Research Letter

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Efficacy and safety of once-daily ritonavir-boosted darunavir plus abacavir/lamivudine for treatment-naïve patients: A pilot study

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The efficacy and safety of once-daily darunavir/ritonavir plus fixed-dose abacavir/lamivudine was examined in 22 treatment-naïve patients with HIV-1 infection. Three patients discontinued antiretroviral therapy due to mild adverse events. Among 18 patients who continued therapy, 66.7% had viral load <50 copies/ml at week 48. Only two patients experienced virologic failure with the emergence of resistant virus. This pilot study demonstrated the viral efficacy and safety of darunavir/ritonavir plus abacavir/lamivudine.

Introduction

Only little information is available on the efficacy and safety of the combination antiretroviral therapy (ART) of ritonavir-boosted darunavir (DRV/r) plus fixed-dose abacavir/lamivudine (ABC/3TC) [1]. DRV/r is a protease inhibitor (PI) with proven efficacy and safety as well as with a high barrier to drug resistance [2,3]. ABC/3TC is an alternative choice of nucleoside reverse transcriptase inhibitor (NRTI) backbone in the American Department of Health and Human Services (DHHS) Guidelines and is the other preferred backbone regimen for treatment-naïve patients in other international guidelines [4,5]. In this pilot study, we evaluated the efficacy and safety of DRV/r plus ABC/3TC for treatment-naïve patients in a single-center, observational cohort.

Methods

The subjects of this retrospective study were all treatment-naïve patients with HIV infection who commenced once-daily DRV/r plus fixed dose ABC/3TC from November 2009 (when the first patient commenced such regimen at our clinic) to November 2010 at our clinic (AIDS Clinical Center, Tokyo, Japan).

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All patients were followed for at least 48 weeks after commencement of treatment at our facility. Baseline data, including age, sex, mode of infection, ethnicity, CD4 count, and HIV viral load, were collected from the medical charts. The Cobas TaqMan HIV-1 real-time PCR version 1.0 assay (Roche Diagnostics, NJ) was used to measure HIV-1 viral load throughout the research period. For those who discontinued either DRV/r or fixed dose ABC/3TC before reaching 48 weeks, the reasons for discontinuation were collected. All patients provided written informed consent for the data to be published. Primary outcomes were the proportion of patients with viral load <50 copies/ml at 24 and 48 weeks. Safety parameters through 48 weeks were also collected.

Results

The study included 22 patients [1 (4.6%) female] of East Asian origin, with a median age of 34.5 years [interquartile range (IQR) 27.5-43.8]. The route of transmission was homosexual intercourse 86.3%, heterosexual 9%, and unknown in one patient. HLA was examined in 20 patients and all were HLA-B*5701-negative. Twenty one patients had HIV-1 drug-resistant testing before commencement of ART and none had resistant mutations related to NRTIs, PIs, or non-NRTIs. At baseline, the median CD4 count was 47/μl (IQR 27.5-187-8) while the HIV viral load was 5.61 log10 copies/ml (IQR 4.57-6.01 log10 copies/ml). In 3 patients, ART was either changed or discontinued during the study due to adverse events [skin rash (n = 1), vomiting (n = 1), and limb paresthesia (n = 1)] and one patient changed the regimen due to concern with drug interactions with antipsychotics before 48 weeks. The skin rash was due to darunavir, because the rash disappeared after switching darunavir to raltegravir, while continuing ABC/3TC. This patient was HLA-B*5701-negative. None presented with ABC-associated hypersensitivity or with grade 3 or 4 liver enzyme elevation.

On-treatment analysis of the 18 patients (excluding the above 4 patients who discontinued the regimen) showed 72.2% had viral load <50 copies/ml at week 24 (88.9% viral load <200 copies/ml), and 66.7% had viral load <50 copies/ml at week 48 (88.9% viral load <200 copies/ml). Intention-to-treatment analysis showed 59.0% with viral load <50 copies/ml at week 24 (77.3% viral load <200 copies/ml), and 54.6% with viral load <50 copies/ml at

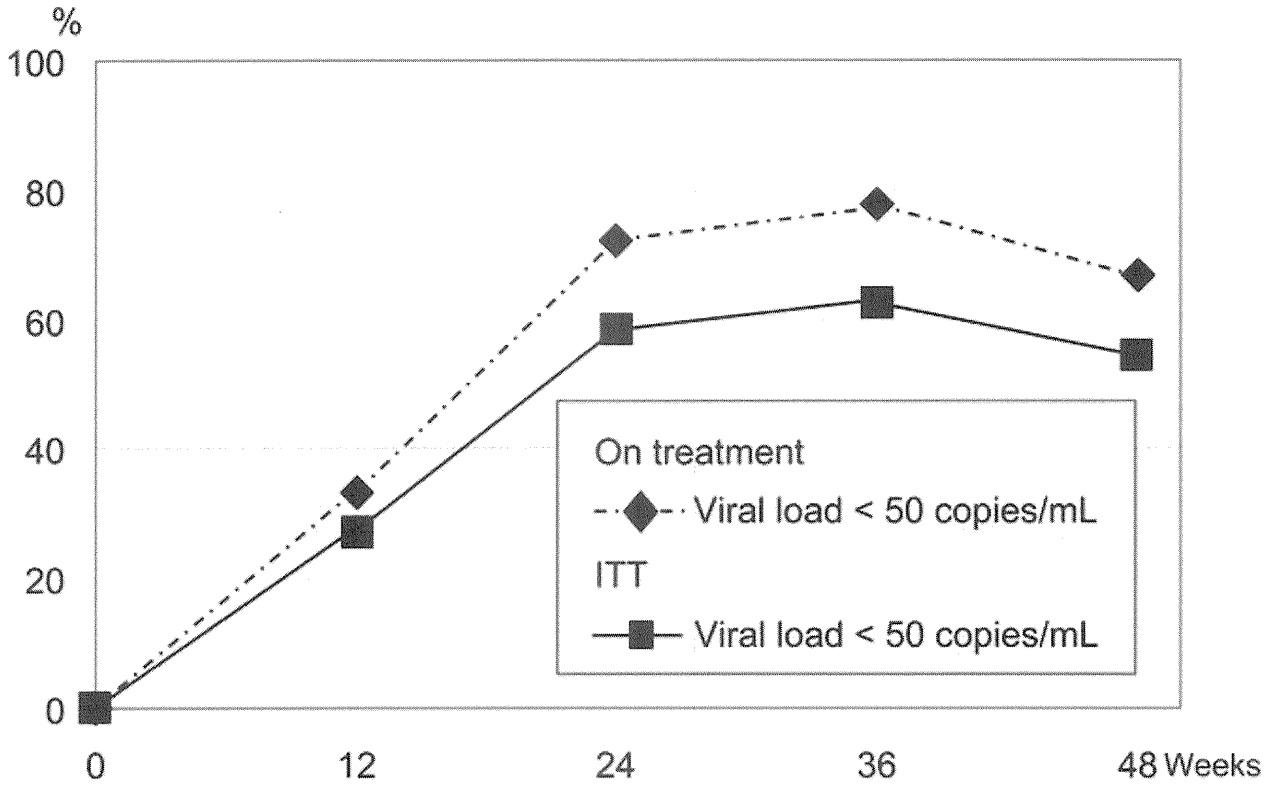

Fig. 1. Proportions of patients with viral load <50 copies/mL at 48 weeks with on-treatment and intention-to-treat (ITT) analysis.

week 48 (72.7% viral load <200 copies/ml) (Fig. 1). Four patients showed rebounds >200 copies/ml (<1000 copies/ml) after 24 weeks; two of them were single rebounds and considered blips. The other two patients showed two consecutive viral load >200 copies/ml, fulfilling the criteria of virological failure (11.1% at 48 weeks). The latter two patients underwent genotypic resistance test that detected in one case the reverse transcriptase mutation M184V and in the other the protease mutation M46I.

In the 12 patients with baseline viral load >100,000 copies/ml, on-treatment analysis showed viral load of < 200 copies/ml at 24 weeks in 10 (83.3%) patients, and <50 copies/ml at both 24 and 48 weeks in 7 (58.3%). In comparison, all 6 patients with baseline viral load <100,000 copies/ml showed suppression of the load to < 50 copies/ml at both 24 and 48 weeks. The median increment in CD4 count at 48 weeks was 187/μl (IQR 82.5-264.5/μl).

Discussion

To our knowledge, this is the first published report on the efficacy and safety of the combination of once-daily DRV/r plus fixed dose ABC/3TC in treatment-naïve patients. This combination ART resulted in viral suppression although the baseline viral load was >100,000 copies/ml in 66.6% of the patients. Only 13.6% discontinued this regimen due to adverse events before 48 weeks and none of the adverse events was serious. Considering that most patients in this cohort were at advanced stage of HIV infection with low median baseline CD4 count of 47/μl, we conclude that DRV/r plus ABC/3TC is a safe and efficacious combination ART.

The DHHS guidelines for the treatment of HIV infection in the U.S. list ABC/3TC as alternative NRTIs since abacavir can potentially cause serious hypersensitivity reaction in 5-8% of the patients and its viral efficacy in patients with baseline viral load of >100,000 copies/mL is inferior to fixed-dose tenofovir/emtricitabine (TDF/FTC) when used with efavirenz or ritonavir-boosted atazanavir as a key drug [4,6]. However, the incidence of ABC-related hypersensitivity is low among HLA-B*5701-negative population, such as the Japanese [7,8]. Moreover, HEAT study demonstrated that the viral efficacy of ABC/3TC was not inferior to that of TDF/FTC when used with lopinavir/ritonavir for treatment-naive patients [9]. Taking this background into account, once-daily DRV/r plus ABC/3TC could be a good alternative, especially in patients with low prevalence of HLA-B*5701 who cannot tolerate tenofovir due to its nephrotoxicity [10].

In conclusion, this single-center pilot study demonstrated the viral efficacy and safety of once-daily DRV/r plus ABC/3TC in treatment-naïve patients with HIV-1 infection. This regimen could be a suitable alternative to DRV/r plus tenofovir/emtricitabine or other first line regimens. Nevertheless, the number of patients in this cohort is too small to allow firm conclusions and further studies of larger samples, ideally a clinical trial that compares the viral efficacy of TDF/FTC to ABC/3TC with once-daily DRV/r, are needed to elucidate this issue.

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Conflict of Interest and Source of Funding

Author contributions: All of the authors contributed to the conception and design of the study and/or the analyses and interpretation of the data. The manuscript was drafted by T.N., H.G.and S.O. and was critically reviewed and subsequently approved by all authors. The authors declare no conflict of interest.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Open-Label Randomized Multicenter Selection Study of Once Daily Antiretroviral Treatment Regimen Comparing Ritonavir-Boosted Atazanavir to Efavirenz with Fixed-Dose Abacavir and Lamivudine

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Abstract

Background The side-effects of anti-retroviral drugs are different between Japanese and Caucasian patients. Severe central nerve system (CNS) side-effects to efavirenz and low rate of hypersensitivity against abacavir characterize the Japanese.

Objective The objective of this study was to select a once daily regimen for further non-inferior study comparing the virological efficacy and safety of the first line once daily antiretroviral treatment regimens in the current HIV/AIDS guideline.

Methods The study design was a randomized, open label, multicenter, selection study. One arm was treated with efavirenz and the other with ritonavir-boosted atazanavir. A fixed-dose lamivudine plus abacavir were used in both arms. The primary endpoint was virologic success (viral load less than 50 copies/mL) rate at 48 weeks. Patients were followed-up to 96 weeks with safety as the secondary endpoint. Clinicaltrials.Gov (NCT 00280969) and the University hospital Medical Information Network (UMIN000000243).

Results A total of 71 participants were enrolled. Virologic success rates in both arms were similar at week 48 [efavirenz arm 28/36 (77.8%); atazanavir arm 27/35 (77.1%)], but were decreased at week 96 to 55.6% in the efavirenz arm and 68.8% in the atazanavir arm (p=0.33). At the 96-week follow-up, 52.8% of the EFV arm and 34.3% of the ATV/r arm reached total cholesterol more than 220 mg/dL and required treatment. None of the patients developed cardiovascular complications in this study by week 96.

Conclusion There was no significant difference in the efficacy of efavirenz and ritonavir-boosted atazanavir combined with lamivudine plus abacavir at 48 weeks. The evaluation of safety was extended to 96 weeks, which also showed no significant difference in both arms.

Key words: HIV, antiretroviral treatment, efavirenz, atazanavir, abacavir, lamivudine

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Introduction

The use of a non-nucleoside transcriptase inhibitor (NNRTI) or ritonavir-boosted protease inhibitor as the key drug, combined with two nucleoside reverse-transcriptase inhibitors (NRTI), as the backbone drugs, is recommended as an initial therapy in human immunodeficiency virus type 1 (HIV-1) infection. For the key drug, when efavirenz (EFV) or ritonavir-boosted atazanavir (ATV/r) is selected, once daily therapy is possible. EFV is a widely used NNRTI, however, in some clinical studies conducted in Asia, a higher rate of adverse events, especially central nervous system-related symptoms, has been noted (1-3).

In terms of backbone drugs, didanosine (ddI), stavudine (d4T) and zidovudine (ZDV) were widely used NRTIs.

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However, their mitochondrial toxicity made long-term use difficult (4-7). Due to HLA-B*5701-related hypersensitivity, abacavir (ABC) is listed as the second line drug under the United States Department of Health and Human Services (DHHS) guidelines. However, HLA-B*5701 is quite rare among Japanese, and thus the incidence of hypersensitivity to ABC in Japanese patients is lower than that of Caucasians (8-10). Although tenofovir (TDF) is widely used as the first line drug, the dose-dependent nephrotoxicity is a major concern in Japanese because Japanese body weight is lighter than that of Caucasians (11, 12).

The present study was designed in 2006, when the combination of TDF, lamivudine (3TC) or entiricitabine (FTC), and EFV was the first line regimen of antiretroviral treatment (13). To explore the optimal antiretroviral combination for the best clinical outcome among Japanese HIV-1 patients (14), a selection study was designed to compare the efficacy and safety of once daily treatment with EFV or ATV/r combined with a fixed-dose ABC and 3TC (ABC/3TC).

Objective

The objective of this study was to select a once daily regimen for further non-inferior study comparing the virological efficacy and safety of the first line once daily antiretroviral treatment regimens in the current HIV/AIDS guideline.

Subjects and Methods

Study design

The study was designed as a randomized, open label, multicenter selection study, which means the superior regimen at the end point is to be selected as alternate arm to compare with the current first line regimen in the next step. Therefore, this study was not to compare superiority or non-inferiority of both arms. As the selection study, the main objective is to select a treatment regimen for further pivotal study and the secondary objective is safety. The primary endpoint was the proportion of patients in each arm who achieved virologic success (HIV-1 RNA less than 50 copies/mL in plasma) at week 48. The secondary endpoints were death, AIDS and serious non-AIDS events, non-AIDS defining cancer, treatment-related serious or grade 3 to 4 adverse events, and discontinuation of antiretroviral treatment before week 96.

The inclusion criteria of this study were those who were treatment-naïve, HIV-1 positive Japanese men with a CD4+ count ranging from 100 to 300 cell/mm^3. The exclusion criteria included current active AIDS, acute retroviral syndrome and persistent active hepatitis B infection (HBs-Ag positive). Patients with a history of 3TC treatment for hepatitis B infection were also excluded. After obtaining informed consent, eligible participants were randomized into once daily 600 mg EFV or 100 mg RTV and 300 mg ATV (EFV arm vs ATV/r arm). All participants received a fixed dose of 600 mg of ABC and 300 mg 3TC (ABC/3TC).

At baseline, the demographic characteristics and a complete medical history were recorded, physical examination was performed, and various laboratory tests were obtained (CD4+ count, HIV-1 RNA, complete blood count, biochemistry, liver and renal function tests, and total cholesterol). Participants were examined at baseline, then every 4 weeks until week 96. Careful clinical examination was provided at each visit, including history taking of any adverse event, adherence to treatment, and physical examination. Furthermore, blood tests were obtained including complete blood count, biochemistry, liver and renal function tests, CD4+ count and HIV-1 RNA. When HIV-1 RNA became less than 50 copies/mL, participants were rescheduled to be seen every 4 to 12 weeks. All participants underwent clinical examination at week 48 as the primary endpoint, then every 12 weeks until week 96 as the secondary follow-up period for evaluation of safety.

The study recruitment period was started on September 1st of 2005 for 2 years. The study protocol was originally designed to follow patients for 48 weeks, however, during the study period, cardiovascular adverse events of ABC-containing regimen were reported (15, 16). Considering the importance of adherence to safety, the follow-up period was extended to 96 weeks.

Independent data and safety monitoring board reviewed virology and safety data by treatment allocation were obtained when all participants had completed 24 weeks of the study. A total of 18 academic medical institutions in Japan participated in this study. The study protocol was approved by the ethics committee of each site and was registered at Clinicaltrials. Gov (NCT00280969) and the University Hospital Medical Information Network (UMIN000000243).

Statistical analyses

The estimated proportion of virologic failure, representing HIV-1 RNA of more than 50 copies/mL at 48 weeks of treatment, was 30% over one year. To choose one treatment group with a probability of 0.90, if it is superior to another treatment by >10%, if any, a sample size of 40 participants per group was necessary according to the selection design (17).

To assess differences in proportions, we used Fisher's exact test and calculated exact confidence intervals (CIs). We conducted intent-to-treat analysis and used the T test to compare the efavirenz arm and the ritonavir boosted atazanavir arm, unless the data showed skewed distribution, in which case the Wilcoxon's test was used. All analyses used a two-sided alfa of 0.05. No adjustment for each test was made for multiple comparisons due to the fact that we have several tests to compare the efficacies and safeties of two groups. All analyses, unless otherwise specified, were determined a priori and were hypothesis driven. Statistical analyses were performed using SAS version 9.1.

Table 1. Baseline Characteristics of Participants

Variable	efavirenz	atazanavir/r	p
Number of patients	36	35	NS
Age (yrs) median	35	36	NS
HIV-RNA (log_{10} copies/mL)			
median	4.6	4.4	NS
range	2.8–5.4	3.0–5.3	
CD4 count (cells/mm^3)			
median	220	226	NS
range	121–323	103–324	
Total Cholesterol (mg/dL)			
median	155.5	159.5	NS
range	122–208	112–215	
Total bilirubin (mg/dL)			
median	0.6	0.5	NS
range	0.3–1.7	0.3–1.5	
ALT (IU/L)			
median	24	20	NS
range	8–71	8–78	
Creatinine (mg/dL)			
median	0.80	0.75	NS
range	0.6–1.03	0.6–1.02	

Results

Participants

In the study recruitment period, 71 participants were randomly assigned to two groups (36 in EFV arm and 35 in ATV/r arm). The baseline characteristics of the subjects are listed in Table 1. Among the 71 participants, 62 (87.3%) for the primary endpoint and 58 (80.6%) for the secondary endpoint completed the study protocol. By week 96, 9 participants had withdrawn due to clinical events, 2 declined to continue the study for personal reasons, one died by accident and 3 were transferred to other non-participating institutions.

Primary endpoint

At week 48, by intent-to-treat, missing-equals-failure analysis, 28 of 36 participants (77.8%, 95% CI: 60.9-89.9) in the EFV arm and 27 of 35 (77.1%, 95% CI: 59.9-89.9) in the ATV/r arm achieved the goal of HIV-1 RNA less than 50 copies/mL. There was no significant difference between the two arms (p=0.95).

Virologic success over time

Figure 1 shows the intent-to-treat analysis of participants who reached virologic success. At week 96, the rates of virologic success in the EFV arm were 55.6% (20 of 36) and 68.6% (24 of 35) in the ATV/r arm (p=0.33). The number of participants with a baseline HIV-1 RNA level of more than 100,000 copies/mL was 5 in the EFV arm and 2 in the ATV/r arm. One participant in each arm withdrew from the study at week 4 due to skin rash. The rest of the participants achieved virologic success in the EFV arm (4 out of 4) and in ATV/r arm (1 out of 1).

Secondary endpoints

In the EFV arm, 7 of 36 participants did not complete the study; 5 of the 7 developed psychiatric symptoms, including suicidal idealization, insomnia and irritation, 2 developed skin rashes and the remaining 2 were lost to follow-up because they were transferred to non-affiliated hospitals. In the ATV/r arm, 6 of 35 patients could not complete the study; one died by accident for unknown reason (the cause of death according to the coroner's report was not related to the cardiovascular system), 2 participants required treatment change (this was due to suicidal idealization in one and to skin rash in the other), one participant withdrew by own wish, one enrolled into another study, and one was transferred to another non-affiliated medical care facility.

Figure 2 shows the change of total cholesterol, liver function and total bilirubin from the baseline. At enrollment in the study, the median total cholesterol in the EFV arm was 155.5 mg/dL (range: 122-208) and in the ATV/r arm was 159.5 mg/dL (range: 112-215). The total cholesterol was not more than 220 mg/dL in any of the participants of both arms at baseline, and there was no significant difference between the two arms. During the study period, the total cholesterol increased to more than 220 mg/dL and required treatment with hypolipidemic agents in 52.8% of the EFV arm and 34.3% of the ATV/r arm. There was a significant increase in total cholesterol from the baseline in both arms ($p < 0.05$). There was no significant change in liver function tests during the study. New onset grade 3 hyperbilirubinemia was noted in 27 of 35 (77.1%) of the ATV/r arm but in none of the EFV arm. None of the hyperbilirubinemia in the ATV/r arm was associated with altered liver function, altered renal function, nephrolithiasis, or cholelithiasis.

Discussion

This study was designed as selection study, which means the superior regimen at the endpoint is to be selected as an alternate arm to compare with the current first line treatment in the next step. By definition of the selection study, the superior arm does not require statistical significance (17). At week 48, 77.8% of ATV/r arm and 77.1% of EFV arm reached HIV-VL of less than 50 copies/mL. Based on the definition of the selection study, the combination ABC/3TC/EFV was selected to compare the current first line treatment while the efficacy of each arm was almost even in this study.

In this clinical trial of 71 participants over a period of 96 weeks, no cardiovascular events or severe hypersensitivity reaction against ABC was observed. In this study, the efficacy of EFV combined with ABC/3TC and ATV/r combined with ABC/3TC was similar. Therefore, ABC based regimen can be selected as a safe combination to compare the efficacy of the first line combinations, such as EFV plus TDF/FTC or ATV/r plus TDF/FTC (18-20), in the next step for the best clinical benefits in Japanese patients.

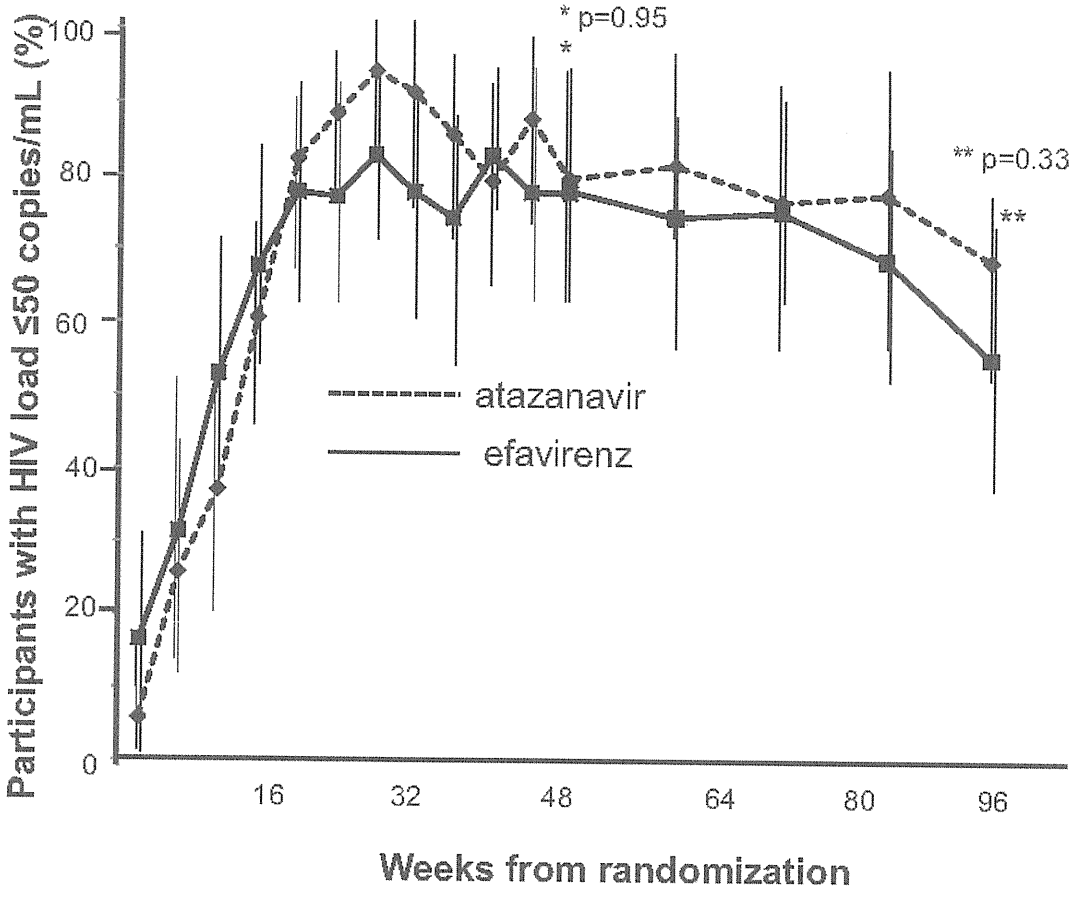

Figure 1. **Proportions of participants with HIV-RNA less than 50 copies/mL.**
The efficacies of the efavirenz arm and ritonavir-boosted atazanavir arm were compared with intent-to-treat analysis. There were no significant difference between arms at both week 48 (p=0.95) and week 96 (p=0.33).

In February 2008, the United States National Institution of Allergy and Infectious Disease announced that the data and safety monitoring board of ACTG 5202 recommended a modification of the study design because they found that among participants with high viral loads (100,000 or more copies/mL) at the time of screening, treatment combinations that included ABC/3TC were not as effective in controlling the virus as those of regimens containing TDF/FTC (19, 21). At that point, all of the present 71 participants were already enrolled in the study and the baseline HIV-1 RNA of 7 participants was more than 100,000 copies/mL. Of these 7 participants, 2 had already withdrawn from the study by week 4, and the rest of participants had reached HIV-1 RNA of less than 50 copies/mL. The safety monitor board made no recommendation to amend the protocol.

As a primary endpoint, 77.8% of the EFV arm and 77.1% of the ATV/r had reached virological success, however, total cholesterol in 58.1% of the EFV arm and 46.9% of the ATV/r arm increased to more than 220 mg/dL, which required treatment. Thus, the overall proportion of participants with good viral suppression and without severe adverse events or treatment modification was 39.6% for the EFV arm and 62.3% for the ATV/r arm. Considering the reasons for treatment modification, the neuro-psychiatric side effects required a regimen change in the EFV arm. Although several studies concluded that the neuro-psychiatric side effects are transient in nature, one study reported that treatment had to be changed in 16% of patients on EFV due to neuro-psychiatric side effects (22-24). Although there was no significant difference even with the small sample size, 5 out of 36 (13.9%) participants on EFV in our study required treatment change, compared with only 1 out of 35 (2.9%) of the ATV/r arm. This aspect of our study was similar to that reported in the Euro SIDA study (24). In the Swiss Cohort study, the treatment-limiting CNS adverse events was 3.8 (95% CI 2.7-5.2) per 100 person-years and it was clearly related to EFV (25). Considered together, these results emphasize the need for close observation of patients treated with EFV.

The incidence of hyperbilirubinemia in the present study was 77.1% in the ATV/r arm but none of these patients was above grade 4. Furthermore, none of the patients in this study developed liver function abnormality, altered renal function, renal stones, or cholelithiasis. As reported by Torti et al and Josephson et al, such clinical outcome can be used as a marker of adherence to ATV therapy (26, 27).

Limitations of this study include a small sample size.

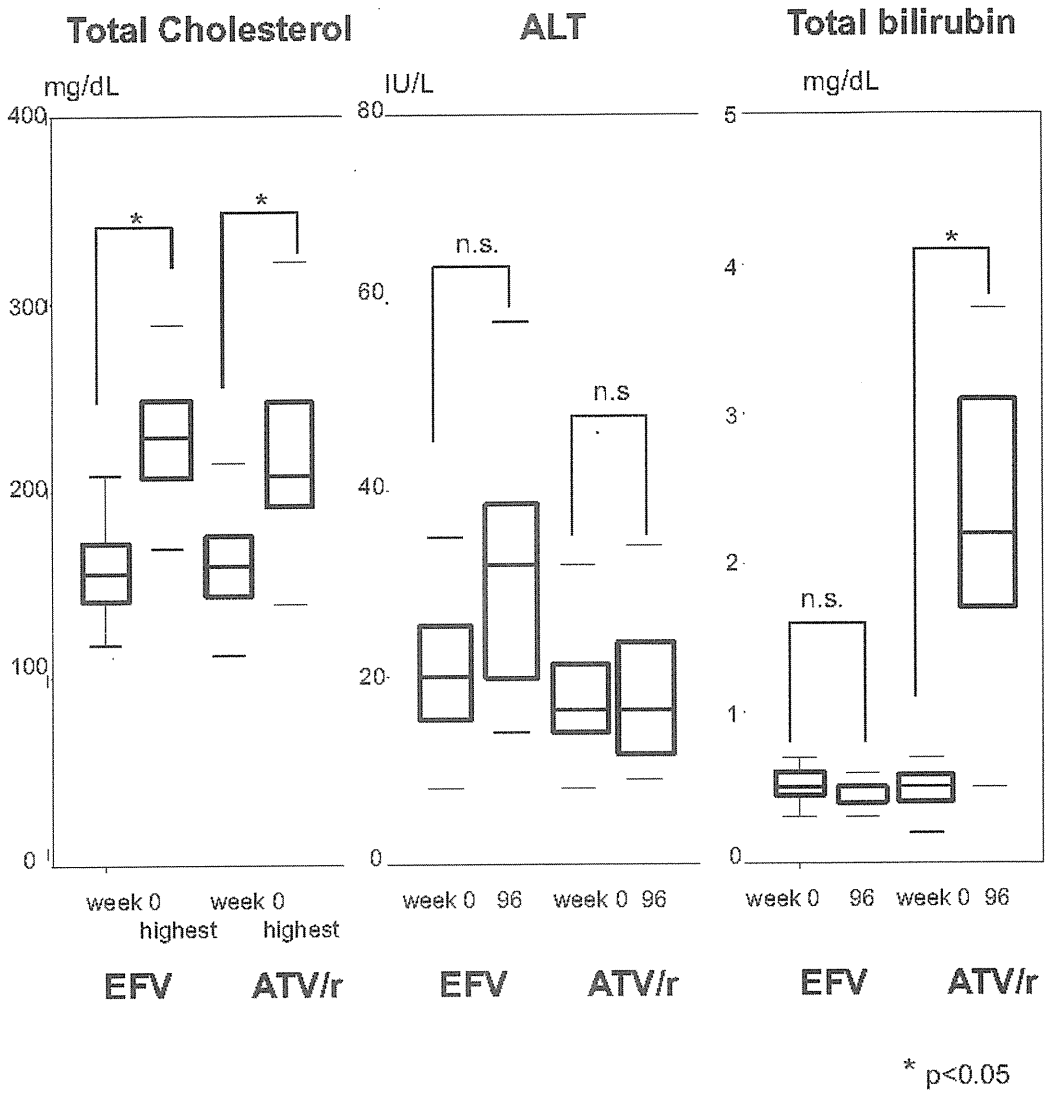

Figure 2. Changes from baseline in total cholesterol, ALT and total bilirubin.
ALT and total cholesterol at week 96 were compared with the baseline values. Since participants who developed hyperlipidemia were treated with lipid-lowering agents during the study period, the highest levels registered in each participant during the follow-up were collected for analysis. There were no significant differences in total cholesterol and ALT between the two arms, while hyperbilirubinemia was significantly higher in the ATV/r arm. Modification of treatment due to hyperbilirubinemia was not required in any of the patients of the ATV/r arm. In these box-and-whisker plots, the lines within the boxes represent median values; the upper and lower lines of the boxes represent the 25th and 75th percentiles, respectively; and the upper and lower bars outside the boxes represent the 90th and 10th percentiles, respectively.

Considering many studies on HIV treatment held in western countries that enrolled few Asian HIV-1 patients, it is important to collect data from Asian population. The current United States Department of Health and Human Services guidelines recommend TDF/FTC as the first line regimen, while the European AIDS Clinical Society recommends 3TC and ABC addition to TDF and FTC alone (28, 29). TDF/FTC is a known potent antiretroviral agent, however, its long-term efficacy and safety remain unclear (11, 12). Considering that the combinations of NRTI are limited, the efficacy and safety of ABC in the low HLA-B*5701 population need to be evaluated for wider treatment options for HIV-1 patients (9, 10).

Conclusion

This study was designed as a selection study to compare the virologic efficacy and treatment safety of EFV and ATV/r, both with ABC/3TC, in Japanese patients. The results showed no significant differences in efficacy between the two regimens at week 48. The evaluation of safety was extended to 96 weeks, which also showed no significant difference in both arms. The results of the present study have already been applied as the basis of a follow-up study that is

currently being conducted in Japan to compare NRTI combinations of ABC/3TC and TDF/FTC with ATV/r as key drugs.

The authors state that they have no Conflict of Interest (COI).

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Impact of Small Body Weight on Tenofovir-Associated Renal Dysfunction in HIV-Infected Patients: A Retrospective Cohort Study of Japanese Patients

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Abstract

Background: Treatment with tenofovir is sometimes associated with renal dysfunction. Limited information is available on this side effect in patients with small body weight, although the use of tenofovir will spread rapidly in Asia and Africa, where patients are likely to be of smaller body weight.

Methods: In a single-center cohort, Japanese patients with HIV infection who started tenofovir-containing antiretroviral therapy were retrospectively analyzed. The incidence of tenofovir-associated renal dysfunction, defined as more than 25% decrement of estimated glomerular filtration rate (eGFR) from the baseline, was determined. The effects of small body weight and body mass index (BMI) on tenofovir-associated renal dysfunction, respectively, were estimated in univariate and multivariate Cox hazards models as the primary exposure. Other possible risk factors were evaluated by univariate analysis and those found significant were entered into the multivariate analysis.

Results: The median weight of 495 patients was 63 kg. Tenofovir-related renal dysfunction occurred in 97 (19.6%) patients (incidence: 10.5 per 100 person-years). Univariate analysis showed that the incidence of tenofovir-related renal dysfunction was significantly associated with smaller body weight and BMI, respectively (per 5 kg decrement, HR = 1.23; 95% CI, 1.10–1.37; $p<0.001$)(per 1 kg/m^2 decrement, HR = 1.14; 95% CI, 1.05–1.23; $p = 0.001$). Old age, high baseline eGFR, low serum creatinine, low CD4 count, high HIV viral load, concurrent nephrotoxic drugs, hepatitis C infection, and current smoking were also associated with tenofovir-related renal dysfunction. Multivariate analysis identified small body weight as a significant risk (adjusted HR = 1.13; 95% CI, 1.01–1.27; $p = 0.039$), while small BMI had marginal significance (adjusted HR = 1.07; 95% CI 1.00–1.16; $p = 0.058$).

Conclusion: The incidence of tenofovir-associated renal dysfunction in Japanese patients was high. Small body weight was identified as an independent risk factor for tenofovir-associated renal dysfunction. Close monitoring of renal function is advocated for patients with small body weight treated with tenofovir.

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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, TDF is known to cause renal proximal tubular dysfunction, and several case reports have been published with TDF-related Fanconi syndrome, diabetes insipidus, and acute tubular necrosis, which sometimes lead to acute renal failure [7–10]. Long-term TDF use also reduces glomerular filtration rate more than other NRTIs [11–14]. To date, the nephrotoxic effect of TDF is regarded as mild and tolerable. A recently published meta-analysis has reported that the use of TDF is associated with a statistically significant but only modest renal dysfunction, and recommended that TDF use should not be restricted even when regular monitoring of renal function and serum phosphate levels is impractical [15]. However, the TDF-related renal dysfunction has hardly been evaluated in patients with small body weight, who are potentially at higher risk for larger drug exposure and thus, more severe toxicity [16–19].

The 2010 WHO guideline on antiretroviral therapy for HIV infection in adults and adolescents, usually applied to resource-constrained settings, recommends TDF as one of the components of first line therapies (URL:http://whqlibdoc.who.int/publications/

2010/9789241599764_eng.pdf). It is expected that the use of TDF will spread rapidly in Asia and Africa in the near future, where patients are more likely to be of small body weight. Thus, at this stage, it is important to establish the relationship between TDF-associated renal dysfunction and body weight. A small body weight is considered a risk factor for TDF-associated renal dysfunction, in addition to old age, high baseline serum creatinine level, low CD4 count, concurrent use of ritonavir-boosted protease inhibitor, and concurrent use of nephrotoxic drugs [4,17,19–21]. To our knowledge, there is almost no report that primarily analyzed the influence of body weight on TDF-associated renal dysfunction. Since Japanese are generally of smaller stature and have a lower median body weight than Whites and African Americans, who mostly comprise the cohorts of studies published to date, it is important to investigate the impact of TDF-associated renal dysfunction in Japanese patients.

Based on the above background, the present study was designed to determine the incidence of TDF-associated renal dysfunction in Japanese patients and analyze the impact of small body weight on TDF-associated renal dysfunction.

Methods

Ethics Statement

This study was approved by the Human Research Ethics Committee of National Center for Global Health and Medicine (Text S1). All patients included in this study provided a written informed consent for their clinical and laboratory data to be used and be published for research purposes. This study has been conducted according to the principles expressed in the Declaration of Helsinki.

Study Design and Settings

We performed a single-center, retrospective cohort study of HIV-infected Japanese patients using medical records at the National Center for Global Health and Medicine, Tokyo, Japan. Our facility is one of the largest clinics for patients with HIV infection in Japan with more than 2,700 registered patients.

Study Subjects

The study population were patients >17 years of age who commenced treatment with standard 300 mg/day of TDF-containing antiretroviral regimen at our clinic between January 1, 2002 to March 31, 2009. Both treatment-naive and patients with experience in antiretroviral treatment but not TDF, with an estimated glomerular filtration rate (eGFR) of >60 ml/min/1.73 m^2 were enrolled. Patients were followed up until September 31, 2009. Patients were excluded if their follow-up period at our facility was less than 24 weeks after commencement of TDF-based therapy, if they had started TDF at other facilities, or if there was evidence of prior TDF use. We only included Japanese patients in order to examine a population with comparatively homogenous basic demographics and background.

Measurements

Outcome measure: TDF-associated renal dysfunction. We defined TDF-associated renal dysfunction as more than 25% decrease in eGFR relative to the baseline [17,22,23]. Baseline eGFR was estimated for each patient from the average of two successive serum creatinine measurements made closest to and preceding the commencement of TDF by no more than 90 days. Changes in eGFR were plotted from the baseline measurement until the value diminished to less than 75% of the baseline or at the end of the follow-up period. The eGFR values at occurrence of TDF-associated renal dysfunction, at censoring, and closest to and preceding 24, 48, and 96 weeks to the diagnosis were collected. Patients generally visited our clinic between every month to every 3 months, and measurement of eGFR was usually conducted on every visit. eGFR was calculated using the equation from the 4-variable Modification of Diet in Renal Disease (MDRD) study [24].

Primary exposure variable. Our primary exposure variables were body weight and body mass index (BMI) at the time of commencement of TDF-containing antiretroviral therapy (ART). BMI was calculated by the equation: BMI = [body weight (kg)/height $(m)^2$].

Other variables: potential risk factors. Potential risk factors for TDF-associated renal dysfunction were determined according to previous studies and collected together with the basic demographics from the medical charts [4,19,20,25]. They included sex, age, baseline laboratory data: CD4 cell count, HIV viral load, and serum creatinine, and other medical conditions (antiretroviral treatment-naïve or experienced, concurrent ritonavir-boosted protease inhibitors, concurrent nephrotoxic drugs such as ganciclovir, sulfamethoxazole/trimethoprim, ciprofloxacin, and NSAIDs, diabetes mellitus, co-infection with hepatitis B defined by positive hepatitis B surface antigen, co-infection with hepatitis C defined by positive HCV viral load, hypertension defined by current treatment with antihypertensive agents, dyslipidemia defined by current treatment with lipid-lowering agents, and current smoking) [26]. We used the data on or closest to and preceding the day of starting TDF-containing ART by no more than 90 days. The data on weight change from the baseline to the end of follow-up period and the frequency of eGFR monitoring for each patient were collected.

Statistical analysis

The time to 25% decline in eGFR from the baseline was calculated from the date of treatment initiation to the date of occurrence of TDF-associated renal dysfunction. Censored cases represented those who discontinued TDF, dropped out, referred to other facilities, or at the end of follow-up period. The time from TDF initiation to 25% decrease in eGFR was analyzed by the Kaplan Meier method for the whole cohort. To estimate the impact of body weight on the incidence of TDF-associated renal dysfunction, we calculated the impact of every 5 kg decrement from the median weight using Cox proportional hazards regression analysis. The impact of every 1 kg/m^2 decrement in BMI on the incidence of TDF-associated renal dysfunction was estimated by the same method. The impact of each basic 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 weight on TDF-associated renal dysfunction, we conducted three models using multivariate Cox proportional hazards regression analysis. Model 1 was the aforementioned univariate analysis for every 5 kg decrement. Model 2 included sex, age plus model 1 in order to adjust for basic characteristics. In model 3, we added variables with P values<0.05 in univariate analysis for adjustment (these included age per 10 years, serum creatinine >0.8 mg/dl, CD4 count <200/μl, HIV viral load per log10/ml, concurrent nephrotoxic drugs, co-infection with hepatitis C, and current smoking). Concurrent ritonavir-boosted protease inhibitors were also added in Model 3 although their p value was 0.116 in the univariate analysis. This was based on the results of several studies suggesting that concurrent use of ritonavir-boosted protease inhibitors is a risk factor for TDF-associated renal dysfunction

[19,20]. The eGFR was excluded from multivariate analysis because of its multicollinearity with sex, age, and serum creatinine, since eGFR was gained by the equation of those variables. The impact of every 1 kg/m^2 decrement in BMI on the incidence of TDF-associated renal dysfunction was estimated by the same method with Model 1 to Model 3.

Four other analyses were conducted to further examine the relationship between low body weight and TDF-associated renal dysfunction. First, the time from initiation of TDF therapy to 25% decrease in eGFR was analyzed by the Kaplan Meier method for intertertile baseline body weight categories: <59, 59–67, and >67 kg. The log-rank test was used to determine statistical significance. Second, to investigate the impact of changes in muscle mass on changes in the eGFR as calculated by MDRD, we compared weight changes with one-way ANOVA among intertertile baseline weight categories. We also conducted the sensitivity analysis by adding the variable "weight change" in multivariate analysis. Third, the median and interquartile value for the actual fall in eGFR from the baseline to 24, 48, and 96 weeks for the whole cohort and three baseline weight categories, respectively, were calculated. The eGFR value at 24, 48, and 96 weeks included those that were censored before reaching 24, 48, and 96 weeks, respectively, so that we could interpret the data for actual fall in eGFR, including not only survived cases but also censored cases. Fourth, we counted the number of patients whose eGFR decreased to <60 and <10 ml/min/1.73 m^2, and who discontinued TDF with the clinical diagnosis of renal dysfunction due to TDF. Chi-square test was used to determine whether the difference among the weight categories was statistically significant.

Statistical significance was defined at two-sided p values< 0.05. We used hazard ratios (HRs) and 95% confidence intervals (CIs) to estimate the impact of each variable on TDF-associated renal dysfunction. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, IL).

Results

Between January 1, 2002 to March 31, 2009, 599 patients started TDF-containing ART (Figure 1). Of these, 104 patients were excluded based on the abovementioned criteria. Thus 495 patients were included in the present study (Dataset S1). Table 1 shows the demographics, laboratory data, and medical conditions of the study population at baseline. Two patients received ART with 3 NRTIs, 3 patients received ART with one protease inhibitor (PI), one non-NRTI (NNRTI), and tenofovir/emtricitabine, and the remaining patients had a standard ART with 2 NRTIs and either PI, NNRTI, or integrase inhibitor (INI). The median body weight and BMI were 63 kg and 21.9 kg/m^2, respectively. The median age of the patients was 38 years and 95.2% were males. The eGFR was well maintained (median: 120.9 ml/min/1.73 m^2), and the median baseline CD4 count was 247/μl. Of the total, 208 patients (42%) were antiretroviral treatment naïve, while 287 were treatment-experienced patients. Viral load was suppressed to <50 copies/ml in 162 (32.7%) patients. 403 (81.4%) were on concurrent PIs as the key drug, 367 (74.1%) were on ritonavir-boosted PIs, and only 83 (16.8%) had NNRTIs as the key drug. Smoking was prevalent among the study population, as 240 (48.5%) were identified as a current smoker.

TDF-associated renal dysfunction defined by more than 25% decrease of eGFR from baseline occurred in 97 patients (19.6%), with an estimated incidence of 10.5 per 100 person-years. The median time from commencement of TDF to occurrence of TDF-associated renal dysfunction was 39 weeks (IQR 13.5–99.4 weeks) (range: 1–1,841 days). The total observation period was 924.7 patient-years (median 72 weeks, IQR 38.6–139.3 weeks). Figure 2 shows the Kaplan-Meier survival curve for the occurrence of TDF-associated renal dysfunction for the whole cohort.

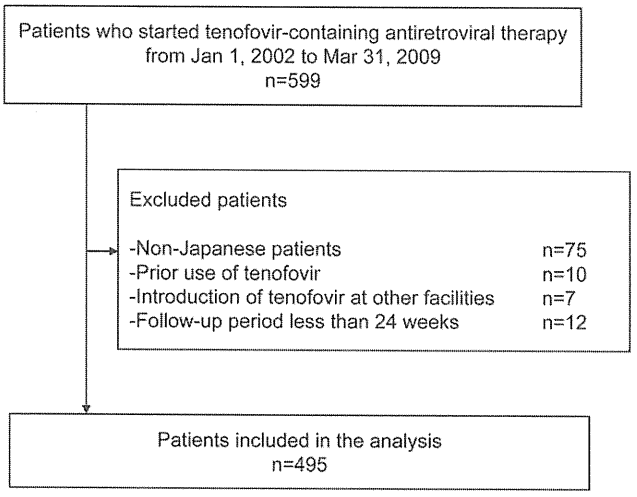

Figure 1. Flow diagram of patient selection.
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Univariate analysis showed a significant relationship between TDF-associated renal dysfunction and every 5 kg less than the median body weight (HR = 1.23; 95% CI, 1.10–1.37; p<0.001), and 1 kg/m^2 less BMI than the median BMI (HR = 1.14; 95% CI, 1.05–1.23; p = 0.001) (Table 2). Furthermore, old age, high eGFR, low serum creatinine, low CD4 counts, high HIV viral load, concurrent use of nephrotoxic drugs, presence of chronic hepatitis C, and smoking were associated with TDF-related renal dysfunction. On the other hand, concurrent use of PIs, ritonavir boosted PIs, and LPV/r tended to be associated with TDF-related renal dysfunction, albeit statistically insignificant. Treatment-naïve or Treatment-experienced was not associated with TDF-related renal dysfunction.

Multivariate analysis showed that every 5 kg less than the median body weight was a significant risk for TDF-associated renal dysfunction after adjustment for sex and age (adjusted HR = 1.21; 95% CI, 1.07–1.36; p = 0.002) (Table 3, Model 2), and also after adjustment for other risk factors (adjusted HR = 1.13; 95% CI, 1.01–1.27; p = 0.039) (Table 3, Model 3). Similarly, every 1 kg/m^2 less than the median BMI was also a significant risk factor for TDF-associated renal dysfunction even after adjustment for sex and age (adjusted HR = 1.13; 95% CI 1.05–1.22; p = 0.002) (Table 4, Model 2), and tended to be a significant factor after adjustment for other variables (adjusted HR = 1.07; 95% CI 1.00–1.16; p = 0.058) (Table 4, Model 3). Old age and current smoking were also independent risk factors in both multivariate analysis for body weight and BMI (Table 3, Model 3 and Table 4, Model 3).

In complementary analyses, First, Figure 3 shows the relation between probability of TDF-associated nephrotoxicity and time from initiation of TDF therapy to 25% decrease in eGFR analyzed by the Kaplan Meier method for intertertile baseline weight categories. Compared to patients with baseline body weight >67 kg, patients with baseline weight <59 kg were significantly more likely to develop >25% decline in eGFR (p = 0.002). On the other hand, the difference in this probability between patients with baseline weight 59–67 kg and those >67 kg was only marginally significant (p = 0.073, log-rank test). Secondly, one-way ANOVA

Table 1. Baseline demographics and laboratory data.

Characteristics		
Median (IQR) weight (kg)	63	(57–69)
Median (IQR) BMI (kg/m^2)	21.9	(20.3–23.8)
Male, n (%)	471	(95.2)
Median (IQR) age	38	(33–46)
Median (IQR) eGFR (ml/min/1.73 m^2)	120.9	(104.8–138.2)
Median (IQR) serum creatinine (mg/dl)	0.72	(0.64–0.81)
Median (IQR) CD4 count (/μl)	247	(159–371)
Median (IQR) HIV viral load (log10/ml)	3.73	(1.60–4.81)
HIV viral load <50 copies/ml, n (%)	162	(32.7)
Antiretroviral therapy naïve, n (%)	208	(42.0)
Key drugs, n (%)*		
PIs	403	(81.4)
Ritonavir-boosted PIs	367	(74.1)
LPV/r	175	(35.4)
ATV/r	131	(26.5)
FPV/r	52	(10.5)
DRV/r	9	(1.8)
FPV	14	(2.8)
ATV	4	(0.8)
NFV	15	(3)
SQV	2	(0.4)
IDV	1	(0.2)
NNRTIs	83	(16.8)
EFV	65	(13.1)
NVP	17	(3.4)
ETR	1	(0.2)
INI		
RAL	10	(2.0)
Concurrent use of nephrotoxic drug, n (%)	131	(26.5)
Diabetes mellitus, n (%)	30	(6.1)
Hepatitis B, n (%)	75	(15.2)
Hepatitis C, n (%)	52	(10.5)
Hypertension, n (%)	28	(5.7)
Dyslipidemia, n (%)	40	(8.1)
Smoking, n (%)	240	(48.5)
Median (IQR) weight change (kg)	0.0	(−2.0–2.25)
Median (IQR) frequency of eGFR monitoring	16	(9.0–27)

(n = 495).

*Two patients did not take any key drugs. Three patients took both PI and NNRTI.

IQR: interquartile range, BMI: body mass index, eGFR: estimated glomerular filtration rate, PI: protease inhibitor, LPV/r: lopinavir/ritonavir, ATV: atazanavir, FPV: fosamprenavir, DRV: darunavir, NFV: nelfinavir, SQV: saquinavir, IDV: indinavir, NNRTI: non-nucleos(t)ide reverse transcriptase inhibitor, EFV: efavirenz, NVP: nevirapine, ETR: etravirine, INI: integrase inhibitor, RAL: raltegravir.

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showed that weight changes among the three baseline weight categories were not significantly different (p = 0.206). Sensitivity analysis after adding the variable "weight change" in Model 3 multivariate analysis (Table 3) showed that adjusted hazard ratio for weight per 5 kg decrement hardly changed (adjusted HR 1.131; 95% CI, 1.007–1.271; p = 0.038). Thirdly, Table 5 shows

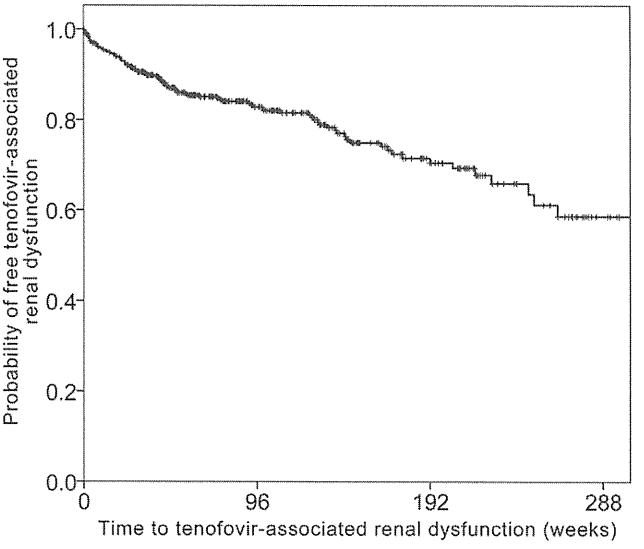

Figure 2. Kaplan-Meier curve showing the time to 25% reduction in eGFR for the whole cohort. eGFR: estimated glomerular filtration rate.

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the median and interquartile values for the actual falls in eGFR from the baseline to 24, 48, and 96 weeks. The eGFR decreased gradually in all categories, except for patients with baseline weight

Table 2. Univariate analysis for TDF-associated renal dysfunction.

	HR	95%CI	P value
Weight per 5 kg decrement	1.23	1.10–1.37	<0.001
BMI per 1 kg/m^2 decrement	1.14	1.05–1.23	0.001
Male gender	0.54	0.26–1.11	0.094
Age per 10 years	1.22	1.02–1.45	0.027
eGFR per 10 ml/min/1.73 m^2	1.10	1.05–1.15	<0.001
Serum creatinine >0.8 mg/dl	0.51	0.30–0.88	0.014
CD4 count <200/μl	1.97	1.32–2.93	0.001
HIV viral load per log10/ml	1.15	1.01–1.30	0.037
Antiretroviral therapy naïve	0.98	0.63–1.52	0.927
Concurrent key drugs			
Any PIs	1.52	0.89–2.59	0.124
Ritonavir boosted PIs	1.46	0.91–2.33	0.116
LPV/r	1.45	0.97–2.17	0.072
ATV/r	1.05	0.66–1.68	0.826
Concurrent nephrotoxic drug	1.59	1.04–2.42	0.031
Diabetes mellitus	1.57	0.76–3.24	0.220
Hepatitis B	1.36	0.82–2.24	0.231
Hepatitis C	1.80	1.07–3.04	0.028
Hypertension	1.18	0.51–2.69	0.702
Dyslipidemia	0.97	0.47–2.00	0.932
Smoking	1.57	1.05–2.36	0.028

TDF: tenofovir, HR: hazard ratio, CI: confidence interval, BMI: body mass index, eGFR: estimated glomerular filtration rate, PI: protease inhibitor, LPV/r: lopinavir/ritonavir, ATV: atazanavir.

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Table 3. Multivariate analysis to estimate the effect of lower body weight on TDF-associated renal dysfunction.

	Model 1 Crude		Model 2 Adjusted		Model 3 Adjusted	
	HR	95%CI	HR	95%CI	HR	95%CI
Weight per 5 kg decrement¶	1.23	1.10–1.37	1.21	1.07–1.36	1.13	1.01–1.27
Male gender			0.88	0.41–1.89	0.57	0.26–1.26
Age per 10 years¶			1.16	0.98–1.38	1.24	1.04–1.49
Serum creatinine >0.8 mg/dl					0.62	0.35–1.07
CD4 count <200/μl					1.65	0.97–2.79
HIV viral load per log10/ml					1.05	0.90–1.23
Boosted PIs					1.54	0.93–2.54
Concurrent use of nephrotoxic drug					1.23	0.77–1.97
Hepatitis C					1.57	0.92–2.69
Smoking¶					1.65	1.09–2.48

¶P<0.05 in Model 3.
TDF: tenofovir, HR: hazard ratio, CI: confidence interval, PI: protease inhibitor.
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>67 kg. Fourthly, the number (percentage) of patients whose eGFR decreased to <60 ml/min/1.73 m^2 was not different among the baseline weight categories (p = 0.229), whereas the number of patients who discontinued TDF with a clinical diagnosis of renal dysfunction due to TDF varied significantly according to body weight (p = 0.001, chi-square test, Table 6). None of the patients showed reduction of eGFR to <10 ml/min/1.73 m^2.

Discussion

In this Japanese cohort, 19.6% of the patients experienced eGFR decline of more than 25% from the baseline after commencement of TDF. The incidence of TDF-associated renal dysfunction was 10.5 per 100 person-years. Multivariate analysis identified smaller body weight and smaller body mass index as significant and almost significant factors, respectively, for TDF-associated renal dysfunction.

The incidence of TDF-associated renal dysfunction in patients with small body weight might be higher than previously reported in studies of patients with larger statures. Studies from North America, Europe, and Australia reported an incidence of <1% to 4.3% for TDF-related renal dysfunction, although the definition used for the diagnosis of renal impairment was different among the studies and varied from an increase in serum creatinine from >0.5 to >2 mg/dL from baseline [1–3,5,13]. Several studies conducted in these regions indicated that the range of patients' mean body weight was 69–74 kg, indicating that their patients were heavier than those of the present study with a median weight of 63 kg [2,6,12,14]. The impact of the comparatively lower body weight seems stronger in our patients probably because they do not appear to have many of the other established risk factors for TDF-associated renal dysfunction despite the high incidence of 10.5 per 100 person-years. For example, they were comparatively young with a median age of 38 years, CD4 count was relatively maintained, and approximately 30% had suppressed HIV viral load at baseline (Table 1). Furthermore, they were less likely to have hypertension, dyslipidemia, and diabetes mellitus.

Table 4. Multivariate analysis to estimate the impact of BMI decrement on TDF-associated renal dysfunction.

	Model 1 Crude		Model 2 Adjusted		Model 3 Adjusted	
	HR	95%CI	HR	95%CI	HR	95%CI
BMI per 1 kg/m^2 decrement	1.14	1.05–1.23	1.13	1.05–1.22	1.07	1.00–1.16
Male gender			0.67	0.32–1.38	0.48	0.23–1.03
Age per 10 years¶			1.20	1.01–1.43	1.27	1.06–1.52
Serum creatinine >0.8 mg/dl					0.60	0.35–1.04
CD4 count <200/μl					1.64	0.97–2.79
HIV viral load per log10/ml					1.05	0.90–1.23
Boosted PIs					1.49	0.90–2.45
Concurrent use of nephrotoxic drugs					1.22	0.76–1.94
Hepatitis C					1.62	0.94–2.76
Smoking¶					1.63	1.08–2.46

¶P<0.05 in Model 3.
BMI: body mass index, TDF: tenofovir, HR: hazard ratio, CI: confidence interval, PI: protease inhibitor.
doi:10.1371/journal.pone.0022661.t004

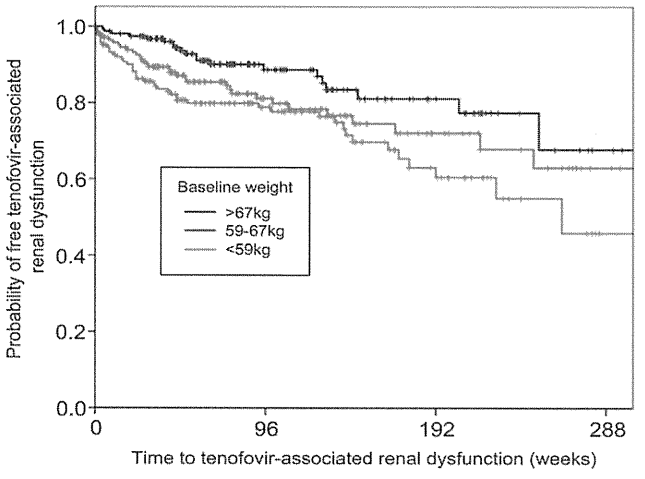

Figure 3. Kaplan-Meier curve showing the time to 25% reduction in eGFR according to baseline weight categories. Compared to patients with body weight >67 kg, those with weight <59 kg were more likely to develop >25% decline in eGFR (P = 0.002), whereas those with weight 59–67 kg showed only a marginal significance (P = 0.073, log-rank test). eGFR: estimated glomerular filtration rate.
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The results of multivariate analysis that each 5 kg decrement in body weight was significantly associated with TDF-associated renal dysfunction but not each 1 kg/m^2 decrement in BMI suggests that weight might be more useful and handy information to estimate the risk for TDF-associated renal dysfunction than BMI. Thus, patient's body weight is an important risk factor to consider at the time of TDF prescription.

Our study is one of a few that have examined the impact of TDF-associated renal dysfunction in patients with small body weight, but is the first to examine the impact of small body weight as a primary exposure by creating the model used for multivariate analysis [16–18]. One study from Thailand that included patients with a median weight of 56.5 kg reported a similar incidence of 16.2 per 100 person-years for developing TDF-associated renal dysfunction [17]. They concluded that the small body weight of their patients was probably associated with the high incidence of TDF-associated renal dysfunction. Our study confirmed that conclusion and provided statistically-backed evidence that small body weight is a significant risk factor of TDF-associated renal dysfunction by using a multivariate model with least multi-collinearity to evaluate the impact of small body weight. The results of the present study could be applied to many countries in Asia and Africa, where stature and body weight of the population are comparatively smaller.

This study adopted a decrease in eGFR of >25% as a definition for TDF-associated renal dysfunction. This criterion is one of common methods in evaluating renal function [22,23]. Using this definition, however, does not mean that all patients with >25% fall in eGFR have severe renal dysfunction. However, the definition of renal dysfunction based on a fall in eGFR of >25% is probably more sensitive than that based on eGFR <60 ml/min/1.73 m^2, in patients with comparatively good baseline renal function, such as patients of our study. Adopting this definition could be useful in detecting early renal dysfunction and in the clinical decision making regarding the need for certain interventions, for example, discontinuation of TDF. Early detection of renal dysfunction is particularly important in patients with HIV infection, because kidney disease may be associated with AIDS and death, and TDF-associated renal dysfunction might be irreversible [27,28].

Since the calculation of eGFR using the MDRD formula is based on serum creatinine, age, race and gender, any fall in eGFR is influenced by hypercreatininemia caused by increased muscle mass [29]. It is possible that the muscle mass increases in patients on ART, especially those with low weight at baseline compared to those with higher weight, reflecting reversal of wasting in those patients who were most malnourished. Such increase in muscle mass could then result in a fall in eGFR despite no change in actual renal elimination of creatinine. However, complementary analysis showed that weight change throughout the follow-up period was not significantly different among patients with different baseline weight, and the sensitivity analysis demonstrated that weight change did not alter the significance of every 5 kg decrement.

In the present study, high eGFR and low serum creatinine levels at baseline were identified as risk factors for falls in eGFR of more than 25%, in contrast to several previous studies that showed high serum creatinine and low eGFR were risk factors [4,10,25]. While the exact reason for this discrepancy is unknown at present, it could be related to differences in the definition of TDF-associated renal dysfunction. The aforementioned Thai study used the same definition applied in the present study and a Canadian study that used the definition of 1.5-fold increase in serum creatinine from baseline also reported high eGFR and low serum creatinine level at baseline as risk factors [17,30]. Thus, it is plausible to observe a fall in eGFR when the baseline value is high, since Horberg et al. reported that patients with baseline eGFR of >80 ml/min/1.73 m^2 were likely to show a pronounced fall in eGFR with TDF use [31].

Multivariate analysis also suggested that old age and current smoking are significant risks for TDF-associated renal dysfunction

Table 5. Median and interquartile range of the actual fall in eGFR from the baseline to 24, 48, and 96 weeks, according to body weight.

	Total (n = 495)		<59 kg (n = 167)		59–67 kg (n = 168)		>67 kg (n = 160)	
	fall in eGFR(ml/min/1.73 m^2)		fall in eGFR		fall in eGFR		fall in eGFR	
	median	IQR	median	IQR	median	IQR	median	IQR
to 24 weeks	7.8	(−1.7–18.1)	9.8	(−3.6–22.6)	6.8	(−1.5–17.3)	7.3	(−1.8–15.4)
to 48 weeks	9.0	(−0.7–21.9)	13.0	(−0.2–29.3)	7.2	(−1.2–20.0)	8.1	(−0.6–18.6)
to 96 weeks	9.3	(−0.5–23.1)	13.4	(1.2–33.2)	8.6	(−0.2–21.7)	7.5	(−2.4–19.8)

eGFR: estimated glomerular filtration rate, IQR: interquartile range.
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Table 6. Number of patients whose eGFR decreased to <60 ml/min/1.73 m^2 and who discontinued tenofovir with clinical diagnosis of renal dysfunction due to tenofovir.

	<59 kg (n = 167)	59–67 kg (n = 168)	>67 kg (n = 160)	p value
eGFR <60 ml/min/1.73 m^2	4 (2.4%)	1 (0.6%)	1 (0.6%)	0.229
Discontinued tenofovir	16 (9.6%)	8 (4.8%)	1 (0.6%)	0.001
Reasons for discontinuation				
>25% eGFR decrement	8 (4.8%)	4 (2.4%)	0 (0%)	
Urine β2 microglobulin >5000 μg/l	11 (6.6%)	4 (2.4%)	1 (0.6%)	

Among the patients who discontinued tenofovir, both >25% fall in eGFR and urine β2 microglobulin >5000 μg/l were registered in six patients with body weight <59 kg, and in three patients with body weight 59–67 kg.
eGFR: estimated glomerular filtration rate.
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(Table 3, Model 3 and Table 4, Model 3). However, these results have to be interpreted with caution, because these multivariate analyses were formulated to primarily evaluate weight decrement, not age or smoking.

The mechanism of TDF-associated renal dysfunction is not fully understood. TDF-associated renal dysfunction probably develops as a result of complex interaction of pharmacological, environmental, and genetic factors, rather than small body weight only [32]. It should be noted, however, that small body weight has been identified as a risk factor for TDF-associated renal dysfunction not only in clinical trials, but also in *in vitro* and pharmacokinetic studies [33–36]. TDF is the prodrug of acyclic nucleotide analog tenofovir, which is excreted by both glomerular filtration and active tubular secretion. *In vitro* studies showed that tenofovir exhibits mitochondrial toxicity in renal proximal tubular cells, and animal studies demonstrated that renal tubular dysfunction was associated with the dose and plasma drug concentrations of TDF [34,35]. Furthermore, pharmacokinetic studies showed that small body weight is associated with reduced plasma TDF clearance and thus high plasma TDF concentrations, which could result in renal tubular dysfunction. [33,36].

There are several limitations to our study. First, because of the retrospective nature of the study, patients with possible risks for TDF-associated renal dysfunction could have not been prescribed TDF. Because of this selection bias, the incidence of TDF-associated renal dysfunction might be underestimated. Second, the study did not compare the incidence of renal dysfunction in a control group (TDF-free ART). Due to the small body weight in Japanese or other factors such as genetics, the use of ART without TDF might cause higher incidence of renal dysfunction as well. Third, as discussed above, the definition of TDF-associated renal dysfunction, especially the criteria used to evaluate proximal renal tubular damage, is not uniformly established in the field and is different in the published studies. Accordingly, we decided to adopt changes in eGFR, instead of parameters for proximal renal tubular damage. Using the eGFR as a marker for TDF-associated renal dysfunction, our results might have underestimated the incidence of TDF-associated renal dysfunction.

In conclusion, the present study demonstrated a high incidence of TDF-associated renal dysfunction among Japanese patients, a potentially high-risk group due to the low median body weight. The results also identified small body weight as a risk for TDF-associated renal dysfunction in a statistical model that included small body weight as a primary exposure. TDF is certainly a drug of choice for one of the components of the first line therapies for HIV infection. However, the importance of close monitoring for renal function in patients with small body weight should be emphasized for early detection of TDF-associated renal dysfunction.

Supporting Information

Text S1 Letter of Approval from Human Research Ethics Committee of National Center for Global Health and Medicine.
(PDF)

Dataset S1 Raw data of the target population.
(XLS)

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Author Contributions

Conceived and designed the experiments: AN TH KU TN MKS. Performed the experiments: AN H. Sakai. Analyzed the data: AN TH KU KO IK H. Sakai. TN MKS. Contributed reagents/materials/analysis tools: H. Suemori H. Sakai. Wrote the manuscript: AN TN MKS.

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Review
Future Microbiology

Clinical significance of HIV reverse-transcriptase inhibitor-resistance mutations

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In this article, we summarize recent knowledge on drug-resistance mutations within HIV reverse transcriptase (RT). Several large-scale HIV-1 genotypic analyses have revealed that the most prevalent nucleos(t)ide analog RT inhibitor (NRTI)-resistance mutation is M184V/I followed by a series of thymidine analog-associated mutations: M41L, D67N, K70R, L210W, T215Y/F and K219Q/E. Among non-nucleoside RT inhibitor (NNRTI)-resistance mutations, K103N was frequently observed, followed by Y181C and G190A. Interestingly, V106M was identified in HIV-1 subtype C as a subtype-specific multi-NNRTI-resistance mutation. Regarding mutations in the HIV-1 RT C-terminal region, including the connection subdomain and RNase H domain, their clinical impacts are still controversial, although their effects on NRTI and NNRTI resistance have been confirmed *in vitro*. In HIV-2 infections, the high prevalence of the Q151M mutation associated with multi-NRTI resistance has been frequently observed.

More than 25 years have passed since the discovery of HIV-1 and HIV-2 as the pathogens of AIDS [1–4]. According to the latest report from the United Nations Programme on HIV/AIDS, the number of HIV infections worldwide in 2008 was estimated at 33.4 million, with 2.7 million new infections and 2.0 million HIV-related deaths [201]. The data clearly indicate that HIV/AIDS still remains a major worldwide health issue.

In the absence of an effective vaccine, the only hope for HIV-infected individuals has been antiretroviral chemotherapy. The prognosis of HIV-infected patients has been greatly improved by the advent of HAART, a combination therapy of at least three antiretrovirals [5,6]. However, the virus has not been eradicated owing to the persistence of latently infected $CD4^+$ T cells, which are recognized as a viral reservoir [7–9]. Therefore, after HAART is initiated, therapy should be continued throughout the patient's lifetime to maintain undetectable viral replication [10]. However, lifelong treatment is a challenge owing to obstacles, such as adverse effects of HAART and the emergence of drug-resistant viruses.

In this article, we focus on the first developed class of antiretroviral drugs and the backbone of HAART, HIV reverse transcriptase (RT) inhibitors. To date, 11 RT inhibitors are available, of which the latest (approved in 2008) is etravirine (Table 1). We review the recent knowledge on drug-resistance mutations within HIV RT and discuss their clinical significance, molecular mechanisms and differences in HIV-1 subtypes or in HIV-2 infections.

Major characteristics of HIV-1 RT

HIV-1, a plus-strand RNA virus, is a member of lentiviruses within the *Retroviridae* family. After the viruses attach and enter into targeted $CD4^+$ cells, viral genomic ssRNAs are converted to dsDNAs by HIV-1 RT through reverse transcription, the processes of RNA-dependent DNA polymerization, template RNA digestion and DNA-dependent DNA polymerization.

HIV-1 RT is well characterized as an error-prone DNA polymerase owing to the lack of exonucleolytic proofreading activity [11,12], and the HIV-1 mutation rate has been estimated at 1.4×10^{-5}–5.4×10^{-5} mutations/base pair/cycle in cell assay systems [13–17]. In addition to this high mutation rate, the robust production of viral particles, at least 1.03×10^{10} virions/day/individual, enables HIV-1 to generate sufficient genetic diversity to evade host immune responses and antiretroviral drug pressure [18,19]. HIV-1 RT is a heterodimer enzyme with two related subunits, p66 and p51, produced from gag-pol polyproteins through proteolytic cleavages (Figure 1) [20–22]. Whereas p66 (residues 1–560) contains both a DNA polymerase domain and an RNase H domain and exhibits enzymatic activities, p51 (residues 1–440) contains only

Keywords
▫ antiretroviral ▫ drug resistance ▫ HIV-1 ▫ HIV-2 ▫ inhibitor ▫ mutation ▫ reverse transcriptase ▫ review ▫ subtype

Table 1. Approved antiretroviral drugs for treatment in HIV infections†.

Generic name	Trade name	Approval (year)	Note
NRTIs			
Zidovudine	Retrovir®	1987	
Didanosine	Videx	1991	
Zalcitabine	Hivid	1992	Discontinued in 2006
Stavudine	Zerit	1994	
Lamivudine	Epivir	1995	
Abacavir	Ziagen	1998	
Tenofovir	Viread®	2001	Supplied as a prodrug, tenofovir disoproxil fumarate
Emtricitabine	Emtriva®	2003	
NNRTIs			
Nevirapine	Viramune®	1996	
Delavirdine	Rescriptor	1997	
Efavirenz	Sustiva®, Stocrin	1998	
Etravirine	Intelence®	2008	
Protease inhibitors			
Saquinavir	Invirase	1995	
Indinavir	Crixivan®	1996	
Ritonavir	Norvir	1996	
Nelfinavir	Viracept®	1997	
Amprenavir	Agenerase®	1999	Discontinued in 2007
Lopinavir/ritonavir	Kaletra®, Aluvia®	2000	
Atazanavir	Reyataz®	2003	
Fosamprenavir	Lexiva®, Telzir	2003	Prodrug of amprenavir
Tipranavir	Aptivus®	2005	
Darunavir	Prezista®	2006	
Integrase inhibitors			
Raltegravir	Isentress®	2007	
Fusion & entry inhibitors			
Enfuvirtide	Fuzeon®	2003	
Maraviroc	Selzentry, Celsentri®	2007	

†Drugs combined with NRTIs or NRTIs/NNRTIs are not listed.
NNRTIs: Non-nucleoside reverse-transcriptase inhibitors; NRTIs: Nucleos(t)ide analog reverse-transcriptase inhibitors.

the DNA polymerase domain and functions as a structural component. Specifically, the DNA polymerase domain contains four subdomains: fingers, palm, thumb and connection (Figure 1). The fingers, palm and thumb subdomains of p66 form a large cleft for binding to a template-primer, the palm subdomain of p66 has the DNA polymerase catalytic site defined as a triad of aspartic acid residues at positions 110, 185 and 186 [20–22], and the outer part of the fingers subdomain (fingertips) functions as a crucial part of the deoxynucleotide triphosphate (dNTP)-binding site [22].

Since the discovery of HIV-1, RT has been recognized a key target for developing antiretroviral therapy (ART). All 12 approved RT inhibitors belong to one of two classes, nucleos(t)ide analog RT inhibitors (NRTIs) or non-nucleoside RT inhibitors (NNRTIs). The two classes of RT inhibitors differ distinctly in the mechanisms by which they inhibit RT and in inducible patterns of drug-resistance amino acid mutations.

NRTIs & their mechanism of RT inhibition

Many of the eight NRTIs developed from 1987 to 2003 are still used today. They are zidovudine [23], didanosine [24], zalcitabine [24], stavudine [25], lamivudine [26], abacavir [27], tenofovir [28] and emtricitabine [29] (Figure 2). Among these RT inhibitors, seven are nucleoside analogs, whereas tenofovir is a nucleotide analog RT inhibitor. This drug has a unique acyclic structure linked with a phosphate group, and thus requires only two phosphorylation steps for conversion to its active 5′-triphosphate form [28]. Today, zalcitabine is not available, and a few