriptase inhibitor (NNRTI) therapy. In this HIV STAR (The HIV Second-line Therapy Anti-Retroviral) study, we found that more patients on mLPV/r had low level plasma viraemia than those treated with tenofovir (TDF), lamivudine (3TC) and LPV/r [11]. Here, we report the results of the genital substudy of HIV STAR in which we compare the HIV RNA in the genital compartment between arms.

Methods

This is a substudy of the HIV STAR study (clinical trial.gov identification number NCT00627055) [11]. From May 2008 to November 2010, Thai adults failing NNRTI-based regimens from five hospitals were enrolled. HIV-infected adults aged ≥18 years who had been treated with NNRTI-based HAART for at least 6 months and had HIV RNA≥1,000 copies/ml without active opportunistic infections were included. The protocol was approved by the Thai Ministry of Public Health and local ethics committees. All subjects gave informed consent.

At enrolment, subjects were randomized to mLPV/r versus TDF/3TC/LPV/r. The dosages were LPV/r 400 mg/100 mg orally every 12 h, TDF 300 mg orally every 24 h and 3TC 150 mg orally every 12 h or 3TC 300 mg orally every 24 h. The formulations of LPV/r were soft gel capsules LPV/r (Kaletra®, 133/33 mg; Abbott Laboratories, Abbott Park, IL, USA) and/or generic LPV/r 200/50 mg tablet (Government Pharmaceutical Organization, Bangkok, Thailand). CD4%, $CD4^+$ T-cell count and plasma HIV RNA were assessed at baseline then every 12 weeks until week 48. Plasma HIV RNA levels were measured centrally at the College of American Pathologists accredited HIV-NAT laboratory in Bangkok, Thailand by the Cobas Ampliprep/Cobas AMPLICOR HIV-1 Ultrasensitive test, version 1.5 (Roche Molecular Systems, Inc., Branchburg, NJ, USA) with a level of detection of 50 copies/ml.

Genital secretion collection and genital HIV RNA measurement

This was an optional study for subjects in the HIV STAR study. Those who accepted to participate in this substudy underwent a genital samples collection at baseline and week 48.

Male participants were asked to refrain from ejaculation for at least 3 days before the date of semen collection. Semen samples were self-collected by masturbation and ejaculation into an empty sterile container, and processed within 4 h. After centrifugation (1,500 g for 10 min), the seminal plasma (supernatant) was carefully removed and aliquotted into 0.6 ml per aliquot tube and frozen at -75°C.

Female participants were asked to refrain from vaginal sexual intercourse for at least 3 days before sample collection. Women who were menstruating on the day of vaginal swab collection were rescheduled. Vaginal swabs were collected by trained staff during a pelvic exam by gently rolling a plastic-handled Dacron swab against the lateral fornix of vagina for one rotation. The Dacron swabs (tips) were placed in a sterile Eppendorf tube and frozen at -75°C. Vaginal swabs were then thawed and carefully mixed for 3–5 min in a mixture of 1 ml normal human plasma. The 1 ml of eluted vaginal secretion was then centrifuged and processed as described for the seminal plasma samples. HIV RNA levels in semen/vaginal secretions were measured at HIV-NAT using the Cobas Ampliprep/Cobas TaqMan HIV-1 test, version 1.0 (Roche Molecular Systems) with 1 ml of genital sample per analysis. The level of detection was 40 copies/ml. Because the volume of vaginal swab fluid may differ between patients, the HIV RNA level in vaginal secretion was reported as copies/ml of vaginal swab extract.

Statistical procedures

Descriptive statistics (means, standard deviations and percentages) were used to summarize demographic and clinical characteristics of patients. The non-parametric Wilcoxon rank-sum test was used to compare continuous variables between treatment arms. The Wilcoxon signed rank test was used to compare continuous variables between baseline and week 48. The χ^2 test was used with categorical variables. Univariate and multivariate logistic regression analyses were performed to identify predictive factors of genital HIV RNA>50 copies/ml. Covariates tested in univariate models included age, gender, HIV transmission route, Centers for Disease Control and Prevention (CDC) clinical classification, time on NNRTI, nadir $CD4^+$ T-cell count, $CD4^+$ T-cell count, plasma HIV RNA, genital HIV RNA, history

of having a sexually transmitted disease (STD) in the past year and number of partners. All variables associated with genital HIV RNA>50 copies/ml at the level of $P<0.10$ in the univariate analysis were used to build the multivariate models. *P*-value less than 0.05 was considered statistically significant. All analyses were conducted using Stata version 11.2 (Stata Corp., College Station, TX, USA).

Results

Of 200 adults in the main HIV STAR study, 40 elected to enrol in this genital compartment substudy with participation from 15 of 100 adults in the mLPV/r arm and 25 of 100 in the TDF/3TC/LPV/r arm. The median age was 37.8 years. Baseline CDC clinical classification A:B:C was 20:38:42%. Median $CD4^+$ T-cell count and plasma HIV RNA were 222 cells/mm^3 and 4.1 $\log_{10}$ copies/ml, respectively. The median (IQR) duration of the first-line NNRTI-based HAART was 2.6 years. The other characteristics are shown in Table 1. There was no statistical difference of baseline characteristics between treatment arms. By gender, the only significant difference was for the lower median body weight in males who were randomized to mLPVr versus those in the TDF/3TC/LPV/r arm (57.6 [54.2–62] versus 67.3 [65.5–70.7] kg; $P=0.03$). Prior to switching to PI, the median genital secretion HIV RNA was 2.3 $\log_{10}$ copies/ml (2.1 $\log_{10}$ copies/ml in

Table 1. Baseline characteristics

Characteristics	*n*	All (*n*=40)	mLPV/r (*n*=15)	TDF/3TC/LPV/r (*n*=25)	*P*-value
Median age, years (IQR)	40	37.8 (34.5–41.4)	37.9 (33.9–46.5)	37.7 (35.6–41.0)	0.856
Male, *n* (%)	40	14 (35)	6 (40)	8 (32)	0.608
Educational level	39				0.945
No certificate or primary school, *n* (%)		15 (38)	6 (40)	9 (36)	
Less than high school, *n* (%)		13 (33)	5 (33)	8 (32)	
High school or higher, *n* (%)		7 (18)	3 (20)	4 (16)	
Bachelor degree or higher, *n* (%)		4 (10)	1 (7)	3 (12)	
Missing, *n* (%)		1 (3)	0	1 (4)	
Monthly income	36				0.112
≤150 USD, *n* (%)		17 (43)	4 (27)	13 (52)	
151–450 USD, *n* (%)		14 (35)	8 (53)	6 (24)	
≥451 USD, *n* (%)		5 (13)	3 (20)	2 (8)	
Missing, *n* (%)		4 (10)	0	4 (16)	
HIV transmission route	40				0.586
Heterosexual, *n* (%)		36 (90)	14 (93)	22 (88)	
Homosexual, *n* (%)		4 (10)	1 (6.7)	3 (12)	
Median weight, kg (IQR)	40	55.4 (48.4–66.6)	56 (53–62)	55.1 (47.1–67.5)	0.922
Median height, cm (IQR)	40	161.5 (155–165.5)	160 (155–167)	163 (155–165)	0.866
CDC clinical classification A:B:C, %	40	20:38:42	13:40:47	24:36:40	0.715
Nevirapine:efavirenz, *n* (%)	40	34 (85):6 (15)	15 (100):0	19 (76):6 (24)	0.067
Median duration of NNRTIs, years (IQR)	40	2.6 (1.2–4.2)	2.9 (1.8–4.7)	2.2 (1.05–4.0)	0.167
Median $CD4^+$ T-cell count nadir, cells/mm^3 (IQR)	37	68 (12–150)	100 (30–152)	35.5 (9–133)	0.258
Median $CD4^+$ T-cell count before switch to PI regimen, cells/mm^3 (IQR)	40	222 (118–292)	196 (143–279)	230 (106–292)	0.944
Median plasma HIV RNA $\log_{10}$ copies/ml before switching to PI regimen (IQR)	40	4.1 (3.8–4.6)	4.4 (3.6–4.6)	4.1 (3.9–4.7)	0.922
Median genital HIV RNA $\log_{10}$ copies/ml before switching to PI regimen (IQR)	40	2.3 (1.6–2.7)	2.5 (1.9–2.7)	2.1 (1.6–2.8)	0.479
History of ever being diagnosed with other sexually transmitted diseases, *n* (%)	31	6 (19)	2 (18)	4 (20)	0.90
Gonorrhoea, *n* (%)		2 (6.5)	0	2 (10)	
Warts, *n* (%)		1 (3.2)	0	1 (5)	
Herpes simplex, *n* (%)		3 (9.7)	2 (18)	1 (5)	
Lifetime sexual partners	31				0.87
1, *n* (%)		5 (16)	2 (18)	3 (15)	
2–5, *n* (%)		16 (52)	5 (45)	11 (55)	
>5, *n* (%)		10 (32)	4 (37)	6 (30)	

mLPV/r, lopinavir/ritonavir monotherapy; PI, protease inhibitor; TDF/3TC/LPV/r, tenofovir/lamivudine/lopinavir/ritonavir.

females and 2.6 $\log_{10}$ copies/ml in males). There was no difference of baseline semen HIV RNA between treatment arms (P=0.6) and baseline vaginal swab extract HIV RNA between treatment arms (P=0.8).

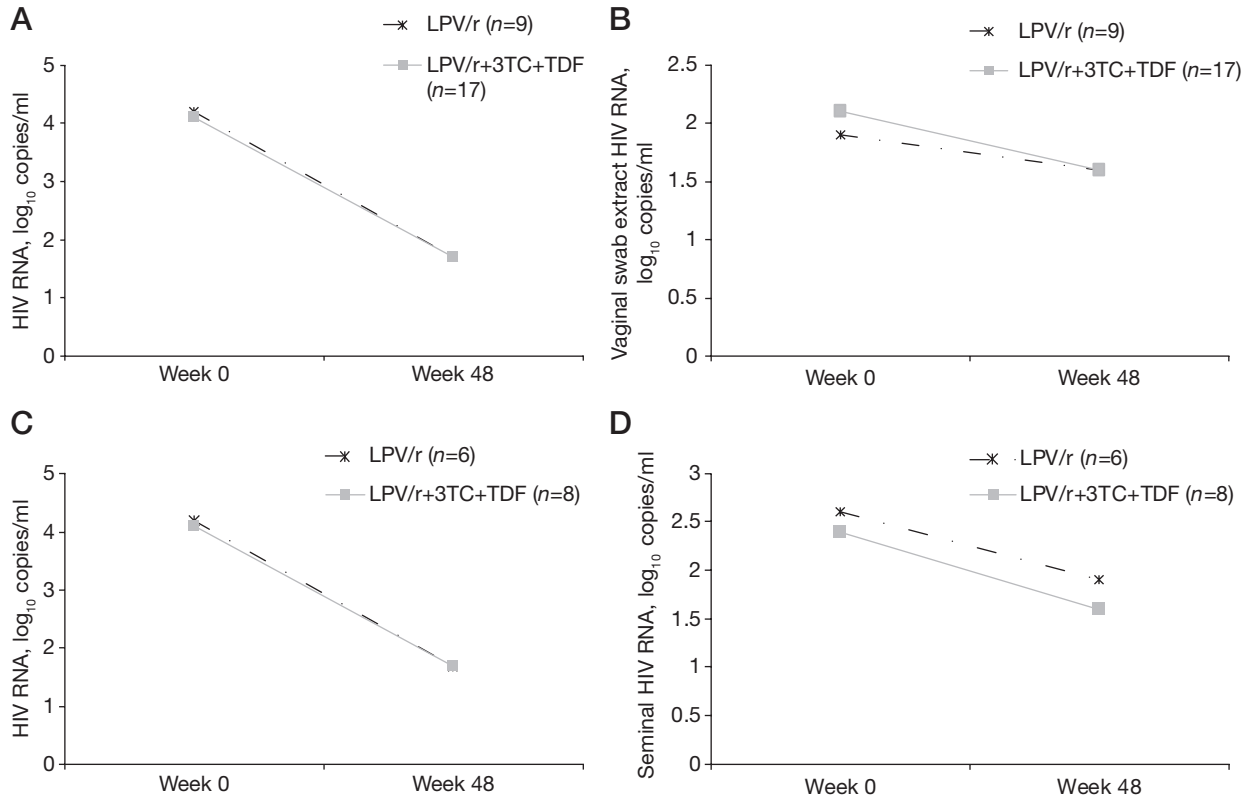
During the 48 weeks of follow-up, no death or loss to follow-up was reported. At week 48, the mean (SD) $CD4^+$ T-cell change was 129 (111) cells/mm^3 without a significant difference between arms (P=0.93). The proportion of patients who had plasma HIV RNA<50 copies/ml was 13/15 (87%) in the mLPV/r and 21/25 (84%) in the TDF/3TC/LPV/r arms (P=0.82). For the genital secretions, 9/15 (60%) had genital HIV RNA<50 copies/ml in the mLPV/r arm and this was 15/25 (60%) in the TDF/3TC/LPV/r arm (P=1.00).

Figure 1 compares the median HIV RNA after 48 weeks of treatment between randomized arms for plasma and for genital secretions by gender (Figure 1A and 1B for females, Figure 1C and 1D for males, respectively); there were no differences of median plasma HIV RNA and genital HIV RNA at week 48 between treatment arms (all P>0.05).

In Table 2, we compare the changes in HIV RNA from baseline to week 48 within each treatment arm as a whole group and by gender. There was a significant decline in plasma HIV RNA at week 48 for both regimens and for all subgroups (P<0.05). For the genital secretion HIV RNA, similar declines after treatment were observed for the whole group (P=0.009 for mLPV/r and P=0.001 for TDF/3TC/LPV/r). However, females on mLPV/r did not have an appreciable difference in vaginal swab extract HIV RNA between baseline and week 48 (median 1.9 versus 1.6 $\log_{10}$ copies/ml, respectively; P=0.18), whereas the females on TDF/3TC/LPV/r did (median 2.1 versus 1.6 $\log_{10}$ copies/ml, respectively; P=0.001). Yet, the opposite is observed for males where there was significant decline in seminal plasma HIV RNA with mLPV/r (median 2.6 versus 1.9 $\log_{10}$ copies/ml at weeks 0 and 48 respectively; P=0.01), but not with TDF/3TC/LPV/r (median 2.4 versus 1.6 $\log_{10}$ copies/ml respectively; P=0.10).

We looked for correlations between plasma and genital secretions HIV RNA, and saw a weak correlation at baseline (0.36, 95% CI -0.02, 0.74; P=0.06) and

Figure 1. Comparison of plasma and genital compartment HIV RNA between treatment arms at baseline and week 48 after treatment



(A) Plasma HIV RNA for female participants. **(B)** HIV RNA of vaginal swab extract. **(C)** Plasma HIV RNA for male participants. **(D)** Seminal HIV RNA for male participants. P-value >0.05 at both weeks 0 and 48 for all panels. Vaginal swab extract (female) and semen (male) samples were used for HIV RNA testing. LPV/r, lopinavir/ritonavir; LPV/r+3TC+TDF, lopinavir/ritonavir/lamivudine/tenofovir.

Table 2. Comparison of plasma HIV RNA and genital HIV RNA between baseline and week 48 within each treatment arm

	All participants (*n*=40)			Female (*n*=26)			Male (*n*=14)		
	Baseline	Week 48	*P*-value	Baseline	Week 48	*P*-value	Baseline	Week 48	*P*-value
mLPV/r	*n*=15			*n*=9			*n*=6		
Median plasma HIV RNA log_{10} copies/ml (IQR; min-max)	4.4 (3.6–4.6; 3.2-5.1)	1.7 (1.7–1.7; 1.6-1.8)	0.001	4.4 (3.6–4.6; 3.2-5.1)	1.7 (1.7–1.7; 1.60-1.8)	<0.01	4.2 (3.9–4.6; 3.3-4.9)	1.7 (1.7–1.7; 1.6-1.7)	<0.001
Subjects with plasma HIV RNA<50 copies/ml, *n* (%)	0	13 (87)	–	0	7 (78)	–	0	6 (100)	–
Median genital HIV RNA log_{10} copies/ml (IQR; min-max)	2.5 (1.9–2.7; 1.6-3.9)	1.6 (1.6–2.1; 1.6-3.0)	0.009	1.9 (1.6–2.5; 1.6-3.3)	1.6 (1.6–2.1; 1.6-2.4)	0.18	2.6 (2.5–3.9; 1.9-3.9)	1.9 (1.6–2.8; 1.6-3.0)	0.01
Subjects with genital HIV RNA≥50 copies/ml, *n* (%)	12 (80)	6 (40)	0.034	6 (67)	3 (33)	0.18	6 (100)	3 (50)	0.37
Subjects with plasma HIV RNA <50 copies/ml and genital HIV RNA≥50 copies/ml, *n*/total *n* (%)	0	6/13 (46)	–	0	3/7 (43)	–	0	3/6 (50)	–
TDF/3TC/LPV/r	*n*=25			*n*=17			*n*=8		
Median plasma HIV RNA log_{10} copies/ml (IQR; min-max)	4.1 (3.9–4.7; 3.10-5)	1.7 (1.7–1.7; 1.60-5)	<0.001	4.1 (3.7–4.8; 3.3-5)	1.7 (1.6–1.7; 1.6-4.8)	<0.01	4.1 (3.9–4.5; 3.1-5)	1.7 (1.6–1.7; 1.6-5)	0.003
Subjects with plasma HIV RNA<50 copies/ml, *n* (%)	0	21 (84)	–	0	14 (82)	–	0	7 (88)	–
Median genital HIV RNA log_{10} copies/ml (IQR; min-max)	2.1 (1.6–2.8; 1.6-4.6)	1.6 (1.6–1.9; 1.6-4.5)	0.001	2.1 (1.6–2.5; 1.6-3.6)	1.6 (1.6–1.9; 1.6-2.6)	0.001	2.4 (1.6–3.3; 1.6-4.7)	1.6 (1.6–1.9; 1.6-4.5)	0.10
Subjects with genital HIV RNA≥50 copies/ml, *n* (%)	15 (60)	10 (40)	0.059	11 (65)	7 (41)	0.045	4 (50)	3 (38)	0.57
Subjects with plasma HIV RNA <50 copies/ml and genital HIV RNA≥50 copies/ml, *n*/total *n* (%)	0	6/21 (29)	–	0	4/14 (29)	–	0	2/7(29)	–

Vaginal swab extracts were used to measure genital HIV RNA in female participants. mLPV/r, lopinavir/ritonavir monotherapy; TDF/3TC/LPV/r, tenofovir/lamivudine/lopinavir/ritonavir.

no correlation at week 48 (0.77, 95% CI 0.26, 1.27; *P*=0.004). When the vaginal swab extract and semen were analysed separately, the only correlation found was between plasma and seminal plasma HIV RNA at week 48 (coefficient 0.84, 95% CI 0.36, 1.33; *P*=0.002).

We further investigated the discordances between HIV RNA suppression in the peripheral blood and genital compartments, and observed 12 of 34 (35%) of the participants with suppressed plasma HIV RNA having detectable HIV RNA in the genital secretions; 6/13 (46%) in mLPV/r and 6/21 (29%) in TDF/3TC/LPV/r arms at week 48 (*P*=0.41). Table 3 provides details of these 12 participants with discordant results with similar proportions of females and males. At baseline, all had higher HIV RNA levels in the plasma than in the genital secretions. At week 48, in the presence of suppressed plasma viraemia, all had detectable genital secretion HIV RNA but the levels were below 1,000 copies/ml (ranges were 113–957 copies/ml in the mLPV/r arm and 59–379 copies/ml in the TDF/3TC/LPV/r arm). No one reported abnormal symptoms consistent with a concurrent STD or had an abnormal genital examination. The only predictor for having detectable genital secretion HIV RNA despite undetectable plasma HIV RNA by multivariate analysis was having genital secretion HIV RNA>50 copies/ml at baseline (odds ratio 5.83, 95% CI 1.01, 33.64; *P*=0.049). Age, gender, transmission route, monthly income, CDC clinical classification, time on failing regimen, history of STD, number of lifetime sexual partners, treatment arm, nadir $CD4^+$ T-cell count, and $CD4^+$ T-cell count and HIV RNA log_{10} at baseline and week 48, were not predictors.

Discussion

In patients who were randomized to second-line mLPV/r versus TDF/3TC/LPV/r, our study observed no differences in plasma and genital secretion HIV RNA suppression between arms at 48 weeks. We documented one-third of patients having detectable genital secretion HIV RNA despite achieving HIV RNA suppression in the peripheral blood. These results illustrated two important points. First, although the triple therapy arm has a theoretical benefit of superior tissue penetration, there was no significant difference in genital shedding in both arms. Second, subjects in both arms experienced discordant HIV RNA results between the genital and peripheral blood compartments. The level of detectable

Table 3. List of participants who had plasma HIV RNA<50 copies/ml but genital HIV RNA>50 copies/ml at week 48

Participant number/regimen	Gender	Baseline		Week 48	
		Genital	Plasma	Genital	Plasma
mLPV/r					
079-001-004	Male	7,820	79,600	957	<50
079-001-014	Male	7,169	43,700	136	<50
079-004-010	Female	362	3,620	158	<50
079-004-017	Female	335	29,700	113	<40
079-006-004	Male	534	1,980	684	<50
079-011-002	Female	40	11,400	252	<50
TDF/3TC/LPV/r					
079-004-005	Male	1,528	30,500	125	<50
079-004-008	Female	676	100,000	59	<50
079-004-018	Female	134	9,540	153	<40
079-010-025	Female	4,409	13,700	379	<50
079-010-042	Male	40	9,490	74	<40
079-011-001	Female	434	11,800	166	<50

Vaginal swab extracts were used to measure genital HIV RNA in female participants. mLPV/r, lopinavir/ritonavir monotherapy; TDF/3TC/LPV/r, tenofovir/lamivudine/lopinavir/ritonavir.

genital HIV RNA was low as 10 of 12 had genital HIV RNA<400 copies/ml.

To our knowledge, our study is the first to evaluate the impact of second-line mLPV/r on HIV-1 quantification in NNRTI-based HAART-failing patients. In recent years, such studies have mainly been in virologically controlled or in HAART-naive patients. PI has a lesser capacity than other antiretroviral drug classes to penetrate the genital tracts [12–14]. The presence of detectable HIV RNA in the genital compartment despite suppressed plasma viraemia is documented, with rates varying between studies, but in general, the viraemia is of low levels [15–17]. In our study, one-third of patients still had low levels of genital HIV RNA shedding with successful therapy. Gutmann *et al*. [18] reported no marked elevation of genital secretion HIV RNA in pretreated with fully suppressed viral load patients randomized to mLPV/r compared with continuing triple therapy; detectable genital secretion HIV RNA was seen in 1 of 34 with mLPV/r and 1 of 37 with triple therapy. Ghosn *et al*. [13] reported no detectable seminal HIV RNA in men treated with either mLPV/r or LPV/r-based HAART despite undetectable LPV/r levels in that compartment. Swindells *et al*. [19] reported undetectable HIV RNA in all semen samples of 8 virologically controlled patients after switching to boosted atazanavir monotherapy for 24 weeks. Vernazza *et al*. [15] reported detectable semen HIV RNA in 2 of 15 (13%) virologically controlled patients after switching to boosted atazanavir monotherapy. Lambert-Niclot *et al*. [16] reported 1 of 23 (4%) virologically controlled patients using darunavir/ritonavir monotherapy for one year had seminal HIV RNA of 270 copies/ml.

The implication of having a low but detectable genital secretion HIV RNA on HIV transmission is unclear and, so far, no threshold for transmission exists. Higher genital HIV-1 RNA concentration however, has been documented as an independent risk for HIV transmission risk in African HIV-1 serodiscordant couples [6]. From previous reports, 33–37% of HIV-infected women with undetectable plasma HIV RNA after receiving potent antiretroviral therapy for 36 weeks had genital HIV RNA shedding [20,21]. Politch *et al*. [17] reported that 25% of 83 HIV-infected men who have sex with men with undetectable plasma HIV RNA still had detectable HIV in semen ranging from 80–2,560 copies/ml. Venkatesh *et al*. [22] reported that 11% of virologically suppressed women had detectable HIV-1 in the genital secretion after changing to second-line HAART. The HPTN 052 study had conclusively shown that early and successful HAART as defined by having plasma viraemic suppression can almost eliminate HIV transmission in discordant heterosexual couples [23]. Strong epidemiological evidence also supports lack of transmission under HAART even in the absence of confirmed viral load suppression and lack of increased transmission risk during episodes of STDs while on suppressive HAART [24,25]. There are, however, reports of risk disinhibiting among patients on suppressive therapy [26]. With the possibility of low level viraemia in the genital secretions, consistent condom use even in patients on successful HAART whose partner is uninfected or do not know their status should be recommended [27].

In our study, there is no difference in genital HIV RNA quantity between genders at baseline and week 48. However, we found a significant correlation of HIV RNA in plasma and semen at week 48. Lambert-Niclot *et al*. [28] reported no association between HIV RNA in plasma and semen in HIV-infected men with repeatedly undetectable blood viral load. The lack of a significant difference in vaginal swab extract HIV RNA after mLPV/r compared with TDF/3TC/LPV/r in females may be due to the lower baseline HIV RNA values in the mLPV/r arm, while the opposite is seen in males and could be a result of a small sample size in the TDF/3TC/LPV/r arm, as a trend for declining seminal HIV RNA was observed (2.4 at baseline to 1.6 $\log_{10}$ copies/ml at week 48).

Similar to many published studies, our sample size was small, thus limiting the ability to observe small differences of HIV RNA values between arms and time points. Our study is also limited by the lack of sexual risk behaviour information and importantly testing for common STDs that could directly impact genital secretion HIV RNA [17,29,30]. There were two semen samples with HIV RNA 400–1,000 copies/ml (Table 3), which may be due to concomitant STD but

we did not investigate this. Finally, there were several technical limitations that could have under- or over-estimated the genital secretion HIV RNA. The semen samples were collected by an undiluted method; therefore, the HIV RNA values could be falsely low due to semen inhibitory factors [31,32]. Although no vaginal swab collection was done during menses, we did not test the vaginal swab specimen for blood and did not collect information on timing of specimen collection relative to the menstrual cycle [33]. The vaginal swab was also not tested for contaminated semen. We collected the swab at the lateral fornix of the vagina but more saturated vaginal fluid could be collected from the posterior fornix of vagina. We did not perform PCR for human DNA to exclude cell-associated RNA contamination. Additionally, a second centrifugation of the seminal plasma samples would have been preferable in limiting contamination of HIV-infected cells. HIV-1 spiking experiments with plasma containing known levels of HIV-1 RNA was not performed before testing the genital samples from our participants.

From the main HIV STAR study, we concluded that mLPV/r was inferior to the TDF/3TC/LPV/r arm because low-level plasma viraemia was more common [11]. Therefore, mLPV/r is not generally recommended in clinical practice without frequent HIV RNA monitoring. In this genital substudy analysis, the median genital HIV RNA in both arms was decreased significantly from baseline to week 48 without statistical difference between randomized arms. One-third of patients with suppressed plasma viraemia had low detectable levels of HIV RNA in the genital secretion.

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Additional file

Additional file 1: A list of the HIV STAR Study team members can be found at http://www.intmedpress.com/uploads/documents/AVT-13-OA-3126_Bunupuradah_Additional_file_1.pdf

References

1. Saksena NK, Potter SJ. Reservoirs of HIV-1 *in vivo*: implications for antiretroviral therapy. *AIDS Rev* 2003; **5:**3–18.
2. Lafeuillade A, Solas C, Halfon P, Chadapaud S, Hittinger G, Lacarelle B. Differences in the detection of three HIV-1 protease inhibitors in non-blood compartments: clinical correlations. *HIV Clin Trials* 2002; **3:**27–35.
3. Ghosn J, Viard JP, Katlama C, *et al*. Evidence of genotypic resistance diversity of archived and circulating viral strains in blood and semen of pre-treated HIV-infected men. *AIDS* 2004; **18:**447–457.
4. Baeten JM, Overbaugh J. Measuring the infectiousness of persons with HIV-1: opportunities for preventing sexual HIV-1 transmission. *Curr HIV Res* 2003; **1:**69–86.
5. Ananworanich J, Kerr SJ, Vernazza P, *et al*. Genital shedding of HIV after scheduled treatment interruption. *Int J STD AIDS* 2011; **22:**61–66.
6. Baeten JM, Kahle E, Lingappa JR, *et al*. Genital HIV-1 RNA predicts risk of heterosexual HIV-1 transmission. *Sci Transl Med* 2011; **3:**77ra29.
7. Pulido F, Matarranz M, Rodriguez-Rivera V, Fiorante S, Hernando A. Boosted protease inhibitor monotherapy. What have we learnt after seven years of research? *AIDS Rev* 2010; **12:**127–134.
8. Bierman WF, van Agtmael MA, Nijhuis M, Danner SA, Boucher CA. HIV monotherapy with ritonavir-boosted protease inhibitors: a systematic review. *AIDS* 2009; **23:**279–291.
9. Cohen MS, Gay C, Kashuba AD, Blower S, Paxton L. Narrative review: antiretroviral therapy to prevent the sexual transmission of HIV-1. *Ann Intern Med* 2007; **146:**591–601.

10. Arribas JR, Delgado R, Arranz A, *et al*. Lopinavir-ritonavir monotherapy versus lopinavir-ritonavir and 2 nucleosides for maintenance therapy of HIV: 96-week analysis. *J Acquir Immune Defic Syndr* 2009; **51:**147–152.
11. Bunupuradah T, Chetchotisakd P, Ananworanich J, *et al*. A randomized comparison of second-line lopinavir/ritonavir monotherapy versus tenofovir/lamivudine/lopinavir/ritonavir in patients failing NNRTI regimens: the HIV STAR study. *Antivir Ther* 2012; **17:**1351–1361.
12. Ghosn J, Chaix ML, Peytavin G, *et al*. Penetration of enfuvirtide, tenofovir, efavirenz, and protease inhibitors in the genital tract of HIV-1-infected men. *AIDS* 2004; **18:**1958–1961.
13. Ghosn J, Chaix ML, Peytavin G, *et al*. Absence of HIV-1 shedding in male genital tract after 1 year of first-line lopinavir/ritonavir alone or in combination with zidovudine/lamivudine. *J Antimicrob Chemother* 2008; **61:**1344–1347.
14. Launay O, Tod M, Louchahi K, *et al*. Differential diffusions of indinavir and lopinavir in genital secretions of human immunodeficiency virus-infected women. *Antimicrob Agents Chemother* 2004; **48:**632–634.
15. Vernazza P, Daneel S, Schiffer V, *et al*. The role of compartment penetration in PI-monotherapy: the Atazanavir-Ritonavir Monomaintenance (ATARITMO) Trial. *AIDS* 2007; **21:**1309–1315.
16. Lambert-Niclot S, Peytavin G, Duvivier C, *et al*. Low frequency of intermittent HIV-1 semen excretion in patients treated with darunavir-ritonavir at 600/100 milligrams twice a day plus two nucleoside reverse transcriptase inhibitors or monotherapy. *Antimicrob Agents Chemother* 2010; **54:**4910–4913.
17. Politch JA, Mayer KH, Welles SL, *et al*. Highly active antiretroviral therapy does not completely suppress HIV in semen of sexually active HIV-infected men who have sex with men. *AIDS* 2012; **26:**1535–1543.
18. Gutmann C, Cusini A, Gunthard HF, *et al*. Randomized controlled study demonstrating failure of LPV/r monotherapy in HIV: the role of compartment and CD4-nadir. *AIDS* 2010; **24:**2347–2354.
19. Swindells S, DiRienzo AG, Wilkin T, *et al*. Regimen simplification to atazanavir-ritonavir alone as maintenance antiretroviral therapy after sustained virologic suppression. *JAMA* 2006; **296:**806–814.
20. Kovacs A, Wasserman SS, Burns D, *et al*. Determinants of HIV-1 shedding in the genital tract of women. *Lancet* 2001; **358:**1593–1601.
21. Cu-Uvin S, DeLong AK, Venkatesh KK, *et al*. Genital tract HIV-1 RNA shedding among women with below detectable plasma viral load. *AIDS* 2010; **24:**2489–2497.
22. Venkatesh KK, Delong AK, Kantor R, *et al*. Persistent genital tract HIV-1 RNA shedding after change in treatment regimens in antiretroviral-experienced women with detectable plasma viral load. *J Womens Health* 2013; **22:**330–338.
23. Cohen MS, Chen YQ, McCauley M, *et al*. Prevention of HIV-1 infection with early antiretroviral therapy. *N Engl J Med* 2011; **365:**493–505.
24. Vernazza P, Hirschel B, Bernasconi E, Fiepp M. HIV-infected patients under HAART without any other sexually transmitted infection do not transmit HIV by sexual intercourse. *Bull Med Suisses* 2008; **89:**165–169.
25. Nosyk B, Audoin B, Beyrer C, *et al*. Examining the evidence on the causal effect of HAART on transmission of HIV using the Bradford Hill criteria. *AIDS* 2013; **27:**1159–1165.
26. Ndziessi G, Cohen J, Kouanfack C, *et al*. Susceptibility to transmitting HIV in patients initiating antiretroviral therapy in rural district hospitals in Cameroon (Stratall ANRS 12110/ESTHER Trial). *PLoS ONE* 2013; **8:**e62611.
27. Hasse B, Ledergerber B, Hirschel B, *et al*. Frequency and determinants of unprotected sex among HIV-infected persons: the Swiss HIV cohort study. *Clin Infect Dis* 2010; **51:**1314–1322.
28. Lambert-Niclot S, Tubiana R, Beaudoux C, *et al*. Detection of HIV-1 RNA in seminal plasma samples from treated patients with undetectable HIV-1 RNA in blood plasma on a 2002-2011 survey. *AIDS* 2012; **26:**971–975.
29. Gitau RW, Graham SM, Masese LN, *et al*. Effect of acquisition and treatment of cervical infections on HIV-1 shedding in women on antiretroviral therapy. *AIDS* 2010; **24:**2733–2737.
30. Anderson BL, Wang CC, Delong AK, *et al*. Genital tract leukocytes and shedding of genital HIV type 1 RNA. *Clin Infect Dis* 2008; **47:**1216–1221.
31. Pasquier C, Anderson D, Andreutti-Zaugg C, *et al*. Multicenter quality control of the detection of HIV-1 genome in semen before medically assisted procreation. *J Med Virol* 2006; **78:**877–882.
32. Pasquier C, Andreutti C, Bertrand E, *et al*. Multicenter assessment of HIV-1 RNA quantitation in semen in the CREAThE network. *J Med Virol* 2012; **84:**183–187.
33. Reichelderfer PS, Coombs RW, Wright DJ, *et al*. Effect of menstrual cycle on HIV-1 levels in the peripheral blood and genital tract. WHS 001 Study Team. *AIDS* 2000; **14:**2101–2107.

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