Table 2. Uni-and multi-variate analyses to estimate the risk of ATV/r use over other PIs-containing antiretroviral therapies for cholelithiasis.

	Model 1 crude (n = 1,242)			Model 2 adjusted (n = 1,203)		
	HR	95%CI	P value	HR	95%CI	P value
ATV/r use	1.365	0.275–6.775	0.702	1.390	0.276–7.017	0.689
Age per 1 year	1.072	1.021–1.127	0.006			
Male gender	0.446	0.052–3.831	0.463			
Race (East Asian origin)	0.285	0.033–2.444	0.252			
Weight per 1 kg increment	0.990	0.914–1.073	0.807			
BMI per 1 kg/m^2 increment	0.997	0.780–1.274	0.980			
CD4 count per 10/μl increment	0.987	0.938–1.038	0.605			
HIV viral load per log10/ml increment	0.917	0.541–1.557	0.750			
Baseline eGFR 10 ml/min/1.73 m^2 decrement	1.140	0.842–1.557	0.394			
Hepatitis B or Hepatitis C	0.040	0.000–1138.5	0.538			

Model 2 was adjusted for age and body weight.
HR: hazard ratio, CI: confidential interval, ATV/r: ritonavir-boosted atazanavir, BMI: body mass index, eGFR: estimated glomerular filtration rate.
doi:10.1371/journal.pone.0069845.t002

mentioned drugs as the preferred choice, at least for 3 years during the study period (http://www.haart-support.jp/guideline2011.

pdf. in Japanese). The attending physician also selected the concurrent drugs, including nucleoside reverse transcriptase inhibitors (NRTI), non-NRTI, integrase inhibitors, and CCR5 inhibitors. None of the patients received two PIs during the study period.

Measurements

Complicated cholelithiasis was defined as follows: 1) cholelithiasis diagnosed by computed tomography or abdominal ultrasonography, together with cholecystitis, cholangitis, or pancreatitis, or 2) symptomatic cholelithiasis or choledocholithiasis requiring invasive procedures, such as cholecystomy or endoscopic retrograde cholangiopancreatography. Before the initiation of ART and until suppression of HIV-1 viral load, patients visited our clinic every month. However, after viral load suppression, the visit interval was extended up to every three months.

In this study, the primary exposure variable was ATV/r use over other PIs (FPV, FPV/r, LPV/r, and DRV/r). The potential risk factors for cholelithiasis were determined according to previous studies and collected from the medical records, together with the basic demographics [4,9,10]. They included age, sex, body weight, body mass index (BMI), baseline laboratory data [CD4 cell count, HIV viral load, estimated glomerular filtration rate (eGFR)], and presence or absence of other medical conditions [concurrent use of tenofovir (TDF), co-infection with hepatitis B, defined by positive hepatitis B surface antigen, and co-infection with hepatitis C, defined by positive hepatitis C viral load]. eGFR was calculated as described previously [11]. At our clinic, weight was measured on every visit whereas other variables were measured in the first visit and at least once annually. We used the data on or closest to and preceding the day of starting ART by no more than 180 days.

Statistical analysis

Baseline characteristics were compared using the unpaired Student's *t*-test or χ^2test (Fisher's exact test) for quantitative or qualitative variables, respectively. The time to the diagnosis of complicated cholelithiasis was calculated from the date of commencement of pre-defined PI-containing ART to the date of diagnosis of cholelithiasis. Censored cases represented those who discontinued the PIs, dropped out, were referred to other facilities, or at the end of follow-up period. The time from the start of ART to the diagnosis of cholelithiasis was analyzed by the Kaplan Meier method for patients who started ATV/r (ATV/r group) or other PIs (other PIs group), and the log-rank test was used to determine the statistical significance. The Cox proportional hazards regression analysis was used to estimate the impact of ATV/r use over other PIs on the incidence of cholelithiasis. The impact of each parameter listed above was also estimated by univariate Cox proportional hazards regression. We conducted multivariate analysis adjusted for age and body weight only, because of the small number of cases that were diagnosed with complicated cholelithiasis.

Statistical significance was defined as two-sided p value <0.05. We used the hazard ratio (HR) and 95% confidence interval (95%CI) to estimate the impact of each variable on cholelithiasis. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, IL).

Results

A total of 1,498 patients commenced or switched key drugs (PIs, non-NRTIs, or integrase inhibitor) between January 1, 2004 and June 30, 2010. Of the 1,242 patients who were included in the analysis, 466 (37.5%) started ATV/r-containing ART while 776 (62.5%) started other PIs-containing ART (Figure 1). Table 1 shows the demographics, laboratory data, and medical conditions of the study population at baseline. The majority of the study population was males, of East Asian origin, and comparatively young. The ATV/r group included significantly more patients of East Asian origin ($p = 0.015$) with significantly higher body weight ($P<0.001$), higher CD4 count ($p<0.001$), lower viral load ($p<0.001$), and lower eGFR ($P = 0.012$), compared with other PI groups. In contrast, patients of the other PIs group were significantly more likely to be treatment naïve ($p<0.001$). However, all other major background parameters were similar in the two groups.

Cholelithiasis was diagnosed in 3 patients (0.64%) of the ATV/r group and 3 (0.39%) in the other PIs group, with an estimated

incidence of cholelithiasis of 2.23 and 1.65 per 1000 person-years, respectively. The incidence was not statistically different in the two groups by log-rank test (P = 0.702). Univariate analysis showed that ATV/r use was not associated with the development of cholelithiasis (HR = 1.365; 95% CI, 0.275–6.775; p = 0.704) (Table 2). Furthermore, other variables, including gender, body weight, race, BMI, co-infection with hepatitis B or C, eGFR, CD4 count, and viral load were not associated with cholelithiasis. On the other hand, older age was associated with increased risk of cholelithiasis (per one year, HR = 1.072; 95% CI, 1.021–1.127; p = 0.006). Multivariate analysis adjusted for age and body weight indicated that ATV/r use was not associated with the development of cholelithiasis (HR = 1.390; 95% CI, 0.276–7.017; p = 0.690) (Table 2).

Table 3 shows the clinical characteristics of the patients diagnosed with cholelithiasis in the present study. For the three patients of the ATV/r group, the time to the diagnosis of cholelithiasis was 18, 34, and 39 months, respectively. They were diagnosed with gallstone pancreatitis, symptomatic choledocholelithiasis, and cholecystitis, respectively, and all patients required invasive therapies. The median observation period was 31.7 months (IQR 16.0–49.7 months) for the ATV/r group and 23.0 months (IQR 10.4–42.5 months) for the other-PIs group.

Discussion

To our knowledge, this is the first study that compared the incidence of complicated cholelithiasis between patients receiving ATV/r and those on other PIs. The incidence of cholelithiasis in the ATV/r group was low at 2.23 per 1000 person-years and was not statistically different from that in the other PIs groups based on uni- and multi-variate analyses.

Previous reports suggested the association between ATV/r use and cholelithiasis [4–6]. However, the association was not demonstrated in this cohort study of 1,242 patients. Rakotondravelo et al. reported 14 cases of PI-related cholelithiasis [4]. Although their study was not designed to calculate the incidence, the estimated incidence was 2.3 cases per 1000 person-years, which is similar to our result. This incidence is 10 times lower than that of ATV/r-associated renal stones reported in our previous study [3]. In fact, only 16 cases with ATV/r-induced cholelithiasis have been reported to date [4–6], compared with substantial number of ATV/r-associated renal stone reported by several groups [3,12–16]. Thus, the potential risk of cholelithiasis in patients on PIs seems low compared to urolithiasis and may not be a major factor in the selection of ART.

Siveke et al. suggested that all PIs could cause cholelithiasis based on 3 cases that developed cholelithiasis while on PIs-containing ART. It is possible that PIs other than ATV/r also contribute to the development of cholelithiasis [8]. However, this cannot be confirmed at this stage and further studies are needed to address this issue.

The exact mechanism of ATV/r-induced cholelithiasis is not fully understood, although several theories have been suggested. One such theory is the precipitation of ATV in the bile with associated ATV-induced hyperbilirubinemia [4]. Another proposed mechanism relates to end-stage liver disease, which results in increased plasma ATV concentration and subsequent ATV/r-induced cholelithiasis [4]. In this study, however, we could not identify any risk factor associated with cholelithiasis.

There are several limitations to our study. First, we could not investigate asymptomatic cholelithiasis and symptomatic gallstone without complications. Thus, the risk of developing cholelithiasis associated with ATV/r might have been underestimated in the

Table 3. Clinical characteristics of patients who developed cholelithiasis.

n	Sex	Age (yrs)	BMI (kg/m^2)	Other conditions	Protease inhibitors	Other antiretroviral agents	Duration of PI therapy (months)	Diagnosis	Invasive procedures
1	F	63	20.0	Breast cancer, hypothyroidism, hypertriglyceridemia	ATV/r	ABC, 3TC	34	Choledocholithiasis	ERCP
2	M	59	25.8	hypertriglyceridemia	ATV/r	TDF, FTC	39	Cholecystitis	PTGBD
3	M	48	29.1	hypertriglyceridemia	ATV/r	ABC, 3TC	18	Gall stone pancreatitis	ERCP
4	M	56	22.7	hypertriglyceridemia	LPV/r	ABC, 3TC	39	Cholecystitis	PTGBD
5	M	37	16.6	hypertriglyceridemia	LPV/r	ABC, 3TC	1	Choledocholithiasis	ERCP
6	M	40	19.3	hypertriglyceridemia	LPV/r	ABC, 3TC	2	Cholelithiasis	Cholecystomy

BMI: body mass index, PI: protease inhibitor, ATV/r: ritonavir-boosted atazanavir, LPV/r: lopinavir/ritonavir, ABC: abacavir, 3TC: lamivudine, ERCP: endoscopic retrograde cholangiopancreatography, PTGBD: percutaneous transhepatic gall bladder drainage.

doi:10.1371/journal.pone.0069845.t003

present study. Second, the prevalence of gallstones is generally lower in East Asians than in European descent and since most of the patients in this study were of East Asian origin, the effect of ATV/r might have been underestimated in our study [17]. Lastly, although prolonged exposure to ATV has been suggested as a possible etiology of ATV-induced cholelithiasis, the median observation period in our study (31.7 months) was shorter than the median latency between commencement of ATV-based therapy and the development of cholelithiasis reported in a previous study (42 months) [4]. Therefore, the short observation period in our study may have underestimated the risk of cholelithiasis. However, it remains to be determined whether ATV has a cumulative effect on the development of cholelithiasis due to the limited information available.

In conclusion, on the contrary to a substantially higher incidence of renal stones in the ATV/r group (23.7 cases per 1000 person-years) than in other PIs groups reported in the same cohort [3], the incidence of complicated cholelithiasis was low of 2.23 per 1000 person-years in the ATV/r group, and was not different between the two groups of PI-treated patients. Although the number of patients in our study might not have been large enough to show differences in the incidence of complicated cholelithiasis, the study at least suggested that the incidence of ATV/r-related cholelithiasis is low. Thus, on the contrary to ATV/r-associated nephrolithiasis, possible risk of cholelithiasis should not preclude the use of ATV/r.

Acknowledgments

The authors thank Akiko Nakano for supporting this study as a research coordinator, and all other clinical staff at the AIDS Clinical Center for their help in the completion of this study.

Author Contributions

Conceived and designed the experiments: YH. Analyzed the data: YH TN HK. Wrote the paper: YH TN HK KT HG YK SO.

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Illicit Drug Use Is a Significant Risk Factor for Loss to Follow Up in Patients with HIV-1 Infection at a Large Urban HIV Clinic in Tokyo

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Abstract

Background: Loss to follow up (LTFU) is an important prognostic factor in patients with HIV-1 infection. The impact of illicit drug use on LTFU of patients with HIV-1 infection is unknown in Japan.

Methods: A single center observational study was conducted to elucidate the impact of illicit drug use on LTFU at a large HIV clinic in Tokyo. LTFU was defined as those who discontinued their visits to the clinic for at least 12 months and were not known to be under the care of other facilities or have died within 12 months of their last visit. Patients who first visited the clinic between January 2005 and August 2010 were enrolled. Information on illicit drug use was collected in a structured interview and medical charts. Comparison of the effects of illicit drug use and no use on LTFU was conducted by uni- and multi-variate Cox hazards models as the primary exposure.

Results: The study subjects were 1,208 patients, mostly Japanese men, of relatively young age, and infected through homosexual contact. A total of 111 patients (9.2%) were LTFU (incidence: 24.9 per 1,000 person-years). Among illicit drug users and non users, 55 (13.3%) and 56 (7.1%) patients, respectively, were LTFU, with incidence of 35.7 and 19.2 per 1,000 person-years, respectively. Uni- and multi-variate analyses showed that illicit drug use was a significant risk for LTFU (HR=1.860; 95% CI, 1.282-2.699; p=0.001) (adjusted HR=1.544; 95% CI, 1.028-2.318; p=0.036). Multivariate analysis also identified young age, high CD4 count, no antiretroviral therapy, and no health insurance as risk factors for LTFU.

Conclusions: The incidence of LTFU among illicit drug users was almost twice higher than that among non users. Effective intervention for illicit drug use in this population is warranted to ensure proper treatment and prevent the spread of HIV.

Citation: Nishijima T, Gatanaga H, Komatsu H, Takano M, Ogane M, et al. (2013) Illicit Drug Use Is a Significant Risk Factor for Loss to Follow Up in Patients with HIV-1 Infection at a Large Urban HIV Clinic in Tokyo. PLoS ONE 8(8): e72310. doi:10.1371/journal.pone.0072310

Editor: Dimitrios Paraskevis, University of Athens, Medical School, Greece

Received May 13, 2013; **Accepted** July 9, 2013; **Published** August 7, 2013

Funding: This work was supported by a Grant-in Aid for AIDS research from the Japanese Ministry of Health, Labour, and Welfare (H23-AIDS-001), and the Global Center of Excellence Program (Global Education and Research Center Aiming at the Control of AIDS) from the Japanese Ministry of Education, Science, Sports and Culture. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: *The authors have declared that no competing interests exist.*

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Introduction

The introduction of highly-active antiretroviral therapy (HAART) has markedly improved the prognosis of patients with HIV-1 infection [1,2]. Patients with HIV-1 infection need to maintain a good level of adherence to antiretroviral therapy (ART) and frequent visits to the health facilities for monitoring treatment efficacy and safety, with regard to the suppression of HIV-1 viral load, recovery of immune function, and improvement of prognosis and survival [3,4]. Those who discontinue medical follow up are likely to develop AIDS-defining illness and die, compared to those who continue their visits [5,6]. Thus, loss to follow up (LTFU) influences prognosis of patients with HIV-1 infection [7–11].

Among patients with HIV-1 infection, those who use illicit drugs are associated with lower ART uptake and inferior adherence to treatment [12–15], which lead to suboptimal treatment outcome, compared with patients with other risk categories [16–18]. However, illicit drug users are one of the "difficult to reach" populations and it is difficult to obtain accurate data on them [19]. It is especially difficult in Japan to collect data on illicit drug users, because of a strong

government policy against illicit drug use and extremely low lifetime prevalence of illicit drug use in the general population (2.9% in 2009 according to the Nationwide General Population Survey on Drug Use and Abuse) [20,21] (http://www.ncnp.go.jp/nimh/pdf/h21.pdf. in Japanese) (http://www.mhlw.go.jp/bunya/iyakuhin/yakubuturanyou/torikumi/dl/index-04.pdf. in Japanese). Thus, there are no data on illicit drug use among patients with HIV-1 infection, and the impact of such use on prognosis of HIV-1 infected patients in Japan [20,22].

Based on the abovementioned background, the aim of the present study was to elucidate the impact of illicit drug use on LTFU among patients with HIV-1 infection at a large urban HIV clinic in Tokyo, Japan.

Methods

Ethics Statement

This study was approved by the Human Research Ethics Committee of the National Center for Global Health and Medicine, Tokyo, Japan. The Committee waived a written informed consent, since this study only uses data of anonymized patients obtained from a routine practice. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Study design

This study was designed and reported according to the recommendations of STROBE (Strengthening the Reporting of Observational studies in Epidemiology) statement [23]. We performed a single center observational study of patients with HIV-1 infection to elucidate whether illicit drug use is a risk factor for LTFU in a large urban HIV clinic in Tokyo. The AIDS Clinical Center is one of the largest clinics for HIV care in Japan with more than 3,300 registered patients. Considering that the total reported number of patients with HIV-1 infection is 21,415 by the end of 2011, this clinic treats approximately 15% of the HIV-1 infected patients in Japan (http://api-net.jfap.or.jp/status/2011/11nenpo/hyo_02.pdf. in Japanese).

Study subjects

The study population was patients with HIV-1 infection, aged >17 years, who visited our clinic for the first time from January 1, 2005 to August 31, 2010. The exclusion criteria were; 1) those who came for the second opinion and 2) those who were referred to other facilities on their first or second visit. They were excluded because the structured interview on social demographics was often not conducted for these patients. Patients who refused to have their data included in the study were also excluded. Patients were followed up until December 31, 2012.

Measurements

Variables were collected through a structured interview conducted at the first visit of each patient as part of routine clinical practice by the nurses specializing at the HIV outpatient care. The interview by these "coordinator nurses" included the following variables: history of illicit drug use and injection drug use (and type of illicit drugs if available), health insurance status, perceived route of transmission, sexuality, and whether living alone or with someone.

Because the interview could underestimate the prevalence of illicit drug use, we also searched the medical records for information on illicit drug use and related variables covering the period from the first visit to December 2012. Information on age, sex, ethnicity, treatment status for HIV infection, and history of AIDS [(defined as history of or concurrent 23 AIDS-defining diseases set by the Japanese Ministry of Health, Labour and Welfare) (http://www.haart-support.jp/pdf/guideline2012.pdf. in Japanese)] were extracted from the medical records. The laboratory data of CD4 cell count, HIV-1 viral load, and hepatitis C antibody on the first visit were also collected, and if these test results were not available on that day, the data within three months from the first visit were used.

Definition of loss to follow up

LTFU was defined according to the literature as follows: patients who discontinued their visits to the AIDS Clinical Center for at least 12 months after the last visit and who were not known to be under the care of other medical facilities or have died within 12 months of their last visit [24]. At our clinic, all patients provide their phone numbers at the first visit, and when they miss the scheduled visit, the abovementioned "coordinator nurse" calls the patient to make another appointment, or leave a message to visit if the patient does not answer the phone. If the patient does not visit the clinic after the first call, the nurses continue calling the patient every three months up to one year. For the majority of lost cases, we checked whether the patient went to seek care in another hospital, because in Japan only a few clinics provide HIV care, due to the low prevalence of HIV-1 infection (0.016%) (http://www.stat.go.jp/english/data/kokusei/pdf/20111026.pdf) (http://api-net.jfap.or.jp/status/2011/11nenpo/hyo_02.pdf. in Japanese). Thus, even if a patient stopped visiting our clinic and started seeking help at other facilities without informing the first health care provider, the new facility almost always contacts the original facility to obtain medical information.

Statistical analysis

Patients' characteristics and social demographics were compared between those who were LTFU and those who continued visiting the clinic by the Student's *t*-test for continuous variables and by either the χ^2 test or Fisher's exact test for categorical variables.

The time to LTFU as defined above was calculated from the date of the first visit to the date of LTFU. Censored cases represented those who were referred to other facilities, or who died within 12 months of their last visit, or at the end of follow-up period. The time from the first visit to LTFU was analyzed by the Kaplan Meier method for patients who experienced illicit drug use and those who did not, and the log-rank test was used to determine the statistical significance. The Cox proportional hazards regression analysis was used to estimate the impact of illicit drug use over non users on the incidence of LTFU as a primary exposure. The impact of each basic

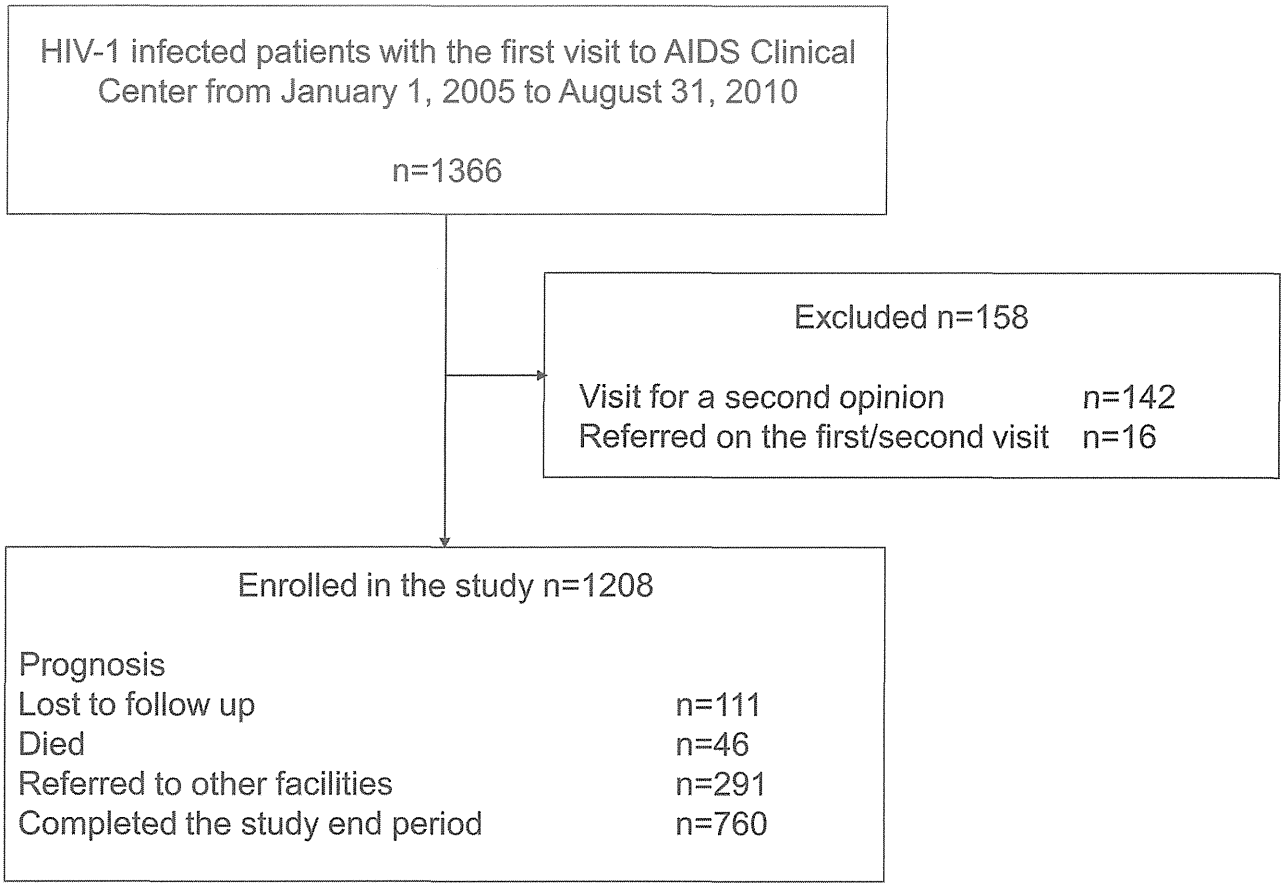

Figure 1. Patient enrollment process.
doi: 10.1371/journal.pone.0072310.g001

demographics, baseline laboratory data, and other medical conditions listed above was also estimated with univariate Cox proportional hazards regression.

To estimate the unbiased prognostic impact of illicit drug use over non-users for LTFU, we conducted three models using multivariate Cox proportional hazards regression analysis. Model 1 was the aforementioned univariate analysis for illicit drug use over non users. Model 2 included basic demographics (age and Japanese) plus model 1. In model 3, we added CD4 count, ART, and health insurance status, because they showed significant relationship with LTFU in univariate analysis and the literatures showed a high CD4 count, without ART and without health insurance is a risk factor for LTFU [11,24,25]. History of AIDS and HIV-1 viral load were not added to the model, based on their multicollinearity with CD4 count and ART, respectively.

To elucidate whether the impact of illicit drug use on LTFU is affected by sexual behavior, we divided patients into MSM and non-MSM groups. Then, the abovementioned multivariate analysis was conducted for each group.

Statistical significance was defined at two-sided p values <0.05. We used hazard ratios (HRs) and 95% confidence intervals (95% CIs) to estimate the impact of each variable on LTFU. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 20.0 (SPSS, Chicago, IL).

Results

A total of 1,366 patients with HIV-1 infection visited the AIDS Clinical Center for the first time during the study period. 142 patients visited for a second opinion and 16 patients were referred to other facilities on their first or second visit. Thus, 158 patients were excluded from the analysis (Figure 1). Table 1 summarizes characteristics of the 1,208 patients included in this study. The perceived route of transmission was homosexual contact in 948 (79%), heterosexual contact in 173 (14%), injection drug use in 22 (2%), contaminated blood product in 11 (1%), vertical transmission in 1 (0.1%), and unknown in 53 (4%). Further analysis indicated that 973 (81%) patients were MSM regardless of the perceived route of transmission (e.g., if a patient considered that they were infected with HIV-1 through injection drug use and they were MSM, they were classified to MSM in this study). The study patients were mostly Japanese men of relatively young age (mean: 36 years). Most patients were ART-naïve, with a median CD4 count of 245/μl.

Table 1. Baseline demographics and laboratory data for all study population, those who were lost to follow up and those who continued the visits.

	All (n=1,208)	Lost follow up (n=111)	Others (n=1,097)	P value
Sex (male), n (%)	1125 (93)	103 (93)	1022 (93)	0.84
Median (IQR) age	36 (29-43)	31 (25-39)	36 (30-43)	<0.01
Illicit drug use, n (%)	415 (34)	55 (50)	360 (33)	<0.01
Injection drug use, n (%)	53 (4)	8 (7)	45 (4)	0.14
Methamphetamine use, n (%)	63 (5)	10 (9)	53 (5)	0.07
Arrested due to illicit drug, n (%)	27 (2)	5 (5)	22 (2)	0.09
Median (IQR) CD4 count (/μl)[a]	245 (101-380)	391 (313-515)	231 (84-359)	<0.01
Median (IQR) HIV-1 viral load (log_{10}/ml)[b]	4.59 (3.89-5.18)	4.32 (3.80-4.75)	4.64 (3.91-5.20)	0.03
AIDS, n (%)	323 (27)	10 (9)	313 (29)	<0.01
On antiretroviral therapy, n (%)	131 (11)	5 (5)	126 (12)	0.02
Positive HCV antibody, n (%)	46 (4)	2 (2)	44 (4)	0.43
Men who have sex with men, n (%)	973 (81%)	89 (80)	884 (81)	0.90
Transmission category, n (%)				0.51
Homosexual contact	948 (79)	84 (76)	864 (79)	
Heterosexual contact	173 (14)	19 (17)	154 (14)	
Injection drug use	22 (2)	4 (4)	18 (2)	
Contaminated blood product	11 (1)	0	11 (1)	
Vertical transmission	1 (0.1)	0	1 (0.1)	
Unknown	53 (4)	4 (4)	49 (5)	
Ethnicity, n (%)[c]				0.02
Japanese	1070 (89)	92 (83)	978 (89)	
Asian	70 (6)	7 (6)	63 (6)	
White	27 (2)	2 (2)	25 (2)	
Black	26 (2)	7 (6)	19 (2)	
Latino	12 (1)	2 (2)	10 (0.9)	
Health insurance status, n (%)				<0.01
Without insurance	55 (5)	13 (12)	42 (4)	
With insurance/public assistance	1153 (95)	98 (88)	1055 (96)	
Working status, n (%)[d]				0.09
Unemployed	230 (19)	23 (21)	207 (19)	
With any job	909 (75)	77 (69)	832 (76)	
Student/housewife	68 (6)	11 (10)	57 (5)	
Living alone, n (%)[e]	532 (44)	46 (41)	486 (44)	0.62
Median (IQR) follow up days	1384.5 (732-1991)	266 (58-800)	1454 (914-2053)	<0.01

Data for [a] two, [b] four, [c] three, [d] one, and [e] fifteen cases, respectively, are missing

Based on the interview and medical records, 34% of the patients were illicit drug users (including injection drug users), 4% were injection drug users and 5% had used methamphetamine. Of the total, 2% were detained or arrested for possession or use of illicit drugs. Among illicit drugs, amyl nitrite and 5-methoxy-diisopropyltryptamine were the most commonly named by study patients (amyl nitrite and 5-methoxy-diisopropyltryptamine became prohibited substance by law in 2006 and 2005, respectively, in Japan) [26]. Methamphetamine, 3,4-methylenedioxymethamphetamine, cannabis, heroin, cocaine, and opium were also mentioned (numbers not counted except for methamphetamine).

LTFU patients were significantly more likely to be illicit drug users and tended to use methamphetamine and be arrested/detained due to illicit drug use than those who continued to visit the clinic. LTFU tended to be non-Japanese, younger age, had higher CD4 count, and less likely to have a history of AIDS, on ART, and covered by health insurance/public assistance, compared to the patients who continued to visit the clinic (Table 1).

Among the 1,208 patients included in the study, 111 (9.2%) were LTFU as defined above, with an incidence of 24.9 per 1,000 person-years. The median time from the first visit to LTFU was 266 days (IQR 58-800 days). Among illicit drug users (n=415) and non-users (n=793), 55 (13.3%) and 56 (7.1%) patients, respectively, were LTFU, with incidence of 35.7 and 19.2 per 1,000 person-years, respectively. Figure 2 shows the time from the first visit to LTFU by the Kaplan Meier method for the two groups. Illicit drug users were significantly more likely to stop visiting the clinic, compared to non-users (p=0.001, Log-rank test). The total observation period was 1,541.4 patient-years [median, 1,405 days, interquartile range (IQR), 674-2,029 days] for illicit drug users and 2,920.4 patient-years (median, 1,371 days, IQR, 759-1943 days) for non users.

Univariate analysis showed a significant relationship between illicit drug use and LTFU (HR=1.860; 95% CI, 1.282-2.699; p=0.001) (Table 2). Furthermore, young age, high baseline CD4 count, low HIV viral load, no history of AIDS, non Japanese, no ART, and no health insurance/public assistance were associated with LTFU. Injection drug use and methamphetamine use, respectively, were marginally associated with LTFU (injection drug use: HR=1.808; 95% CI, 0.880-3.713; p=0.107) (methamphetamine use: HR=1.684; 95% CI, 0.879-3.225; p=0.116).

Multivariate analysis identified illicit drug use as a significant risk for LTFU after adjustment for age and Japanese (adjusted HR=1.802; 95% CI, 1.209-2.686; p=0.004) (Table 3, Model 2), and also after adjustment for other risk factors (adjusted HR=1.544; 95% CI, 1.028-2.318; p=0.036) (Table 3, Model 3). Young age, high baseline CD4 count, no ART, and no health insurance/public assistance also persisted to be risk for LTFU in multivariate analysis.

Subgroup analysis of the patients stratified by sexual behavior showed that among MSM patients (n=973), the impact of illicit drug use on LTFU was slightly more evident (adjusted HR=1.641; 95% CI, 1.061-2.538; p=0.026) (Table 4) than in the total population (adjusted HR=1.544; 95% CI, 1.028-2.318; p=0.036) (Table 3, Model 3). On the other hand, illicit drug use had no significant impact in non-MSM patients (n=233) (adjusted HR=1.119; 95% CI, 0.248-5.053; p=0.883).

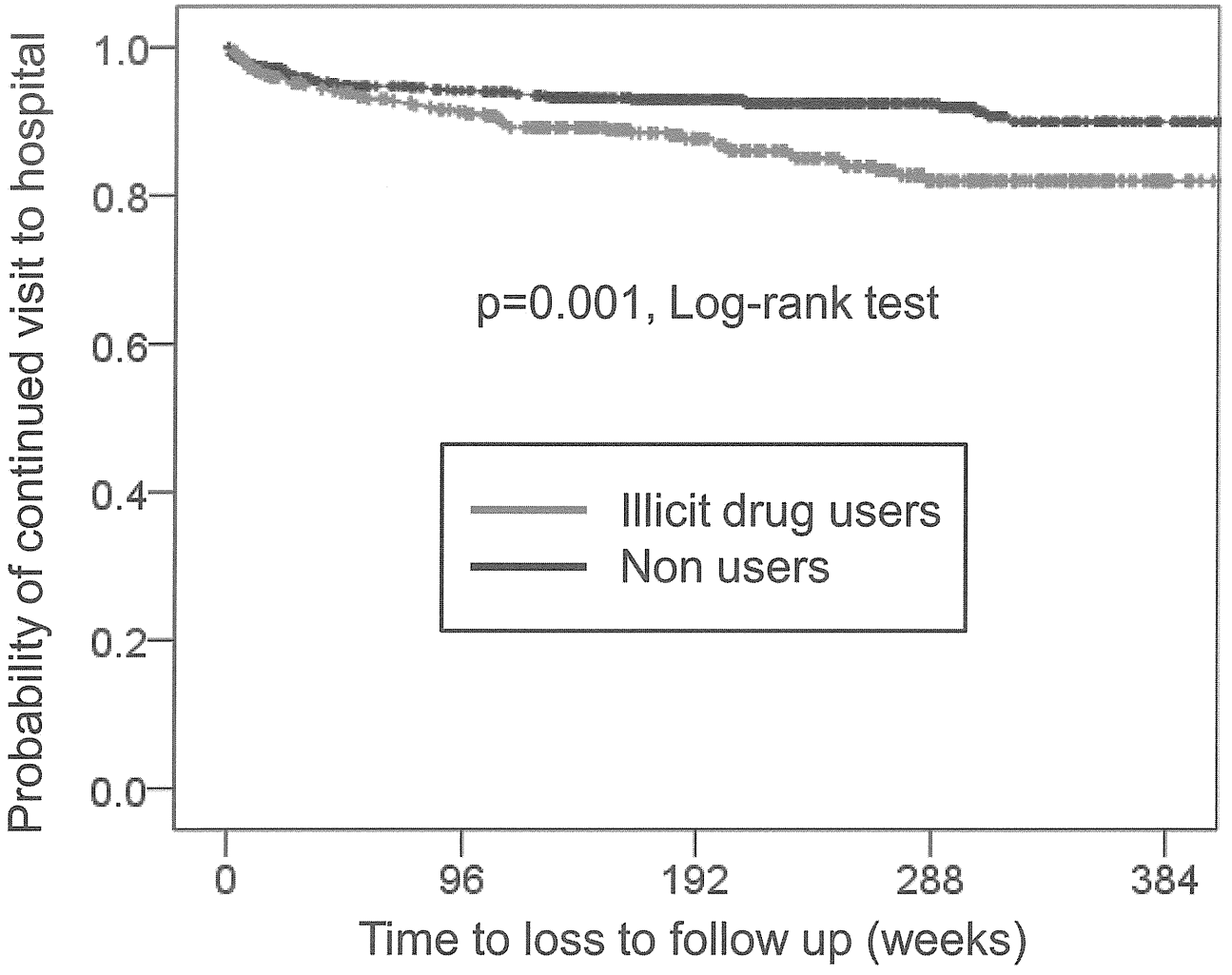

Figure 2. Kaplan-Meier curve showing time to loss to follow up for illicit drug users and non users. Compared to non drug users, illicit drug users were more likely to discontinue their visits to the hospital (p=0.001, Log-rank test).

doi: 10.1371/journal.pone.0072310.g002

Table 3. Multivariate analysis to estimate the risk of illicit drug use for loss to follow up.

	Model 1 Crude (n=1,208)		Model 2 Adjusted (n=1,208)		Model 3 Adjusted (n=1,206)	
	HR	95% CI	Adjusted HR	95% CI	Adjusted HR	95% CI
Illicit drug use†	1.860	1.282-2.699	1.770	1.208-2.592	1.513	1.018-2.248
Age ≤30 years†			Reference		Reference	
30< Age ≤40 years†			0.462	0.304-0.703	0.467	0.303-0.720
Age >40 years†			0.360	0.212-0.609	0.442	0.259-0.752
Japanese			0.472	0.286-0.779	0.798	0.443-1.436
CD4 count ≤200/μl†					Reference	
200< CD4 count ≤350 /μl†					2.221	1.148-4.297
CD4 count >350/μl†					7.087	3.951-12.71
On antiretroviral therapy†					0.366	0.147-0.912
With health insurance/public assistance†					0.204	0.102-0.409

† p<0.05 in Model 3

Table 2. Univariate analysis to estimate the risk of various factors for loss to follow up.

	Hazard ratio	95% CI	P value
Illicit drug use	1.860	1.282-2.699	0.001
Injection drug use	1.808	0.880-3.713	0.107
Methamphetamine use	1.684	0.879-3.225	0.116
Arrested/detained due to illicit drug	1.981	0.808-4.859	0.135
Male gender	0.961	0.468-1.974	0.961
Men who have sex with men	0.926	0.581-1.477	0.747
Age ≤30 years	Reference		
30 < Age ≤40 years	0.455	0.299-0.692	<0.001
Age >40 years	0.320	0.190-0.538	<0.001
CD4 count ≤200/μl	Reference		
200 < CD4 count ≤350/μl	2.536	1.318-4.878	0.005
CD4 count >350/μl	7.651	4.309-13.59	<0.001
HIV-1 viral load per $\log_{10}$/ml	0.846	0.730-0.981	0.027
History of AIDS	0.269	0.140-0.514	<0.001
Positive HCV antibody	0.466	0.115-1.888	0.285
Japanese	0.559	0.337-0.926	0.024
On antiretroviral therapy	0.402	0.164-0.986	0.046
With any job	0.870	0.549-1.376	0.551
On health insurance/public assistance	0.249	0.139-0.444	<0.001
Living alone	0.949	0.649-1.388	0.788

Table 4. Multivariate analysis to estimate the risk of illicit drug use for loss to follow up stratified by sexual behavior.

	Adjusted HR	95% CI	P value
MSM (n=973)	1.641	1.061-2.538	0.026
Non MSM (n=233)	1.119	0.248-5.053	0.883

Adjusted by variables in Table 3, Model 3 (age, Japanese, CD4 count, antiretroviral therapy, and health insurance)

MSM: men who have sex with men

Discussion

At this large urban HIV clinic in Tokyo, 9.2% of the patients were lost to follow up, with an incidence of 24.9 per 1,000 person-years. Furthermore, 34% of the study patients were illicit drug users and the incidence of LTFU for illicit drug users was almost twice higher than that for non users (35.7 and 19.2 per 1,000 person-years, respectively). Illicit drug use was identified as a significant risk for LTFU in uni- and multi-variate analyses (HR=1.860; 95%CI, 1.282-2.699; p=0.001) (adjusted HR=1.544; 95% CI, 1.028-2.318; p=0.036). The impact of illicit drug use on LTFU was slightly more evident among MSM than in the total study population.

To our knowledge, only a few studies have examined the impact of non-injection illicit drug use on LTFU [9,27], and this is the first such study conducted in Asia. The results showed that illicit drug use is a risk factor for LTFU, which is a marker for prognosis in patients with HIV-1 infection [7–11]. The result emphasizes the need for effective prevention and intervention strategies for illicit drug use in patients with HIV-1 infection in Japan. The finding of a more evident impact of illicit drug use in MSM patients also highlights the need for close monitoring of adherence to HIV care in this group of patients.

Among patients with HIV-1 infection, the prognosis of injection drug users is reported to be worse than that of non-injection drug users [28]. However, this study primarily focused on illicit drug use as a whole, rather than injection drug use for two main reasons; First, only a few studies focused on illicit drug use among HIV-1 infected patients, although a large number of studies focused on injection drugs [24,25,27,29,30]. Illicit drug use in patients with HIV-1 infection is an important issue, because not only illicit drug use lead to inferior treatment outcome compared with non users [16–18], but also non injection drug users are prone to practice high risk sexual behaviors, which might lead to transmission of HIV and other infectious diseases [14,31]. Furthermore, illicit drug use, especially opioid use, can be a trajectory into injection drug use [32,33]. Second, because only 0.5% of the patients were infected with HIV-1 through injection drug use by the end of 2011 in Japan (according to a nationwide surveillance conducted by the AIDS Surveillance Committee of the Ministry of Health, Labour and Welfare that covered all reported cases with HIV-1 infection), the anticipated prevalence of injection drug use was very low (http://api-net.jfap.or.jp/status/2011/11nenpo/hyo_02.pdf. in Japanese). Surprisingly, the prevalence of injection drug use was 4% in this study, the number is much higher than what the AIDS Surveillance Committee reported. This suggests a substantial underreporting for injection drug use as a route of transmission from the patients.

In the planning and design of effective prevention and intervention strategies for illicit drug users with HIV-1 infection in Japan, the unique circumstances related to this issue need to be taken into consideration. First, on one hand, the government maintains a strict punitive policy against illicit drug use and this policy has been one of the factors that helped maintain a relatively low prevalence of illicit drug use (lifetime prevalence 2.9%) [21] (http://www.ncnp.go.jp/nimh/pdf/h21.pdf. in Japanese). On the other hand, possibly due in part to severe criminalization of drug use, treatment and rehabilitation schemes for drug users remain poorly developed [20,34].

Second, most injected drugs in Japan are methamphetamine: In 2010, the number of arrested illicit drug users categorized by each drug was the largest for methamphetamine (12,200), while the numbers for other injectable drugs, such as heroin and cocaine were very small (22 and 112, respectively) (http://www.mhlw.go.jp/bunya/iyakuhin/yakubuturanyou/torikumi/dl/index-01.pdf. in Japanese). In the study patients, injection drug users and methamphetamine users also appeared to overlap considerably. Evidence from other countries shows that methamphetamine use has gained popularity among MSM, and methamphetamine use is strongly associated with high-risk sexual behavior [35–38]. Thus, any intervention for injection drug users with HIV-1 infection in Japan needs to take into consideration the frequent use of methamphetamines.

Several limitations need to be acknowledged. First, due to the nature of single-center study, the results of this study do

not necessarily represent all patients with HIV-1 infection in Japan. However, as abovementioned, our clinic treats approximately 15% of the total HIV patients in Japan, and furthermore, characteristics of the patients with HIV-1 infection newly diagnosed and reported to the Japanese National HIV Registry in 2011 (n=1529) is very similar to those of the study population: 94% male, 64% infected through homosexual contact, and 59% in their 20s and 30s of age (http://api-net.jfap.or.jp/status/2011/11nenpo/hyo_02.pdf. in Japanese). Most HIV-1 infected patients reside in urban areas such as Tokyo metropolitan area as well. Thus, the discrepancy between the study patients and all HIV patients in Japan should not be too large. Second, the structured interview designed for data collection does not prevent underreporting of illicit drug use. However, underreporting to a certain degree is unavoidable with regard to issues such as illicit drugs [19].

In conclusion, the incidence of LTFU in illicit drug users was almost twice higher than that in non users among patients with HIV-1 infection in Japan. Multivariate analysis identified illicit drug use as a significant risk factor for LTFU, which influences prognosis of patients with HIV-1 infection. Little data is available for illicit drug use in Japan, especially among patients with HIV-1 infection. However, all relevant parties in relation to this issue need to recognize that illicit drug use has spread among patients with HIV-1 infection, and that illicit drugs worsens adherence to HIV care in Japan. Appropriate measures for prevention and intervention of illicit drug use are urgently needed to ensure proper treatment and prevention of spread of HIV infection.

Acknowledgements

The authors thank Dr. Kiyoshi Wada, Department of Drug Dependence Research, National Institute of Mental Health, National Center of Neurology and Psychiatry, and Dr. Keishiro Yajima, AIDS Medical Center, National Hospital Organization Osaka National Hospital, for valuable comments for the manuscript. The authors also thank "coordinator nurses" who conducted the structured interviews (Ruiko Yakuwa, Beni Ito, Yuko Sugino, Miki Koyama, Kenji Takeda, Megumi Shimada, Jongmi Seo, Yuki Yamada, Kyoko Ishigaki), and all other clinical staff at the AIDS Clinical Center for their help in completion of this study.

Author Contributions

Conceived and designed the experiments: TN HG HK MT SO. Performed the experiments: MO KI. Analyzed the data: TN HK HG MT SO. Contributed reagents/materials/analysis tools: MO KI SO. Wrote the manuscript: TN HG MT SO.

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Switching Tenofovir/Emtricitabine plus Lopinavir/r to Raltegravir plus Darunavir/r in Patients with Suppressed Viral Load Did Not Result in Improvement of Renal Function but Could Sustain Viral Suppression: A Randomized Multicenter Trial

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Abstract

Background: Whether tenofovir nephrotoxicity is reversible after its withdrawal is unknown. Furthermore, there are no data on the viral efficacy of raltegravir (RAL) plus ritonavir-boosted Darunavir (DRV/r) in patients with suppressed viral load.

Methods: This multicenter, randomized trial compared renal function and viral efficacy in patients with suppressed viral load treated with RAL+DRV/r and ritonavir-boosted lopinavir (LPV/r) plus tenofovir/emtricitabine (TVD), who had been previously on LPV/r+TVD. The primary endpoint was the proportion of patients with >10% improvement in estimated glomerular filtration rate (eGFR) at 48 weeks calculated with Cockcroft-Gault equation.

Results: 58 randomized and treatment-exposed patients were analyzed (28 on RAL+DRV/r and 30 on LPV/r+TVD). Greater than 10% improvement in eGFR was noted in 6 (25%) out of 24 with RAL+DRV/r and 3 (11%) of 28 with LPV/r+TVD, and the difference was not statistically significant (p=0.272, 95% CI -0.067 to 0.354). Sensitivity analyses using three other equations for eGFR showed the same results. Urinary β2 microglobulin, a sensitive marker of tenofovir tubulopathy, significantly improved with RAL+DRV/r than with LPV/r+TVD (-271 versus -64 μg/gCr, p=0.026). Per protocol analysis showed that the HIV-RNA was <50 copies/mL at week 48 in all patients of both arms (24 in RAL+DRV and 29 in LPV/r+TVD).

Conclusions: Switching LPV/r+TVD to RAL+DRV/r did not significantly increase the proportion of patients who showed >10% improvement in renal function among those with relatively preserved eGFR. However, the switch improved urinary β2 microglobulin, suggesting that discontinuation of TDF might be beneficial in the long-term. RAL +DRV/r showed favorable viral efficacy in patients with suppressed viral load.

Trial Registration: ClinicalTrials.gov NCT01294761 http://clinicaltrials.gov/ct2/show/NCT01294761?term=SPARE&rank=2, Umin Clinical Trials Registry UMIN000005116 http://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&action=brows&type=summary&recptno=R000006083&language=J)

Citation: Nishijima T, Gatanaga H, Shimbo T, Komatsu H, Endo T, et al. (2013) Switching Tenofovir/Emtricitabine plus Lopinavir/r to Raltegravir plus Darunavir/r in Patients with Suppressed Viral Load Did Not Result in Improvement of Renal Function but Could Sustain Viral Suppression: A Randomized Multicenter Trial. PLoS ONE 8(8): e73639. doi:10.1371/journal.pone.0073639

Editor: D William Cameron, University of Ottawa, Canada

Received April 18, 2013; **Accepted** July 19, 2013; **Published** August 8, 2013

Funding: This work was supported by a Grant-in-Aid for the research on HIV/AIDS (H22-AIDS-001) from the Ministry of Health, Labor, and Welfare of Japan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: T. Naito received research grants from MSD K.K., Janssen Pharmaceutical K.K., and ViiV Healthcare. TF received research grants from ViiV Healthcare and MSD K.K. HM is a co-inventor on a government patent for darunavir as an employee of the U.S. Government under the terms of the Federal Technology Transfer Act. All rights, title, and interest to the patent have been assigned to the Government. SO received research grants from MSD K.K., Abbott Japan, Co., Janssen Pharmaceutical K.K., Pfizer, Co., and Roche Diagnostics K.K. All other authors declare no conflict of interest. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

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¶ Membership of the SPARE study team is provided in the Acknowledgments.

Introduction

Tenofovir disoproxil fumarate (TDF) is one of the most widely used nucleotide reverse transcriptase inhibitors (NRTI) for patients with HIV infection, with proven efficacy and safety [1-6]. However, tenofovir is excreted by both glomerular filtration and tubular secretion, and is known to cause renal proximal tubular dysfunction. Moreover, long-term TDF use reduces glomerular filtration rate more than other NRTIs [7-10]. Although the mechanism of tenofovir-induced kidney damage is not fully understood, mitochondria toxicity, a well-known adverse event of NRTIs [11,12], in the proximal renal tubular cells is considered to be the main mechanism [13,14]. In addition to renal dysfunction, TDF also reduces bone mineral density, and both complications might lead to serious outcomes with long-term use of TDF [9,15-19]. The concurrent use of ritonavir-boosted protease inhibitors (PI/r) is a risk factor for TDF-associated nephrotoxicity, since PI/r modifies tenofovir clearance and thus increases the severity of tenofovir nephrotoxicity [20,21].

Clinical manifestations such as lipoatrophy and neuropathy caused by NRTI-induced mitochondria toxicity are difficult to reverse [22,23], but whether TDF nephrotoxicity is reversible after discontinuation of TDF remains unknown at present. Unfortunately, the results of few small studies that have examined this issue are contradictory [24-26]. Of note, there is no randomized controlled study that has examined the reversibility of TDF-associated nephrotoxicity.

Recently, antiretroviral therapy (ART) not containing NRTIs (NRTI sparing regimens) has gained a wide attention, since these combinations can avoid NRTI toxicity. Despite high expectations, the results of studies on the efficacy and safety of NRTI sparing regimens for treatment-naïve patients showed dismal results. A small single arm study of CCR5 inhibitor maraviroc plus ritonavir-boosted Darunavir (DRV/r) showed a high rate of virologic failure, especially in patients with high baseline viral load of >100,000 copies/mL [27]. Raltegravir (RAL) plus unboosted atazanavir in a small randomized trial showed frequent grade 4 hyperbilirubinemia and emergence of raltegravir resistance [28]. Even the combination of RAL, a well-tolerated integrase inhibitor, and DRV/r, a protease inhibitor with high barrier to drug resistance and favorable lipid profile [29,30], showed a high prevalence of virological failure for patients with high baseline viral load in a single arm study [31].

At this stage, it is important to elucidate the effectiveness of NRTI sparing regimen for patients with suppressed HIV-1 viral load, because longer exposure with NRTIs tends to result in clinically overt NRTI-associated mitochondrial toxicity [22,32], and NRTI sparing regimens may avoid such long-term NRTI toxicity. Of note, the viral efficacy of NRTI-sparing regimen of RAL plus DRV/r has not been evaluated in patients with suppressed viral load [31].

Based on the above background, this multicenter randomized trial was conducted to elucidate 1) the reversibility of tenofovir nephrotoxicity, and 2) efficacy and safety of RAL +DRV/r for patients with suppressed viral load.

Methods

This clinical trial was designed and reported according to the recommendations of the Consolidated Standard of Reporting Trials (CONSORT) statement [33]. The protocol for this trial and supporting CONSORT checklist are available as supporting information; see Checklist S1 and protocol S1.

Ethics Statement

The Research Ethics Committees of Hokkaido University Hospital, Higashisaitama National Hospital, Niigata University Medical and Dental Hospital, the Institute of Medical Science, the University of Tokyo, Juntendo University School of Medicine, Shirakaba Clinic, Saku Central Hospital, Hiroshima University Hospital, Ehime University Hospital, National Hospital Organization Kyushu Medical Center, Kumamoto University Graduate School of Medical Sciences and National Center for Global Health and Medicine approved the study protocol. All patients enrolled in this study provided a written informed consent. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Study Design

The SPARE trial is an on-going phase 3B, multi-center, randomized, open-label, parallel group study conducted in Japan to compare renal function and viral efficacy of NRTI-sparing regimen of RAL+DRV/r and a standard regimen of PI/r + 2NRTIs [(lopinavir/ritonavir (LPV/r) plus fixed dose of tenofovir/emtricitabine (TVD)] for 96 weeks, randomly allocated to patients on LPV/r+TVD with suppressed viral load. With one to one ratio, patients with suppressed viral load on LPV/r (800 mg/200 mg) plus fixed dose of TDF (300 mg)/emtricitabine (200 mg) were randomly assigned to either RAL (800 mg) plus DRV/r (800 mg/100 mg) or to continue LPV/r+TVD. Patient enrollment remained open between February 21, 2011 and December 2011, and the follow-up period is scheduled to end in December 2013. This report summarizes the findings after 48 weeks of treatment, including the primary endpoint.

Randomization was stratified based on baseline body weight of 60 kg because low body weight, especially body weight of <60 kg, is an important risk for tenofovir nephrotoxicity [4,18,34]. Randomization was conducted at the data center with independent data managers, using a computer-generated randomization list prepared by a statistician with no clinical involvement in the trial.

Study Patients

The study population included Japanese patients with HIV-1 infection, aged ≥20 years, who were on LPV/r plus TVD and with suppressed HIV-1 RNA viral load of <50 copies/ml over a period of more than 15 weeks. Patients were screened and excluded if found positive for hepatitis B surface antigen, or had history of virologic failure with regimens including protease inhibitor or integrase inhibitor, or if they were considered inappropriate for the study by the attending physicians. Candidates were also excluded if the level of alanine aminotransferase was 2.5 times the upper limit of normal, estimated glomerular filtration rate (eGFR) calculated by Cockcroft-Gault equation (CG equation) was <60 ml/min, {[(140 - age) × weight (kg)] / (serum creatinine × 72)] (× 0.85 for females)} [35], or on treatment for opportunistic infection. Actual body weight was used for the calculation of eGFR. Patients who provided written informed consent started the allocated regimens within 4 weeks of enrollment.

Study Procedure

Visits for clinical and laboratory assessments were required within 15 weeks before registration for screening, at registration, and every 12 weeks for the duration of the study. Patients of the RAL+DRV/r arm were required to visit within 4 weeks after commencement of the allocated regimen to screen for adverse events. Baseline evaluation and evaluations at each visit covered medical history, including history of AIDS-defining illness and other comorbidities, concurrent medications, concurrent smoking, physical examination, CD4 cell count, HIV-1 RNA viral load, complete blood cell count, blood chemistries (albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, creatine kinase, blood urea nitrogen, serum creatinine, sodium, potassium, calcium, phosphate, triglyceride, total cholesterol, low-density lipoprotein cholesterol, high density lipoprotein cholesterol, glucose), and urine examination (urine dipstick, phosphate, creatinine, β2 microglobulin, N-acetyl-β-D-glucosaminidase (NAG), and albumin). The values of urinary β2 microglobulin, NAG, and albumin were expressed relative to urinary creatinine of 1 g/L (/g Cr). Percent tubular resorption of phosphate was calculated by the following formula: {1 – [(urine phosphate × serum creatinine) / (urine creatinine × serum phosphate)]} × 100 [36]. All data, including HIV-1 RNA viral load, were collected at each participating site and then transferred to a central data center. Grade 3 or 4 serious adverse events were reported to the independent data and safety monitoring board and analyzed for their relation to the study drugs. The grade of adverse events was classified according to the Division of AIDS Table for grading the severity of adult and pediatric events, version 2004 (URL:http://www.mtnstopshiv.org/sites/default/files/attachments/Table_for_Grading_Severity_of_Adult_Pediatric_Adverse_Events.pdf). Independent monitors visited all facilities to conduct source document verification to ensure the accuracy of all submitted data by week 48 and compliance to the protocol. All authors participated in the trial design, data analysis, and preparation of the manuscript, and vouch for the completeness and accuracy of the presented data.

Statistical Analysis

The tested hypothesis was that more patients in the RAL +DRV/r arm will experience >10% improvement in eGFR from the baseline than patients in the LPV/r+TVD arm after switching from LPV/r+TVD to RAL+DRV/r. Sample size calculation was based on the assumption that 50% of the patients of the RAL+DRV/r arm and 10% of the patients of the LPV/r + TVD arm will experience >10% improvement in eGFR from the baseline to week 48. With a 2-sided alpha level of 0.05 and 80% power, the estimated population sample required in this study was 50 patients (25 per single arm). To account for dropouts, we planned to enroll 27 patients per one arm. The study was not fully powered for secondary analysis. Per protocol population while on the initial randomized regimen was used for the analysis of the primary endpoint.

The primary endpoint was the proportion of patients with >10% improvement in eGFR at 48 weeks from the baseline calculated with the CG equation [35]. The baseline eGFR was estimated from the average of serum creatinine measured at baseline and at screening for enrollment. eGFR at week 48 was estimated from the average of serum creatinine at weeks 36 and 48. The proportion of such patients was compared between the two arms by the Fisher exact test. The following three equations for eGFR were also used for sensitivity analysis: 1) A 3-variable equation for the Japanese set by the Japanese Society of Nephrology (JSN equation): [194 × (serum creatinine)$^{-1.094}$× (age)$^{-0.287}$× (0.739 for female patients)][37], 2) the Modification of Diet in Renal Disease (MDRD) equation adjusted with coefficient for the Japanese [0.808 × 175 × (serum creatinine)$^{-1.154}$ × (age)$^{-0.203}$× (0.742 for female patients)] [37], and 3) Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation adjusted for the Japanese [0.813 × 141 × min (serum creatinine/κ, 1)$^{\alpha}$× max (serum creatinine/κ, 1) $^{-1.209}$ × (0.993)^{age}× (1.018 for females)] (where κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, *min* represents the minimum of serum creatinine/κ or 1, and *max* is the maximum of serum creatinine/κ or 1) [38]. Furthermore, the percent improvement in eGFR from baseline to week 48, calculated with all four equations described above, was compared between the two arms by the Student's t-test. Because the percent improvement in eGFR may depend on the baseline value, a correlation between the percent improvement in eGFR and the baseline value was tested, and the results showed very weak correlation (0.001<r<0.2) for all four equations for eGFR. Accordingly, the comparison of the percent improvement was conducted by the t-test as described above.

The secondary renal endpoint was changes in per protocol renal tubular markers from the baseline to week 48, and the results were compared by the Mann-Whitney test. The secondary efficacy endpoint was the proportions of patients with HIV-1 RNA <50 copies/mL at weeks 24 and 48. Data of both per protocol population and the intent-to-treat (ITT) population, comprising all randomized treatment-exposed subjects were used for the assessment of efficacy. With regard to analysis on the viral efficacy in this study, per protocol analyses were more important than ITT analyses, because some patients enrolled in the RAL+DRV/r arm were expected to develop adverse events due to switching to the new medications and subsequent discontinuation of the allocated regimen, whereas new adverse events were not likely in patients of the LPV/r+TVD arm solely by continuing the same regimen as before. Baseline parameters were compared between the two arms by the Student's t-test for continuous variables and by either the χ^2 test or Fisher's exact test for categorical variables. Statistical significance was defined at two-sided *p* values <0.05. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 21.0 (SPSS, Chicago, IL).

Results

Patient disposition and baseline characteristics

Between February and December of 2011, 59 patients from 11 centers were enrolled in the study and randomized. Of these, 29 and 30 patients were allocated to the RAL+DRV/r and the LPV/r+TVD arm, respectively (Figure 1). One patient in the RAL+DRV/r arm withdrew consent before starting the allocated regimen, thus was excluded from the analysis. The baseline demographics and characteristics of the participating patients are listed in Table 1. Most patients were men who have sex with men, with well-maintained CD4 count. Patients of the LPV/r+TVD arm were younger (p=0.040) and had lower CD4 count (p=0.029) than those of the RAL+DRVr arm. All other major variables were similar between the two arms.

Table 1. Baseline characteristics of the enrolled patients.

	RAL+DRV/r (n=28)	LPV/r+TVD (n=30)	P value
Sex (male), n (%)	28 (100)	29 (97)	1.000
Age (years)†	44 (37-51)	39 (34-45)	0.040
CD4 count (/μl)†	549 (384-710)	456 (330-592)	0.029
Route of transmission (homosexual contact), n (%)	27 (96)	24 (80)	0.151
History of AIDS, n (%)	10 (36)	11 (37)	1.000
Body weight (kg)†	66 (59-75)	66 (59-72)	0.502
Body mass index (kg/m²)†	22 (21-25)	22.6 (19.9-24.6)	0.440
eGFR by JSN equation (ml/min/1.73 m²)†	87 (76-103)	85 (70-90)	0.356
eGFR by CG equation (ml/min)†	119 (88-143)	108 (89-120)	0.456
Serum creatinine (mg/dl)†	0.78 (0.70-0.87)	0.76 (0.67-0.83)	0.184
Urinary albumin (mg/g Cre)†	8 (6-27)	7 (5-12)	0.075
Urinary β2 microglobulin (μg/g Cre)†	452 (178-1566)	424 (204-2275)	0.234
Tubular resorption of phosphate (%)†	92 (87-93)	90 (86-94)	0.886
NAG (U/g Cr)†	6.2 (3.7-11.6)	5.2 (3.7-8.3)	0.183
Hypertension, n (%)	2 (7)	1 (3)	0.605
Dyslipidemia, n (%)	17 (61)	8 (27)	0.016
Diabetes mellitus, n (%)	0 (0)	1 (3)	1.000
Current smoking, n (%)	13 (46)	13 (43)	1.000
Hepatitis C, n (%)	0 (0)	0 (0)	N/A
Duration of tenofovir use (weeks)	163 (109-224)	124 (85-212)	0.721

Hypertension was defined by current treatment with antihypertensive agents or systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg. Dyslipidemia was defined by current treatment with lipid-lowering agents or low-density lipoprotein cholesterol >140 mg/dl, high-density lipoprotein cholesterol <40 mg/dl, total cholesterol >240 mg/dl, or triglyceride >500 mg/dl. IQR: interquartile range, AIDS: acquired immunodeficiency syndrome, eGFR: estimated glomerular filtration rate, LDL: low-density lipoprotein, JSN: the Japanese Society of Nephrology equation [37], CG: Cockcroft-Gault equation [35]

† median (interquartile range)

Primary endpoint

At week 48, six patients (25%) out of 24 in the RAL+DRV/r arm and 3 patients (11%) out of 28 in the LPV/r+TVD arm, experienced >10% improvement in eGFR from baseline, and the difference was not statistically significant (p=0.272, 95% CI -0.067 to 0.354). Sensitivity analysis with three other equations for eGFR (JSN, CKD-EPI, and MDRD) showed the same results; no difference in the proportion of patients with improvement of >10% in eGFR was noted between the two arms (JSN equation: 4/24 in RAL+DRV/r, 3/29 in LPV/r+TVD, p=0.688, 95% CI -0.126 to 0.267) (CKD-EPI equation: 2/24 in RAL+DRV/r, 2/29 in LPV/r+TVD, p=1.000, 95% CI -0.148 to 0.197) (MDRD equation: 5/24 in RAL+DRV/r, 3/29 in LPV/r +TVD, p=0.444, 95% CI -0.093 to 0.313) (Table 2).

Additional analysis showed that the percent improvement in eGFR from the baseline to week 48 calculated using all four equations was not significantly different between the two arms [CG equation: difference in mean % improvement (DRV/r+RAL versus LPV/r+TDF/FTC) -8.7%, 95% CI -18.2 to 0.8, p=0.071] (JSN equation: -1.1%, -6.9 to 4.8, p=0.720) (CKD-EPI equation: -1.6%, 95% CI -4.7 to 1.6, p=0.323) (MDRD equation: -1.1%, 95% CI -6.9 to 4.8, p=0.722) (Table 2). Thus, this study demonstrated that switching to NRTI-sparing regimen of RAL+DRV/r did not increase the proportion of patients who showed >10% improvement in eGFR, compared to continuation of LPV/r+TVD.

Secondary renal endpoints

Among the four renal tubular markers used in this study, the improvement in urinary β2 microglobulin from baseline to week 48 was significantly larger in the RAL+DRV/r arm (n=23) than

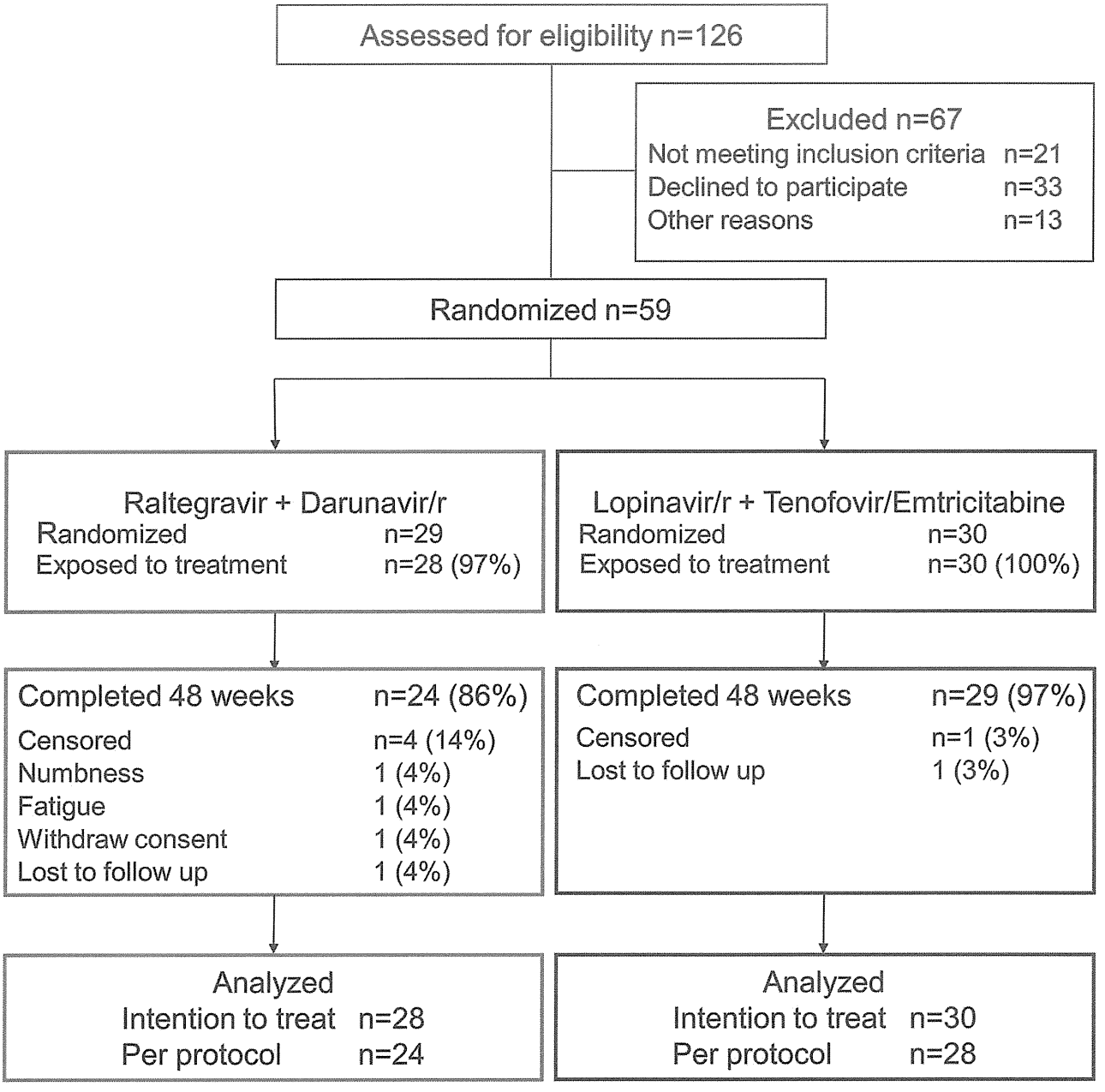

Figure 1. Enrollment, randomization, and disposition of patients. Darunavir/r, ritonavir-boosted darunavir; Lopinavir/r, ritonavir-boosted lopinavir.

doi: 10.1371/journal.pone.0073639.g001

in the LPV/r+TVD arm (n=28) (-271 versus -64 µg/g Cr, p=0.026) (Figure 2A). However, urinary albumin, the percent tubular resorption of phosphate, and NAG showed little change from baseline, and the observed changes were not significantly different between the two arms (Figure 2B, C, D).

Secondary efficacy endpoints

Among the per protocol population, the proportion of patients with HIV RNA <50 copies/mL was 96.2% for the RAL+DRV/r arm and 96.7% for the LPV/r+TVD arm at week 24, with a difference of -0.5% (95% CI, -10% to 9%), and 100% for the both arms at week 48, with a difference of 0% (95% CI -0.1 to 0.1) (Figure 3A). ITT analysis showed that the proportion was 89.3% and 96.7% for the RAL+DRV/r and LPV/r+TVD arms, respectively, at week 24, with a difference of -7% (95% CI, -21% to 6%), and 85.7% and 96.7%, respectively, at week 48, with a difference of -11% (95% CI, -25% to 4%) (Figure 3B). There was no significant difference in viral efficacy between the

Table 2. Proportion of patients with >10% and mean percent improvement in eGFR at 48 weeks from the baseline calculated by the four equations.

	Cases with >10% increase from baseline	P value (95% CI)	Mean % improvement in eGFR from baseline	Difference in mean % improvement (95% CI) (DRV/r + RAL versus LPV/r + TDF/FTC)	P value
CG equation					
DRV/r + RAL	6/24	0.272 (-0.067 to 0.354)	5.4%	-8.7% (-18.2 to 0.8)	0.071
LPV/r + TDF/FTC	3/28		-3.3%		
JSN equation					
DRV/r + RAL	4/24	0.688 (-0.126 to 0.267)	2.5%	-1.1% (-6.9 to 4.8)	0.720
LPV/r + TDF/FTC	3/29		1.5%		
CKD-EPI equation					
DRV/r + RAL	2/24	1.000 (-0.148 to 0.197)	1.9%	-1.6% (-4.7 to 1.6)	0.323
LPV/r + TDF/FTC	2/29		1.7%		
MDRD equation					
DRV/r + RAL	5/24	0.444 (-0.093 to 0.313)	2.7%	-1.1% (-6.9 to 4.8)	0.722
LPV/r + TDF/FTC	3/29		1.7%		

DRV/r: ritonavir-boosted darunavir, RAL: raltegravir, LPV/r: ritonavir-boosted lopinavir, TDF: tenofovir, FTC: emtricitabine, CG: Cockcroft-Gault equation [35], JSN: the Japanese Society of Nephrology equation [37], CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation adjusted for the Japanese[38], MDRD: the Modification of Diet in Renal Disease equation adjusted with coefficient for the Japanese [37]

two arms at weeks 24 and 48. At week 48, all patients of the RAL+DRV/r arm on the allocated regimen (n=24) had a viral load of <50 copies/mL.

Safety and tolerability

One patient from each arm was lost to follow-up. Three patients of the RAL+DRV/r arm discontinued the allocated regimen by week 48 (one discontinued the regimen at week 4 due to weakness in the lower extremities and one at week 24 because of fatigue, which was later found to be related to acute hepatitis B infection). The other patient withdrew consent at week 24, because it was easier for him to maintain a good medication adherence with once-daily LPV/r+TVD (the regimen the patient used before enrollment). None of the patients of the LPV/r+TVD arm discontinued the allocated regimen by week 48. Thus, at week 48, 24 patients (86%) out of 28 in the RAL +DRV/r arm and 29 (97%) of 30 in the LPV/r+TVD arm, were on the allocated regimens.

The following grade 3 or 4 laboratory data or abnormal symptoms that were at least one grade higher than the baseline were encountered in this study: RAL+DRV/r arm: a rise in ALT (due to acute hepatitis B infection, n=1), and elevated LDL-cholesterol (n=3), LPV/r+TVD arm: elevated LDL-cholesterol (n=1), and hypophosphatemia (n=3). The above side effects did not lead to discontinuation of the study drugs.

Discussion

This randomized trial elucidated the recovery of TDF-associated nephropathy after discontinuation of TDF. The results demonstrated no significant increase in the proportion of patients who showed >10% improvement in eGFR after switching to NRTI sparing regimen of RAL+DRV/r, compared to continuation of LPV/r+TVD. This finding could be due to any of the following reasons; 1) Relatively preserved baseline renal function of the enrolled patients, with a median eGFR of 86 ml/min/1.73 m^2 (IQR 75-97, JSN equation), with only one patient with CKD stage 3 due to persistent +1 proteinuria, and no patients with stage 4 or more. Although the number of patients is relatively small, a previous pilot study of 21 patients reported improvement of eGFR (by CG equation) in most patients after switching from PI/r+TVD to PI/r+RAL in patients with proteinuria and suppressed HIV viral load [39]. Thus, improvement of eGFR after discontinuation of TDF might be more significant in patients with severe to moderately impaired renal function. Larger studies are needed to investigate this issue thoroughly. 2) Study patients had been on TDF for a long period of time at enrollment (median: 136 weeks, range 27-370 weeks, 72% were on TDF for more than 2 years), although shorter duration of TDF therapy is likely to be associated with greater eGFR improvement after discontinuation [26]. Furthermore, because TDF-induced renal dysfunction is mainly observed during the first 6 months after commencement of such therapy [18,19,40], it is possible that patients who developed severe renal dysfunction soon after starting TDF might have already discontinued TDF and therefore not included in the study.

Although the present study did not show an increase in eGFR after discontinuation of TDF, it is noteworthy that the value of urinary β2 microglobulin, a sensitive marker for TDF-induced tubulopathy [41,42], improved significantly in the RAL +DRV/r arm compared to LPV/r+TVD, even in patients with relatively preserved eGFR. It is of importance considering that

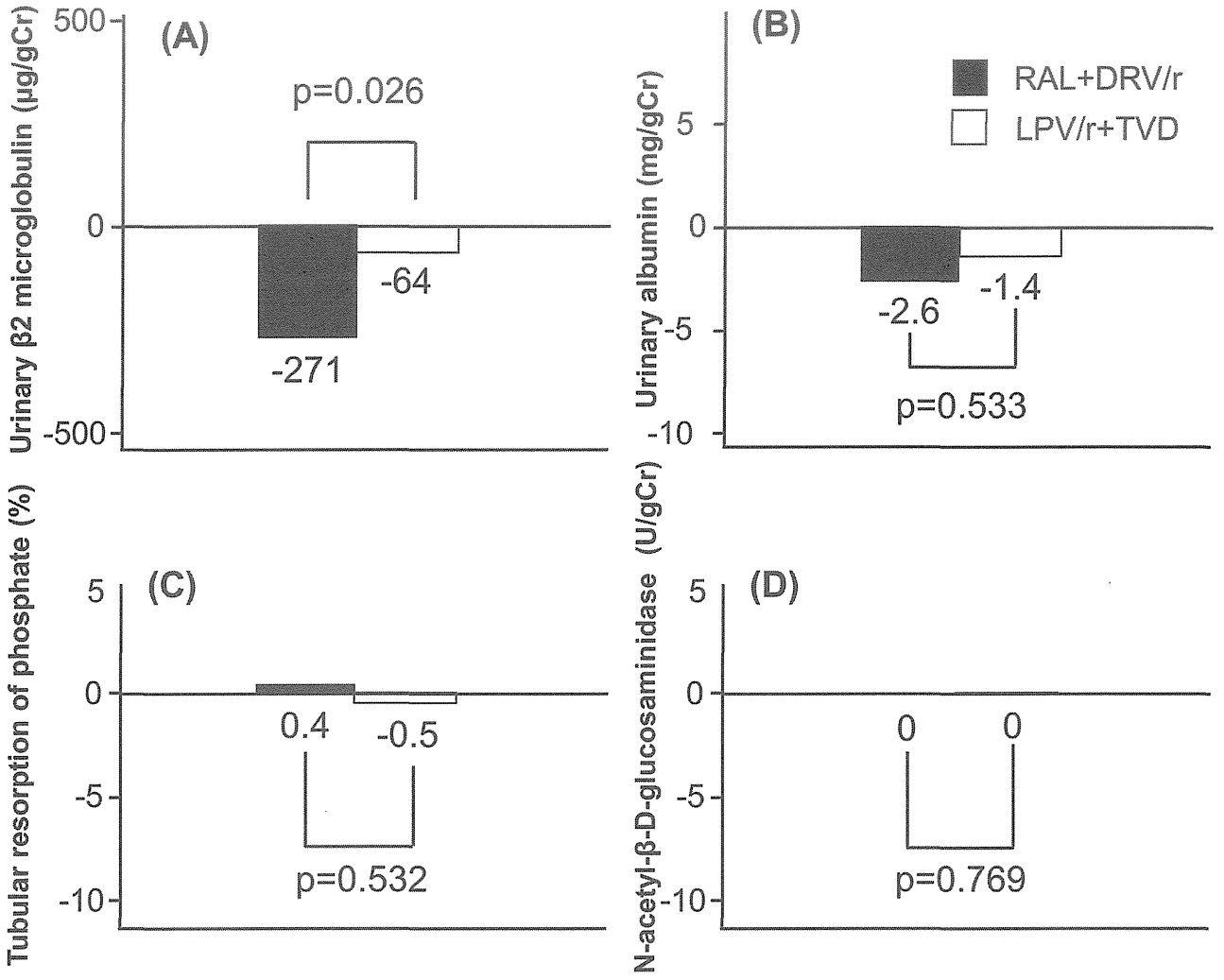

Figure 2. Median changes in markers of renal tubular function between baseline and 48 weeks. (A) Urinary β2 microglobulin, (B) Urinary albumin, (C) Percent tubular resorption of phosphate, (D) Urinary N-acetyl-β-D-glucosaminidase. RAL, raltegravir; DRV/r, ritonavir-boosted darunavir; LPV/r, ritonavir-boosted lopinavir; TVD, fixed dose of tenofovir/emtricitabine.

doi: 10.1371/journal.pone.0073639.g002

proximal tubulopathy is associated with bone mineral density abnormality and possible long-term nephrotoxic effect [17,43-45]. Further large and long-term studies are needed to elucidate the long-term impact of TDF-induced tubulopathy on GFR.

With regard to the viral efficacy and safety of RAL+DRV/r, all patients in that arm who continued the allocated regimen accomplished viral suppression of <50 copies/ml at week 48 (n=24). Only one (3.6%) patient discontinued RAL+DRV/r due to a side effect possibly related to RAL+DRV/r (weakness of the lower extremities), confirming the safety of this combination. To our knowledge, this is the first study to examine the viral efficacy of RAL+DRV/r in patients with suppressed viral load. The KITE study, an industry-sponsored pilot study, examined the viral efficacy of RAL+LPV/r in patients with suppressed viral load [46]. However, LPV/r is placed as an alternative PI in the American Department of Health and Human Services Guidelines, mainly because of the higher rates of gastrointestinal side effects and hyperlipidemia compared with other PIs (URL: http://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf). Because the number of enrolled patients is relatively small and this study does not have sufficient power to elucidate viral efficacy, further studies are needed to confirm the viral efficacy of RAL+DRV/r in patients with suppressed viral load. If the NRTI sparing regimen of RAL+DRV/r is proved to be efficacious in maintaining viral suppression in treatment-experienced patients, switching to this combination for patients with suppressed viral load should become an attractive treatment option for patients who cannot tolerate NRTI toxicity or to prevent further NRTI toxicity.

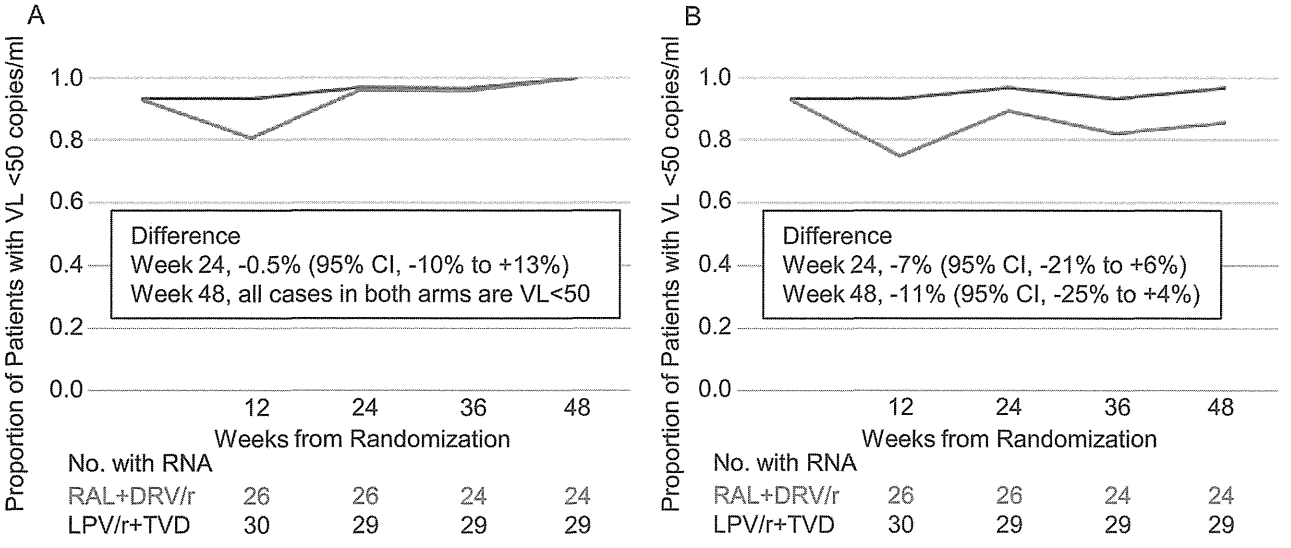

Figure 3. Proportion of patients with HIV RNA <50 copies/ml at 24 and 48 weeks. (A) Per protocol analysis. (B) Intention-to-treat analysis. VL, viral load; RAL, raltegravir; DRV/r, ritonavir-boosted darunavir; LPV/r, ritonavir-boosted lopinavir; TVD, fixed dose of tenofovir/emtricitabine.

doi: 10.1371/journal.pone.0073639.g003

Several limitations must be acknowledged. First, as mentioned above, this trial has sufficient power for the primary endpoint only; other results should be interpreted with caution. Further larger studies are needed to confirm the improvement in urinary β2 microglobulin after switching ritonavir-boosted PI to NRTI sparing regimen of RAL+DRV/r and the viral efficacy of RAL+DRV/r in patients with suppressed viral load. Second, the enrolled patients had relatively preserved renal function. This was a study-design related issue; patients with severely impaired eGFR, the population in whom TDF nephrotoxicity can be reversible is clinically important, were excluded from the study. Based on the study design and need for randomization, patients of one arm needed to continue treatment with TDF, and it was considered ethically inappropriate to have patients with impaired renal function to continue TDF. Third, all study subjects were Japanese and almost exclusively men (mostly men who have sex with men). Further studies are needed to determine whether the findings of this study are also applicable to females, patients with different routes of transmission, and patients of different racial background.

In conclusion, this trial showed that discontinuation of LPV/r +TVD and switching to NRTI-sparing regimen of RAL+DRV/r did not result in improvement of renal function among patients with relatively preserved eGFR and suppressed HIV viral load. However, urinary β2 microglobulin, a sensitive marker of TDF-induced tubulopathy, improved after discontinuation of TDF plus ritonavir-boosted PI, suggesting switching TDF to NRTI sparing regimen might be beneficial in the long-term. RAL +DRV/r showed favorable viral efficacy and safety in patients with suppressed viral load, but further larger studies are needed to confirm the viral efficacy of this combination.

Supporting Information

Protocol S1. Trial protocol.
(DOCX)

Checklist S1. CONSORT checklist.
(DOC)

Acknowledgements

We thank the patients for participation in this study.
The SPARE trial team includes the following members:
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