Research Letters

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Once-daily darunavir/ritonavir and abacavir/lamivudine versus tenofovir/emtricitabine for treatment-naïve patients with a baseline viral load of more than 100 000 copies/ml

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The efficacy and safety of fixed-dose abacavir/lamivudine against tenofovir/emtricitabine, both with once-daily darunavir/ritonavir, was examined in 80 treatment-naïve patients with a baseline HIV-1 viral load of more than 100 000 copies/ml. The time to virologic failure by 48 weeks was not different between the two groups. The percentage of patients with viral suppression was not significantly different with per protocol population. Tenofovir/emtricitabine showed better tolerability; more patients on abacavir/lamivudine changed regimen than those on tenofovir/emtricitabine. A randomized trial to elucidate the efficacy and safety of these two regimens is warranted.

Little information is available on the efficacy and safety of antiretroviral therapy (ART) of ritonavir-boosted darunavir (DRV/r) and fixed-dose abacavir/lamivudine (ABC/3TC) [1,2]. DRV/r is a protease inhibitor with proven efficacy and safety, and with high barrier to drug resistance [3,4]. ABC/3TC is an alternative choice of nucleoside reverse transcriptase inhibitors (NRTIs) in the American Department of Health and Human Services Guidelines [5]. Here, we conducted a single-center, observational pilot study to compare the efficacy and safety of DRV/r and ABC/3TC versus tenofovir/emtricitabine (TDF/FTC) in patients with a baseline HIV-1 viral load of more than 100 000 copies/ml. Patients with such a viral load were chosen because ACTG 5202 demonstrated that the time to virologic failure was significantly shorter with ABC/3TC than with TDF/FTC in patients with a viral load of more than 100 000 copies/ml on efavirenz or ritonavir-boosted atazanavir [6]. All patients were treatment-naïve who commenced once-daily DRV/r and either fixed-dose ABC/3TC or TDF/FTC from November 2009 to August 2011 at the AIDS Clinical Center, Tokyo. Baseline data (basic demographics, CD4 count, and viral load) were collected. Viral load was measured by Cobas TaqMan HIV-1 real-time PCR version 1.0 assay (Roche Diagnostics, NJ) to the end of November 2011, and later by Cobas TaqMan version 2.0 assay. It was the decision of the attending physician to start ART with either TDF/FTC or ABC/3TC, because the Japanese guidelines consider both TDF/FTC and ABC/3TC as the preferred NRTIs [7].

The efficacy outcomes were the time from commencing ART to virologic failure (defined as a viral load >1000 copies/ml at or after 16 weeks and before 24 weeks, or >200 copies/ml at or after 24 weeks) [6], and the proportion of patients with a viral load <50 copies/ml at 48 weeks regardless of previous virologic failure. The tolerability outcome was the time to any regimen modification. Intent-to-treat (ITT) population, comprising all patients, was used for all efficacy and tolerability analyses, whereas per protocol population was used in the efficacy analysis of the suppressed viral load. Censored cases represented those who dropped out, referred to other facilities, or reached 48 weeks. Time-to-event distributions were estimated using the Kaplan–Meier method. Univariate and multivariate Cox hazards models estimated the impact of ABC/3TC use over TDF/FTC on the incidence of virologic failure.

The study included 80 patients [ABC/3TC: 21, TDF/FTC: 59, median age: 37.9 years, men: 74 (92.5%), East Asian origin: 72 (90%)], of whom 66 (82.5%) were infected with HIV-1 through homosexual contact. Patients on ABC/3TC had a lower baseline CD4 count (46/μl versus 100, $P=0.031$), higher viral load (5.75 $\log_{10}$ copies/ml versus 5.58, $P=0.044$), and were more likely to have a history of AIDS (71.4% versus 37.3, $P=0.010$), than patients with TDF/FTC. All subjects were HLA-B*5701-negative, and all underwent HIV-1 drug-resistance tests before commencement of ART and none had resistant mutations.

The time to virologic failure with ABC/3TC [3 patients (14.3%)] was not significantly different from that with TDF/FTC [4 (6.8%)] by 48 weeks (Fig. 1a), by univariate and multivariate analyses adjusted by CD4 count and viral load (HR, 2.651; 95% CI, 0.592–11.88; $P=0.203$, adjusted HR, 1.589; 95% CI, 0.341–7.401; $P=0.555$). At week 48, ITT analysis showed more patients with TDF/FTC had a viral load of less than 50 copies/ml (ABC/3TC: 38.1%, TDF/FTC: 64.4%, $P=0.043$) (Fig. 1c), whereas with per protocol analysis, no difference was noted (ABC/3TC: 57.1%, TDF/FTC: 73.1%, $P=0.328$) (Fig. 1d).

Among the seven patients with virologic failure, three (ABC/3TC: 1, TDF/FTC: 2) achieved sustained viral

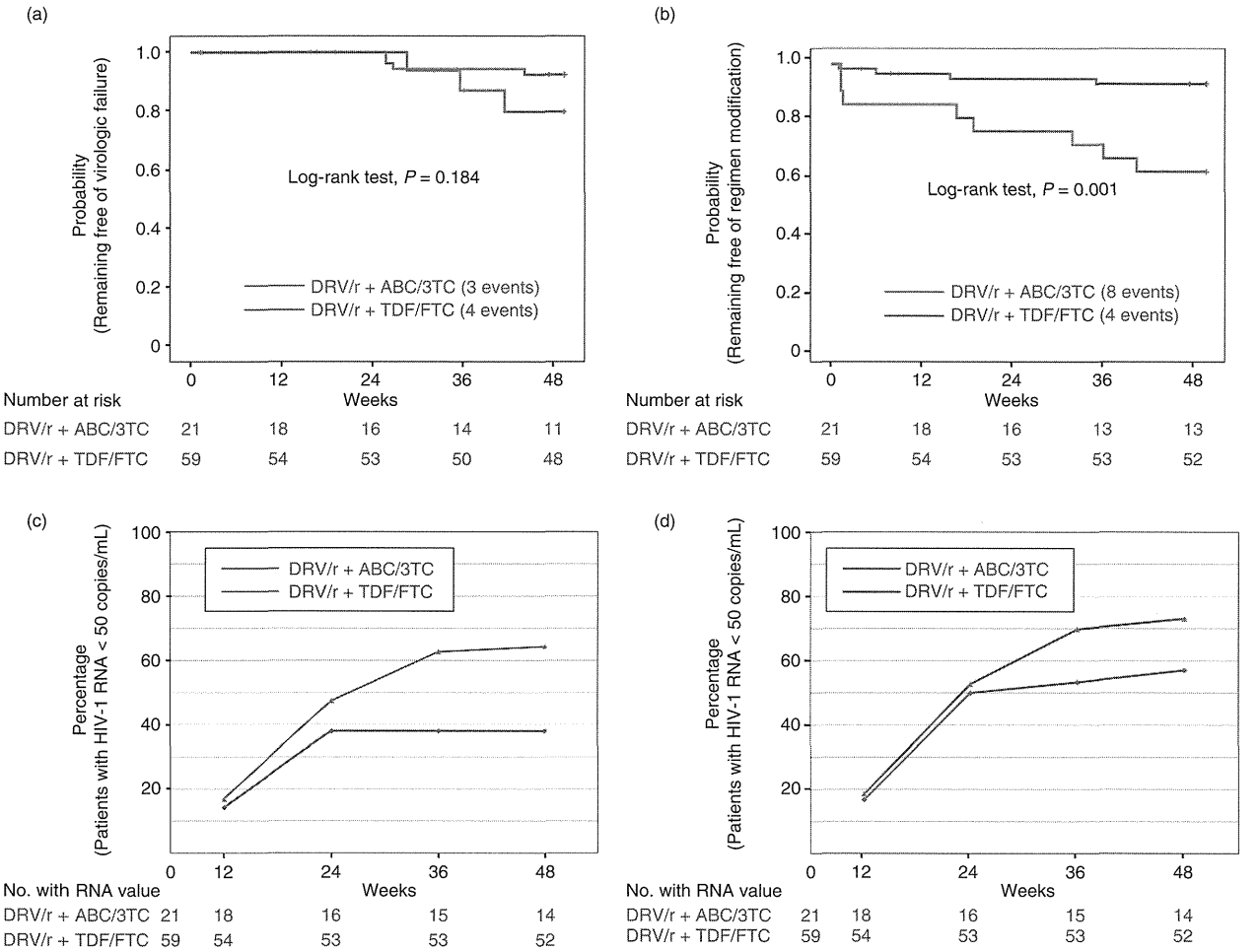

Fig. 1. Efficacy and tolerability results over 48 weeks. (a) Time to protocol-defined virologic failure. (b) Time to tolerability endpoint, defined as first change in treatment regimen. Percentage of patients with HIV-1 RNA less than 50 copies/ml at weeks 12, 24, 36, and 48, regardless of previous virologic failure, with (c) intention-to-treat population, and with (d) per protocol population.

load suppression after week 60 of the initial regimen. The other four underwent drug-resistance tests. One on ABC/3TC was switched to TDF/FTC at week 41; however, viral suppression was not achieved until raltegravir was added at week 74. The other with ABC/3TC was switched to TDF/FTC at week 49 and achieved viral suppression despite the emergence of protease mutation M46I. Another patient on TDF/FTC had persistent viremia (100–200 copies/ml) without mutation. Another patient on TDF/FTC showed the emergence of reverse transcriptase mutation V75L and viremia persisted with 200–500 copies/ml. Reverse transcriptase mutation M184I/T/V did not emerge in any patients.

More patients on ABC/3TC changed or discontinued the initial regimen during the research period [ABC/3TC: 8 (38.1%), TDF/FTC: 4 (6.8%), $P=0.001$] (Fig. 1b). Six [ABC/3TC: 4 (19%), TDF/FTC: 2 (3.4%)] changed ART due to adverse events or virologic failure [ABC/3TC: virologic failure ($n=1$), limb paresthesia ($n=1$), and nausea ($n=2$); TDF/FTC: tenofovir nephrotoxicity ($n=2$)]. None developed ABC-associated hypersensitivity.

This is the first comparison report of the efficacy and safety of ABC/3TC against TDF/FTC with DRV/r in treatment-naïve patients with a viral load of more than 100 000 copies/ml. The time to virologic failure by 48 weeks was not different between the two groups. Although a higher percentage of patients on TDF/FTC showed viral suppression than those on ABC/3TC at week 48 with ITT population, the difference was not significant with per protocol population. TDF/FTC showed better tolerability, as more patients on ABC/3TC changed regimen than those on TDF/FTC.

These results need to be interpreted with caution, because the baseline characteristics of patients of the two groups were not well matched due to the nature of the observational study, and this study did not have sufficient power due to the small number of enrolled patients.

Because our patients had small stature with median body weight of 58.1 kg, a risk factor for TDF nephrotoxicity, it is sometimes our practice to avoid TDF in patients with multiple risks, such as advanced HIV-1 infection, to prevent possible acute kidney injury [8–10]. This is presumably the reason for prescribing ABC/3TC to patients with worse disease condition in this study. This allocation bias might have worked as a disadvantage for the efficacy and tolerability results of ABC/3TC.

The usefulness of ABC/3TC has recently received higher recognition than it did in the past; the FDA meta-analysis did not confirm the association between ABC use and myocardial infarction [11], and it became clear that TDF use is associated with decreased bone mineral density and renal dysfunction, both of which might develop into serious complications with long-term TDF use [12–17]. Thus, once-daily DRV/r, a protease inhibitor with high barrier to drug resistance, and ABC/3TC could be good alternative, especially in patients, who cannot tolerate TDF. A randomized trial to elucidate the efficacy and safety of ABC/3TC and TDF/FTC with once-daily DRV/r is warranted.

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All authors contributed to the concept and design of the study and/or the analyses and interpretation of the data. The article was drafted by T.N., H.K., H.G., and S.O. and critically reviewed and subsequently approved by all authors.

Conflicts of interest

S.O. received research grants from MSD K.K., Abbott Japan, Co., Janssen Pharmaceutical K.K., Pfizer, Co., and Roche Diagnostics K.K. The other authors declare that they have no conflicts of interest.

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Poor outcome of HIV-infected patients with plasmablastic lymphoma: results from the German AIDS-related lymphoma cohort study

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Out of 302 AIDS-related lymphoma (ARL) patients enrolled in the German ARL cohort study, 18 patients had plasmablastic lymphoma (PBL). Twelve out of 18 patients (67%) have died with a median survival of 4 months (range 0–11 months). In univariate analysis, an intermediate or high international prognostic index score was associated with a significantly lower overall survival and progression-free survival. The predominant cause of death was progressive lymphoma (67%). Our data indicate that the outcome of AIDS-related PBL is still very poor.

Since the introduction of combination antiretroviral therapy (cART), the incidence of AIDS-related lymphomas (ARLs) has remarkably declined while the prognosis has considerably improved [1,2]. However, ARLs still remain a serious cause of mortality and morbidity in HIV-infected patients [3]. Plasmablastic lymphomas (PBLs), which are characterized by the absence of B-cell markers (CD20) and the presence of plasma cell markers, comprise a rare entity within ARL [4–8]. The aim of the present study was to describe the clinical characteristics and to analyze the outcome of HIV-infected patients with PBL enrolled in the prospective German ARL-cohort study.

The German ARL-cohort study is a prospective observational multicenter evaluation. HIV-1-infected patients with ARL diagnosed in 30 participating German centers after 1 January 2005, were included in the study. The present analysis consists of 18 patients with the histopathological diagnosis of PBL out of 302 ARL patients enrolled until June 2011. Fifteen out of 18 cases with diagnosis of PBL were confirmed by a review pathologist of one of the German lymphoma reference centers. Overall survival (OS) and progression-free survival (PFS) were calculated from the date of ARL diagnosis until death or until the last follow-up and until lymphoma progression or death as a result of any cause. Kaplan–Meier survivor function was used to evaluate OS and PFS. Prior AIDS-defining illness, CD4 T-cell count at ARL diagnosis, cART before ARL diagnosis, suppressed HIV-RNA, age more than 60, enhanced lactate dehydrogenase (LDH), Eastern Cooperative Oncology Group (ECOG) [9] score >2, stage III/IV disease, extranodal involvement, and the International Prognostic Index (IPI) [10] were considered as potential predictors (definitions of ECOG, IPI, and Ann Arbor score [11] are listed in Table 1). Approval was granted by the ethic committee of the University of Cologne, Germany and of each participating site. Written informed consent was obtained.

All patients were men with a median age of 44 years. Median CD4 T-cell count at ARL diagnosis was 85/μl (range 0–1100/μl). Only five patients had an undetectable HIV-RNA at the time of PBL diagnosis. The baseline characteristics are depicted in Table 1.

With regard to histopathological findings, all PBLs were CD20-negative and at least one plasma cell marker (VS38c, CD38, MUM1, CD138) has been expressed in 82% of cases. Data on KI-67 and Epstein–Barr virus (EBV) are available for 94 and 78% of cases, respectively. A very high proliferation index (KI-67 ≥80%) was found in 13 out of 17 patients (76%) and EBV positivity was observed in 12 out of 14 cases (86%).

Protocols based on CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) were the initial regimen (CHOP-21: $n=6$, CHOP-14: $n=3$, CHOEP: $n=1$) in 10 patients, whereas seven patients were treated according to the high-dose methotrexate-based B-ALL protocol adapted from B-ALL/NHL2002 (ClinicalTrials.gov identifiers NCT00199082/NCT00388193) of the German Multicenter Study Group for the Treatment of Adult Acute Lymphoblastic Leukemia (GMALL). Twelve patients (67%) received at least four cycles of chemotherapy according to the CHOP protocol or B-ALL protocol.

By 30 June, 2011, 12 out of 18 patients (67%) have died after a median survival time of 4 months (range 0–11 months; Table 1). None of these patients achieved a complete remission. Six patients were still alive in their first complete remission with a median follow-up of 32 months (range 21–76 months). The median survival of the entire cohort of patients was 5 months (range 0–76 months). By univariate analysis, an increased LDH, an ECOG performance >2, an age >60 years at lymphoma diagnosis, and an intermediate or high IPI

INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Epstein-Barr Viral Load in Cerebrospinal Fluid as a Diagnostic Marker of Central Nervous System Involvement of AIDS-related Lymphoma

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Abstract

Objective AIDS-related lymphoma (ARL) often involves the central nervous system (CNS). Although the diagnostic value of Epstein-Barr virus (EBV)-DNA in cerebrospinal fluid (CSF) in detecting HIV-positive primary CNS lymphoma (PCNSL) has been established, its usefulness for identifying CNS involvement of systemic ARL remains elusive. In this study, we evaluated the utility of the EBV-DNA load in CSF in identifying CNS involvement in patients with systemic ARL.

Methods We retrospectively reviewed the clinical and pathological data of consecutive ARL patients managed at our clinic between January 1998 and June 2012. Sixty-two patients with ARL, including eight PCNSL patients and 52 systemic ARL patients, and 63 controls underwent CSF EBV-DNA load evaluations before receiving chemotherapy. ARL-related CNS involvement was defined as any lesion diagnosed histologically or radiologically as a lymphoma in the brain, meninges, spine, cranial nerves or oculus.

Results A cut off value of 200 copies/mL predicted the presence of CNS lesions with a sensitivity of 70% and a specificity of 85% in both the PCNSL and systemic ARL patients, while a sensitivity of 75% and a specificity of 93% were obtained for systemic ARL. A cut off value of 2,000 (3.30 log) copies/mL provided the best specificity (100%), with a sensitivity of 50%.

Conclusion Our results support the clinical utility of evaluating the quantitative EBV-DNA load in the CSF for the diagnosis of CNS involvement of systemic ARL as well as PCNSL.

Key words: AIDS-related lymphoma, Epstein-Barr virus

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Introduction

Although the incidence of AIDS-related lymphoma (ARL) has decreased following the advent of highly active antiretroviral therapy (HAART), the morbidity and mortality associated with this complication remain significant due to the aggressive clinical course and high frequency of extra nodal localization especially in the central nervous system (CNS) (1-3). Since the majority of patients with ARLs are diagnosed at the advanced stage of HIV infection, making the differential diagnosis of CNS lesions from other opportunistic diseases is crucial for the management of ARL.

Epstein-Barr virus (EBV) can cause various lymphoproliferative disorders in immunocompromised patients and the detection of EBV-DNA in the cerebrospinal fluid (CSF) is a well-established diagnostic tool for identifying primary CNS lymphoma (PCNSL) in HIV-infected individuals (3-10). However, the diagnostic value of detecting EBV-DNA in CNS involvement of systemic ARL remains to be elucidated. In this study, we retrospectively evaluated the value of EBV-DNA in the diagnosis of CNS lesions of ARL, both PCNSL and systemic ARL.

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Table. Characteristics of the Participating Patients

	PCNSL (n=8)	Systemic ARL		non-ARL control subjects (n=63)	p value
		CNS involvement (+) (n=12)	CNS involvement (-) (n=42)		
Male sex, n	8	10	41	60	0.981
Age, median years (range)	38 (28-53)	52 (27-67)	37 (25-63)	38 (22-70)	0.160
Histology					
DLBCL	3	6	16	-	
Burkitt	0	4	16	-	
Others	2	1	10	-	
Not specified	3	1	0	-	
EBER-positive, % (n/total n)	40 (2/5)	40 (4/10)	58.3 (21/36)	-	0.999
CD4 count, median cells/mm^3 (range)	18 (2-79)	83 (3-652)	117 (3-824)	57 (1-450)	0.006
Plasma HIV viral load, median log copies/mL (range)	5.8 (4.5-6.0)	4.7 (1.6-7.1)	4.7 (1.6-7.5)	4.6 (1.7-6.3)	0.081
Plasma EBV-DNA-positive, % (n/total n)	66.7 (4/6)	63.6 (7/11)	58.3 (21/36)	NA	0.999
CSF EBV-DNA-positive, % (n/total n)	62.5 (5/8)	75.0 (9/12)	7.1 (3/42)	20.6 (13/63)	0.035

PCNSL: primary CNS lymphoma, ARL: AIDS-related lymphoma, DLBCL: diffuse large B-cell lymphoma, EBER: EBV-encoded small RNAs, NA: not assessed. CSF: cerebrospinal fluid. The Kruskal-Wallis test was used for comparisons of continuous variables and the chi-square test was used for comparisons of the categorical data.

Materials and Methods

We reviewed the clinical and pathological data of consecutive cases of ARL managed at the AIDS Clinical Center, National Center for Global Health and Medicine (NCGM), Tokyo between January 1998 and June 2012. CNS involvement of systemic ARL was defined as any lesion histopathologically or radiologically diagnosed as a lymphoma in the brain, meninges, spine, cranial nerves or oculus on either initial diagnosis or recurrence. HIV-infected patients with other opportunistic infections and meningeal or parenchymal brain lesions during the same period were enrolled in the control group for the analysis. Patients who did not have available CSF samples were excluded.

Real-time polymerase chain reaction (RT-PCR) was used to quantify EBV-DNA in CSF samples obtained before chemotherapy and stored at -80℃, using a method previously described (11). Briefly, DNA was extracted using a QIA Symphony Virus/Bacteria Mini kit (Qiagen, Valencia, CA), and the *BNRF1* gene was amplified with the following primers: forward [5'-CCAGTGCTGTGATCGAGCATCT] and reverse [5'-CTGTCGACAAACTGCTGCATTC] and TaqMan probe [5'-(FAM)-TCTGCTGTGTTTCTGTCTCACCTACCG G-(TAMRA)-3']. The cutoff level for detection was 200 copies/mL.

In patients with available results of *in situ* hybridization (ISH) assay of EBV-encoded small RNAs (EBERs), which were performed on paraffin tissue sections using a cocktail of fluorescein-isothiocyanate-labeled oligonucleotides complementary to the two EBERs (types 1 and 2), as previously described (12, 13), we assessed the correlation between the results of EBER and the CNS localization of lymphoma.

Before the analysis, the levels of EBV-DNA were log-transformed and samples with undetectable EBV-DNA were considered to contain 0.0 copies/mL. For continuous variables, the Mann-Whitney U-test was used to compare two groups, while the Kruskal-Wallis test was applied to compare three or more groups. Categorical data were examined using the chi-square test. Differences were considered to be significant at $p<0.05$. The statistical analyses were performed using the SPSS-II software package for Windows, version 17.0J (SPSS Japan Inc, Tokyo, Japan).

Results

During the study period, 76 patients were diagnosed with ARL, including eight patients with PCNSL and 68 patients with systemic ARL. One patient developed ARL twice (diffuse large B-cell lymphoma and plasmablastic lymphoma) within a several year interval and was considered to represent two systemic ARL cases. The frequency of CNS involvement in the systemic ARL patients was 22.1% (15/68). Of the 76 patients with ARL, 62 had available CSF samples and were assigned to the analysis [PCNSL n=8, systemic ARL with CNS involvement (ARL-CNS(+), n=12) and systemic ARL without CNS involvement (ARL-CNS(-), n=42)] (Table). The 63 control subjects with definitive diagnoses of other CNS opportunistic infections during the study period consisted of 18 patients with cryptococcal meningitis, 16 patients with toxoplasmosis, 12 with progressive multifocal leukoencephalopathy (PML), five patients with cytomegalovirus (CMV) encephalitis, three patients with tuberculous meningitis, three patients with neurosyphilis, three patients with Varicella-zoster virus meningitis, two patients with HIV encephalitis, one patient with aseptic meningitis due to acute retroviral syndrome and one patient with CNS candidiasis. Three subjects in the control group had multiple opportunistic infections. There were no significant differences in sex, age or HIV viral load between the two groups. The median CD4 count in the PCNSL group was significantly lower than that observed in the group with systemic ARL with CNS involvement; however, the CD4 counts of the other groups were comparable.

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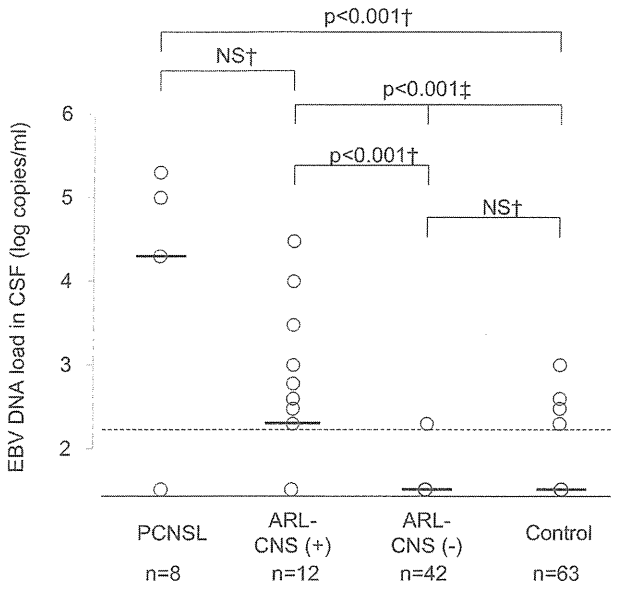

Figure 1. The EBV-DNA loads in the cerebrospinal fluid (CSF) of the patients with AIDS-related lymphoma and the control subjects. PCNSL: primary CNS lymphoma, ARL-CNS (+): systemic AIDS-related lymphoma with CNS involvement, ARL-CNS (-): systemic AIDS-related lymphoma without CNS involvement, NS: not significant. The Mann-Whitney U- test (†) and the Kruskal-Wallis test (‡) were used to compare to the EBV-DNA loads in the CSF. Individual values are plotted, and the horizontal bars represent the median values. The dotted horizontal line indicates the detection limit of the EBV-DNA load assay.

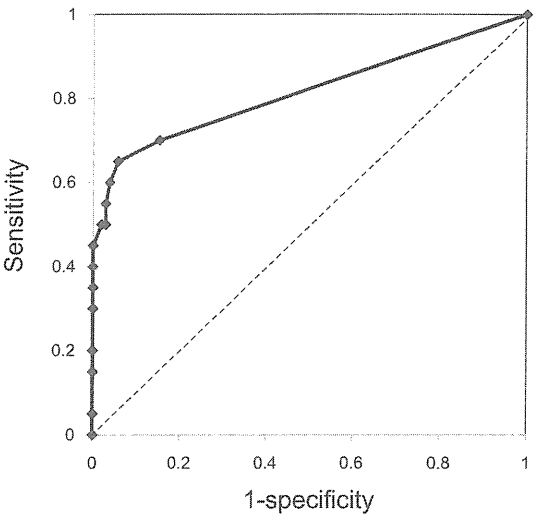

Figure 2. Receiver operating characteristic (ROC) curve for the cutoff values of the EBV-DNA load in the cerebrospinal fluid for the diagnosis of CNS involvement of systemic AIDS-related lymphoma. The dotted line is the reference line. The area under the ROC curve was 0.856 (95% confidence interval, 0.690-1.000). A cutoff value of 200 copies/mL had a sensitivity of 75% and a specificity of 93%.

The proportion of patients positive for EBV-DNA in the CSF (with a detection limit of 200 copies/mL) was 62.5% in the PCNSL, 75.0% in the ARL-CNS(+), 7.1% in the ARL-CNS(-) and 20.6% in the control group. The median (range) EBV-DNA loads in the CSF of the above groups were 4.30 (0-5.30), 2.53 (0-4.48), 0.00 (0-2.30) and 0.00 (0.00-3.00) log copies/mL, respectively (Fig. 1). Both the rate of EBV-DNA-positive cases (Table) and the median EBV-DNA load in the CSF (Fig. 1) were significantly higher in the PCNSL and ARL-CNS(+) groups compared with those observed in the ARL-CNS(-) and control groups; however, these values were not different between the PCNSL and ARL-CNS(+) groups or between the ARL-CNS(-) and control groups. Neither the detection of EBV-DNA in plasma nor histological evidence of EBER in tissue were found to be correlated with the CNS localization of lymphoma (Table). Among nine EBER-negative ARL-CNS(+) cases, CSF EBV-DNA was positive in the five patients who were positive for plasma EBV-DNA, while the remaining four patients were negative for both CSF and plasma EBV-DNA. Six EBER-positive ARL-CNS(+) cases included four patients with positive CSF EBV-DNA and negative plasma EBV-DNA, and one patients with positive and one patients with negative EBV-DNA in both the CSF and plasma. The concordant rate of EBV-DNA detection in the CSF and plasma was 100% in the EBER-negative in ARL-CNS(+) cases and 33% in the EBER-positive in ARL-CNS(+) cases.

With regard to the diagnostic value of the quantitative EBV-DNA load in the CSF, a cut off value of 200 copies/mL provided a sensitivity of 70% and a specificity of 85% for the CNS localization of all ARLs, including the cases of PCNSL and systemic ARL and provided a higher sensitivity of 75% and a specificity of 93% in the systemic ARL cases. A cut off value of 300 copies/mL exhibited a similar sensitivity of 65% and a higher specificity of 94%; however the best specificity (100%) was noted using a cut off value of 2,000 copies/mL, with a sensitivity of 50%. The area under the receiver operating characteristic (ROC) curve in the diagnosis of CNS localization of ARL was 0.816 for all ARLs and 0.856 for systemic ARLs (Fig. 2). Among the EBER-positive ARLs, a cut off value of 200 copies/mL provid a sensitivity of 83.3% and a specificity of 90.4% in the diagnosis of CNS involvement and provide a sensitivity of 55.6% and a specificity of 100% in the EBER-negative ARL cases.

Discussion

The present study demonstrated the usefulness of measuring the EBV-DNA load in the CSF for diagnosing CNS lesions of ARL, regardless of the type of localization of lymphoma, and the presence of PCNSL or CNS involvement of systemic ARL. Although the diagnostic value of EBV-DNA for HIV-positive PCNSL is well-documented (3-10, 18), evidence showing its usefulness for identifying CNS lesions of systemic ARL is limited (3-10). Since the prevalence (21.7%) of CNS involvement in patients with systemic ARL

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is considerably higher (3) than that of non-HIV lymphoma patients (2-7%) (14-16), our results might support the clinical utility of evaluating EBV-PCR with CSF in the management of patients with HIV-positive systemic ARL.

In our study, quantitative EBV-PCR in the CSF with a cut off value of 200 (2.30 log) copies/mL had a sensitivity of 70% and a specificity of 85% for the identification of lymphoma in CNS, while a cut off value of 300 copies/mL provided a similar sensitivity of 65% and a higher specificity of 94%. A previous study that assessed the diagnostic value of quantitative EBV-DNA assays in the CSF for identifying both systemic ARL and PCNSL (10) reported a sensitivity of 75% and specificity of 76% using a cut off value of 100 copies/mL, while the best specificity (100%) was obtained using a cut off value of 3.53 log (3,388) copies/mL. Although our study used a slightly higher detection limit and had a higher specificity and lower sensitivity, the results of the two studies are comparable. In addition, a similar sensitivity (75%) and a higher specificity (93%) were obtained using the cut off value of 200 copies/mL for identifying CNS involvement in systemic ARL than from among all ARLs. Overall, a cut off value of 100-300 seems to be beneficial for identifying CNS lesions of ARL.

In the present study, the prevalence of CSF EBV-DNA in the PCNSL group (62.5%) and the EBER expression (40%) were relatively lower than those reported previously for AIDS-related PCNSL patients (80-100%) (3-10, 18). One possible reason for the low prevalence was the undetectable CSF EBV-DNA load in two patients who had been occasionally treated with anti-herpetic therapy before and during the treatment of PCNSL, including acyclovir for genital herpes in one patient and gancyclovir for CMV retinitis in the other (17). A history of anti-herpetic therapy should be considered when interpreting the results of EBV-PCR. In addition, most previous reports on the high rate of the EBER expression in patients with AIDS-related PCNSL were conducted before or in the early HAART era (18), enrolling severely immunocompromised patients. Since the EBER expression is rare in immunocompetent PCNSL patients (19), our results of low EBER positivity indicate changes in the characteristics of ARL among HIV patients with relatively preserved immunity in the HAART era.

In this study, we found five patients with ARL-CNS(+) who were positive for EBV-DNA in the CSF but negative for the EBER expression in tissue. Notably, among all of the patients with EBER negative ARL-CNS(+), CSF EBV-DNA was detected only when plasma EBV-DNA was detectable, thus suggesting that plasma EBV-DNA transudation into CSF through the blood-brain barrier (BBB) is damaged by CNS involvement of ARL. The presence of plasma EBV-DNA among ARL patients is thought to reflect EBV replication, not in lymphoma tissue, but in other lymphatic tissues such as tonsil endothelial cells, under immunosuppression (20, 21). Although increased EBV activation may lead to ARL development, the increase in the EBV-DNA load in plasma and the EBER expression in tissue are not fully synchronized (20, 21). This may explain our finding of EBER-negative but CSF EBV-DNA positive ARL. Since our study is retrospective, the residue of specimens for EBER ISH was unavailable in 25%of the patients with CNS involvement. Further studies are needed to understand the role of CSF EBV-DNA measurement in the context of EBER-negative ARL.

Conclusion

The EBV-DNA load in the CSF is a marker of CNS involvement of ARL, with 200 copies/mL being a cut off level for the diagnosis of PCNSL and the identification of CNS involvement in patients with systemic ARL. Identifying EBV-DNA may help to differentiate the CNS lesions of ARL from other disorders.

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

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Abacavir/Lamivudine versus Tenofovir/Emtricitabine with Atazanavir/Ritonavir for Treatment-naive Japanese Patients with HIV-1 Infection: A Randomized Multicenter Trial

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Abstract

Objective To compare the efficacy and safety of fixed-dose abacavir/lamivudine (ABC/3TC) and tenofovir/emtricitabine (TDF/FTC) with ritonavir-boosted atazanavir (ATV/r) in treatment-naïve Japanese patients with HIV-1 infection.

Methods A 96-week multicenter, randomized, open-label, parallel group pilot study was conducted. The endpoints were times to virologic failure, safety event and regimen modification.

Results 109 patients were enrolled and randomly allocated (54 patients received ABC/3TC and 55 patients received TDF/FTC). All randomized subjects were analyzed. The time to virologic failure was not significantly different between the two arms by 96 weeks (HR, 2.09; 95% CI, 0.72-6.13; p=0.178). Both regimens showed favorable viral efficacy, as in the intention-to-treat population, 72.2% (ABC/3TC) and 78.2% (TDF/FTC) of the patients had an HIV-1 viral load <50 copies/mL at 96 weeks. The time to the first grade 3 or 4 adverse event and the time to the first regimen modification were not significantly different between the two arms (adverse event: HR 0.66; 95% CI, 0.25-1.75, p=0.407) (regimen modification: HR 1.03; 95% CI, 0.33-3.19, p=0.964). Both regimens were also well-tolerated, as only 11.1% (ABC/3TC) and 10.9% (TDF/FTC) of the patients discontinued the allocated regimen by 96 weeks. Clinically suspected abacavir-associated hypersensitivity reactions occurred in only one (1.9%) patient in the ABC/3TC arm.

Conclusion Although insufficiently powered to show non-inferiority of viral efficacy of ABC/3TC relative to TDF/FTC, this pilot trial suggested that ABC/3TC with ATV/r is a safe and efficacious initial regimen for HLA-B*5701-negative patients, such as the Japanese population.

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Key words: HIV-1 infection, tenofovir/emtricitabine, abacavir/lamivudine, ritonavir-boosted atazanavir, treatment-naïve Asian patients, HLA-B*5701-negative

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Introduction

The fixed-dose combinations of tenofovir disoproxil fumarate 300 mg/emtricitabine 200 mg and abacavir sulfate 600 mg/lamivudine 300 mg are components of antiretroviral therapy for treatment-naïve patients with HIV-1 infection in developed countries (1, 2). The efficacy and safety of tenofovir/emtricitabine (TDF/FTC) and abacavir/lamivudine (ABC/3TC) remain the focus of ongoing debate. The ACTG 5202 trial demonstrated that the viral efficacy of ABC/3TC is inferior to that of TDF/FTC among treatment-naïve patients with a baseline HIV viral load of >100,000 copies/mL receiving efavirenz or ritonavir-boosted atazanavir as a key drug (3). On the other hand, the HEAT study showed that the viral efficacy of ABC/3TC is not inferior to that of TDF/FTC, regardless of the baseline viral load when used in combination with lopinavir/ritonavir (4).

With regard to safety, the occurrence of ABC-associated serious hypersensitivity reactions, the most important adverse effect of ABC affecting 5-8% of patients, has limited its use (5). However, screening for HLA-B*5701 or prescribing ABC in HLA-B*5701-negative populations, such as the Japanese, can reduce the incidence of immunologically-confirmed hypersensitivity to 0% (6, 7). Another negative aspect of ABC use is its association with myocardial infarction, as reported by the D:A:D study (8). However, the possible association of myocardial infarction with ABC was not confirmed by a recent meta-analysis report of the US Food and Drug Administration (9). On the other hand, renal proximal tubular damage leading to renal dysfunction and a loss of phosphate, which can result in decreased bone mineral density, is a well-known adverse effect of TDF (10-14).

Taking this background into account, the American Department of Health and Human Services (DHHS) Guidelines place TDF/FTC as the preferred drug and ABC/3TC as an alternative choice, whereas other international guidelines, including the European AIDS Clinical Society (EACS) Guidelines and the Japanese Guidelines, recommend both TDF/FTC and ABC/3TC as preferred choices (1, 2, 15).

Randomized control trials comparing TDF/FTC and ABC/3TC have been conducted in the US and Europe, but not in other parts of the world (4, 16, 17). The efficacy and safety of these two fixed-dose regimens in patients with different genetic backgrounds and body statures might not be similar to the results of previous trials, especially considering that the prevalence of HLA-B*5701 is zero in the Japanese population (7). Moreover, the degree of decrement in the renal function with TDF is larger in patients with a low body weight, such as the Japanese, which might limit the use of TDF in patients with a high risk for renal dysfunction (18-20).

Based on the above described background, the present randomized trial was originally designed in 2007 to elucidate whether the viral efficacy of ABC/3TC is not inferior to that of TDF/FTC with ritonavir-(100 mg) boosted atazanavir (300 mg) in treatment-naïve Japanese patients, whose body weight is much lower than Whites or Blacks (21). However, the independent data and safety monitoring board (DSMB) recommended that the protocol be modified to examine the efficacy, safety and tolerability among Japanese patients with HIV-1 infection for 96 weeks as a pilot trial because only 109 patients were enrolled and randomized at the end of the enrollment period despite a planned sample size of 240 patients, primarily due to the above mentioned negative reports of ABC use in the D:A:D study and ACTG 5202 (3, 8).

Materials and Methods

This clinical trial was designed and reported according to the recommendations of the Consolidated Standard of Reporting Trials (CONSORT) statement (22). The protocol and supporting CONSORT checklist are available as supplementary files (see Supplementary files 1 and 2).

Ethics statement

The Research Ethics Committee of each participating center approved the study protocol. All patients enrolled in this study provided a written informed consent. This study was conducted according to the principles expressed in the Declaration of Helsinki.

Study design

The Epzicom-Truvada study is a phase 4, multicenter, randomized, open-label, parallel group pilot study conducted in Japan that compared the efficacy and safety of a fixed dose of ABC/3TC and TDF/FTC, both combined with ritonavir-boosted atazanavir (ATV/r) for the initial treatment of HIV-1 infection for 96 weeks. Enrollment of patients began in November 2007 and ended in March 2010, and the follow-up period ended in February 2012. With a one to one ratio, the patients were randomly assigned to receive either a fixed dose of ABC/3TC or TDF/FTC, both administered with ATV/r. The randomization was stratified according to each participating site and conducted at the data center with

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independent clinical research coordinators using a computer-generated randomization list prepared by a statistician with no clinical involvement in the trial.

Study patients

This study population included treatment-naïve Japanese patients aged 20 or over with HIV-1 infection who met the eligibility criteria for the commencement of antiretroviral therapy according to the DHHS Guidelines in place in the U.S. at the time of the writing of the study protocol (a CD4 count <350/μL or a history of AIDS-defining illness regardless of the CD4 count) (23). Patients were screened and excluded if they had previously taken lamivudine, tested positive for hepatitis B surface antigens, had comorbidities such as hemophilia or diabetes mellitus that required medical treatment, congestive heart failure or cardiac myopathy or if they were considered not suitable for enrollment by the attending physicians. Candidates were also excluded if their alanine aminotransferase level was 2.5 times greater than the upper limit of normal, they had an estimated glomerular filtration rate (eGFR) calculated using the Cockcroft-Gault equation of <60 mL/min, {creatinine clearance = [(140- age) × weight (kg)]/(serum creatinine ×72)(×0.85 for females)} or a serum phosphate level <2 mg/dL or had active opportunistic diseases that required treatment (24). Each patient's actual body weight was used for the calculation of eGFR. At screening, a genotypic drug resistant test and screening for the HLA-B*5701 allele were permitted but not required because the prevalence of both the drug resistant virus and the HLA-B*5701 allele are low in Japanese patients (7, 25). Medical history, including a history of AIDS-defining illnesses and other comorbidities, was also collected. Enrollment stopped on March 3, 2008 due to the recommendation from the DSMB of the trial based on the interim analysis of the ACTG5202 that ABC/3TC is less effective than TDF/FTC in patients with a baseline viral load >100,000 copies/mL (3). Accordingly, the DSMB recommended that the trial should be restarted with modified inclusion criteria: to enroll patients with an HIV-1 viral load of <100,000 copies/mL at screening, and the enrollment restarted from April 1, 2008.

Study procedures

Required visits for participants for clinical and laboratory assessments were at screening, enrollment and every 4 weeks until the viral load diminished to <50 copies/mL. For patients with a viral load <50 copies/mL, the required visit interval was every 12 weeks for the duration of the study. The evaluation performed at each visit included a physical examination, CD4 cell count, HIV-1 RNA viral load, a complete blood cell count and blood chemistries (total bilirubin, alanine aminotransferase, lactate dehydrogenase, serum creatinine, potassium, phosphate, triglycerides and low-density lipoprotein (LDL) cholesterol) and a urine examination of the levels of phosphate, creatinine and β2 microglobulin. The values of urinary β2 microglobulin were expressed relative to a urinary creatinine level of 1 g/L (/g Cr). The percent tubular resorption of phosphate was calculated using the following formula: {1-[(urine phosphate × serum creatinine)/(urine creatinine × serum phosphate)]} ×100 (26). All data, including the HIV-1 RNA viral load, were collected at each participating site and sent to the data center. Grade 3 or 4 serious adverse events were reported to the DSMB, which made a judgment whether they were caused by the study drugs. Independent research coordinators at the data center visited at least 10 facilities every year to monitor the accuracy of the submitted data and compliance to the study protocol. All authors vouch for the completeness and accuracy of the reported data.

Statistical analysis

The sample size calculation was originally conducted as follows: Assuming a 90% success rate in the TDF/FTC arm at week 48, a sample size of 224 patients (112 patients per arm) provided 80% power (one sided, α=0.05) to establish non-inferiority of ABC/3TC to TDF/FTC each in combination with ATV/r. Non-inferiority was defined as the lower bound of the two-sided 95% confidence interval (CI) with the treatment difference being above -10%. Based on this assumption, the targeted sample size was set to 240 patients (120 in each arm). However, as previously described, due to the shortage of accrued subjects, this study was underpowered and conducted as a pilot trial.

The primary efficacy endpoint was the time from randomization to virologic failure (defined as a confirmed HIV-1 RNA >1,000 copies/mL at or after 16 weeks and before 24 weeks or >200 copies/mL at or after 24 weeks) (3). The secondary efficacy endpoints included the time from randomization to either virologic failure or ART modification and a comparison of the proportions of patients with HIV-1 RNA <50 copies/mL at weeks 48 and 96 regardless of previous virologic failure. The intent-to-treat (ITT) population comprising all randomized subjects was used to assess the efficacy data; however, a comparison of the proportion of virologically-suppressed patients was conducted with both the ITT and a per protocol population while on the initial randomized regimen.

The safety endpoint was the time from randomization to the first occurrence of grade 3 or 4 laboratory data or abnormal symptoms that were at least one grade higher than the baseline. Isolated hyperbilirubinemia was excluded from the safety endpoints. 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 (27). The tolerability endpoint was the time from randomization to any regimen modification. The safety and tolerability endpoints were calculated in the ITT population. Changes per protocol in the CD4 cell count, lipid markers and renal tubular markers at weeks 48 and 96 were compared using the Mann-Whitney test. A repeated measures mixed model was used to estimate and compare changes in the renal function between the two arms (17). The renal function was calculated using the Modification of Diet in Renal Disease study

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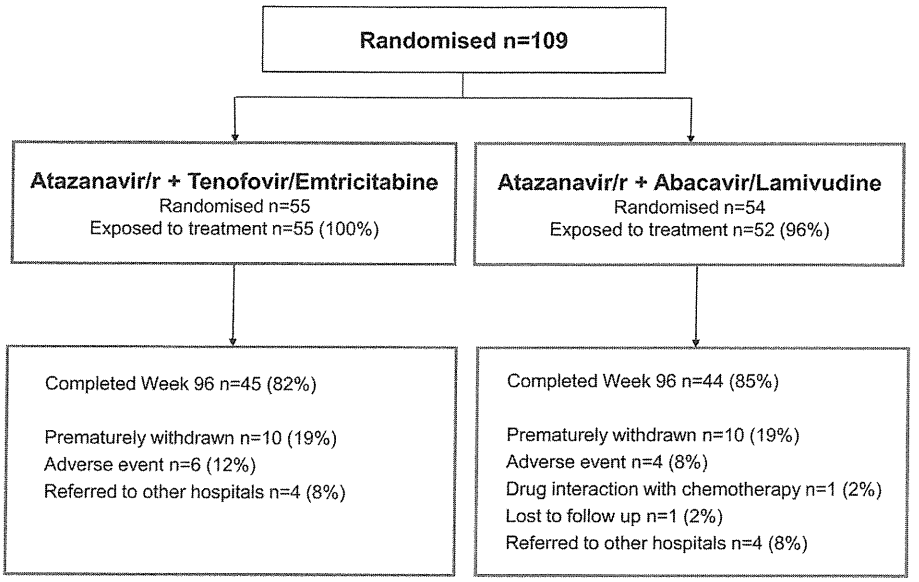

Figure 1. Enrollment, randomization and disposition of patients.

Table 1. Demographic and Baseline Characteristics

	ABC/3TC (n=54)	TDF/FTC (n=55)	Total (n=109)
Sex (male), n (%)	53 (98.1)	54 (98.2)	107 (98.2)
Age (years)†	39 (28.8-44)	35 (29-42)	36 (29-42.5)
CD4 count (/μL)†	236.5 (194-301.3)	269 (177-306)	257 (194-305)
HIV RNA viral load (log_{10}/mL)†	4.29 (3.92-4.67)	4.28 (3.86-4.60)	4.28 (3.89-4.67)
HIV RNA viral load >100,000 log_{10}/mL, n (%)	1 (1.9)	0 (0)	1 (0.9%)
Route of transmission (homosexual contact), n (%)	47 (87)	49 (89.1)	96 (88.1)
History of AIDS n (%)	1 (1.9)	5 (9.1)	6 (5.5)
Body weight (kg)†	64 (59-72.1)	63.1 (58-69)	64 (58.3-70.7)
Body mass index (kg/m^2)†	22.6 (20.4-24.2)	21.9 (20.3-23.6)	22.4 (20.3-23.7)
eGFR (mL/min/1.73 m^2)†	96.9 (82.7-107.3)	94.4 (83.6-105.7)	96.7 (83.0-106.7)
Creatinine clearance (mL/min)†	119.3 (105.4-136.6)	124.6 (103-139.3)	120.3 (104.7-138.3)
Serum creatinine (mg/dL)†	0.76 (0.67-0.83)	0.75 (0.68-0.84)	0.76 (0.68-0.83)
Urinary β2 microglobulin (μg/g Cre)†	195.8 (98.3-505.3)	138.4 (86.8-426.4)	172.9 (88.3-458.7)
Tubular resorption of phosphate (%)†	92.9 (90-95.1)	92.3 (87.7-95.2)	92.7 (89.3-95.1)
LDL-cholesterol (mg/dL)†	91.5 (75-125.5)	94 (72.5-111.5)	94 (74.5-114)
Triglycerides (mg/dL)†	132 (98-170.5)	114 (73-184)	127 (85.5-175)
Hypertension, n (%)	3 (5.6)	1 (1.8)	4 (3.7)
Diabetes mellitus, n (%)	0 (0)	0 (0)	0 (0)
Concurrent use of nephrotoxic drugs, n (%)	10 (18.5)	10 (18.2)	20 (18.3)
Hepatitis C, n (%)	0 (0)	0 (0)	0 (0)

†median (interquartile range)

IQR: interquartile range, AIDS: acquired immunodeficiency syndrome, eGFR: estimated glomerular filtration rate, LDL: low-density lipoprotein

equation adjusted for the Japanese population (28), and a sensitivity analysis was conducted using the above mentioned Cockcroft-Gault equation.

Time-to-event distributions were estimated using the Kaplan-Meier method and compared using the two-sided log-rank test. Hazard ratios (HRs) and 95% confidence intervals (95% CIs) were estimated using the Cox proportional hazards model. For grade 3 or 4 serious adverse events caused by the study drugs, the description and severities were recorded. Statistical significance was defined at two-sided p values <0.05. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, IL).

Results

Patient disposition and baseline characteristics

109 patients from 18 centers were enrolled and randomized between November 2007 and March 2010. Of these patients, 54 and 55 were allocated to the ABC/3TC and TDF/FTC arms, respectively (Fig. 1). The baseline demographics and characteristics are shown in Table 1. Most patients were men, with a median body weight of 64 kg. The median CD4 cell count was 257/μL (IQR: 194-305). One patient in the ABC/3TC arm had a baseline HIV-1 RNA level of >100,000

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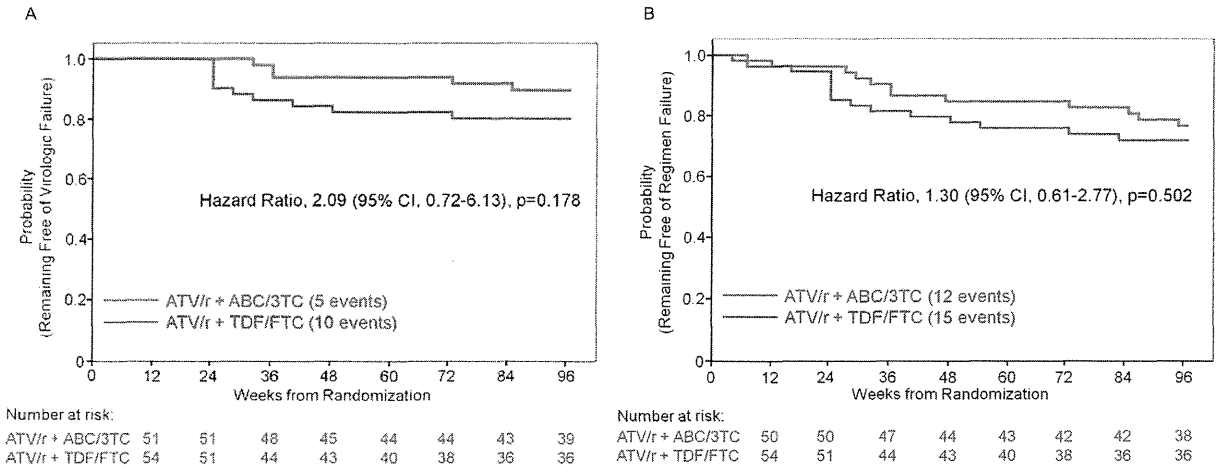

Figure 2. Efficacy results over 96 weeks. (A) Time to protocol-defined virologic failure. (B) Time to the first occurrence of either virologic failure or discontinuation of the initially randomized regimen. ATV/r: ritonavir-boosted atazanavir, ABC/3TC: abacavir/lamivudine, TDF/FTC: tenofovir/emtricitabine

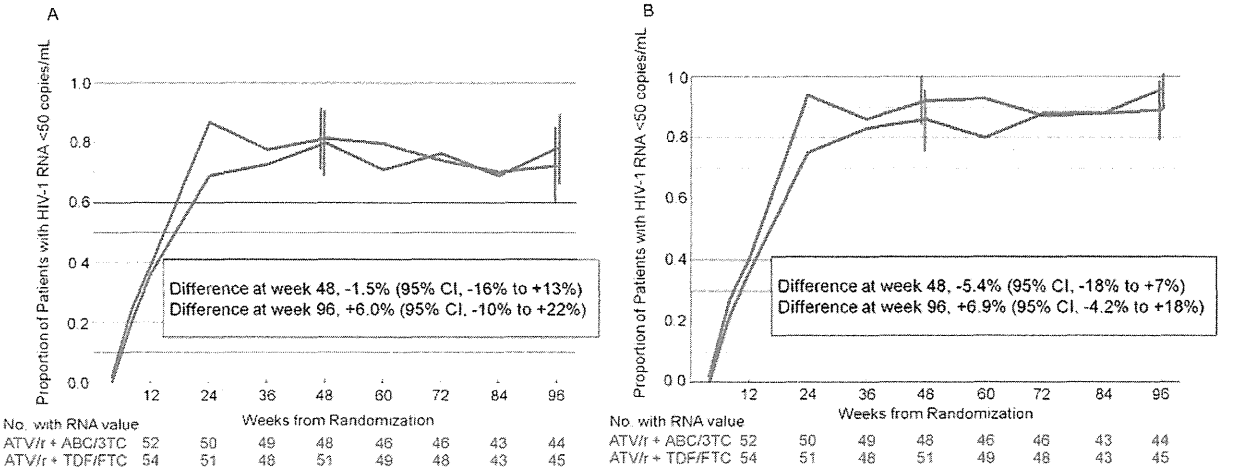

Figure 3. Efficacy results at 48 and 96 weeks. Proportion of patients with an HIV RNA level <50 copies/mL regardless of previous virologic failure with 95% binomial confidence intervals at 48 and 96 weeks. (A) Intention-to-treat analysis. (B) Per protocol analysis. ATV/r: ritonavir-boosted atazanavir, ABC/3TC: abacavir/lamivudine, TDF/FTC: tenofovir/emtricitabine

copies/mL. This patient was enrolled before the announcement of the interim analysis of ACTG5202 in March 2008 and achieved an HIV-1 RNA level of <50 copies/mL by the end of that month. One patient in the TDF/FTC arm had a history of lamivudine use. That patient was included in the analysis because this aspect of the medical history was identified after randomization and initiation of the allocated treatment.

Efficacy results

In the primary efficacy analysis, the time to virologic failure was not significantly different in the ABC/3TC arm from that observed in the TDF/FTC arm by 96 weeks (HR, 2.09; 95% CI, 0.72-6.13; p=0.178). Virologic failure occurred in 5 and 10 patients in the ABC/3TC and TDF/FTC arms, respectively (Fig. 2A). In the secondary efficacy analysis, the times to the first occurrence of confirmed virologic failure or discontinuation of the initially allocated regimen were not different between the two arms (HR, 1.30; 95% CI, 0.61-2.77; p=0.502) (Fig. 2B). Among the ITT population, the proportion of patients with an HIV RNA level <50 copies/mL at week 48 regardless of previous virologic failure was 81.5% in the ABC/3TC group and 80% in the TDF/FTC group, for a difference of -1.5% (95% CI, -16% to 13%), and at week 96, 72.2% and 78.2% for the ABC/3TC and TDF/FTC groups, respectively, for a difference of 6% (95% CI, -10% to 22%) (Fig. 3A). The per protocol analysis showed that the proportions at week 48 were 91.7% and 86.3% for the ABC/3TC and TDF/FTC groups, respectively, for a difference of -5.4% (95% CI, -18% to 7%). At week 96, the proportions were 88.6% and 95.6% for the ABC/3TC and TDF/FTC groups, respectively, for a

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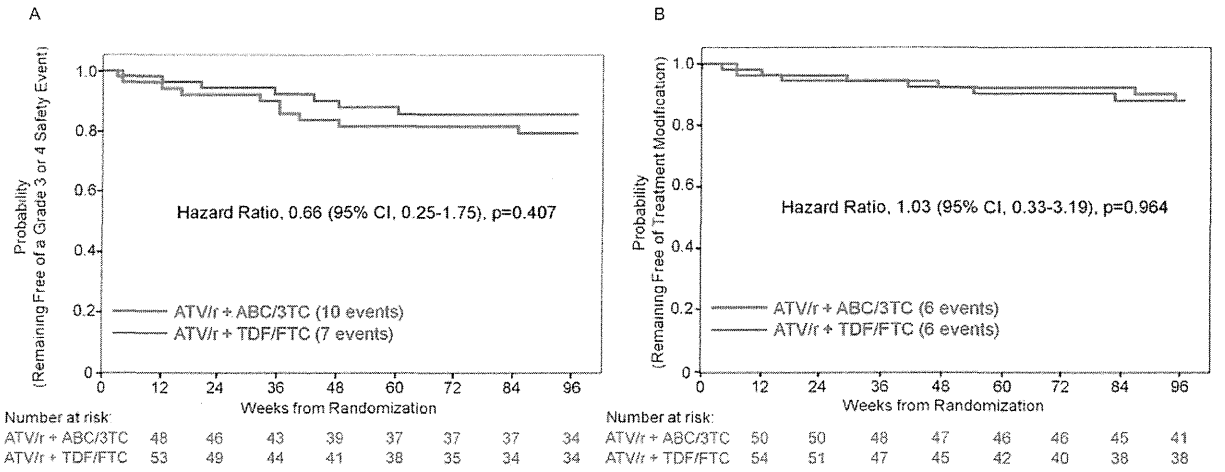

Figure 4. Safety and tolerability results over 96 weeks. (A) Time to first primary safety endpoint, defined as the first grade 3 or 4 event on the initial randomized regimen, which was at least one grade higher than baseline. (B) Time to tolerability endpoint, defined as the first change in regimen. ATV/r: ritonavir-boosted atazanavir, ABC/3TC: abacavir/lamivudine, TDF/FTC: tenofovir/emtricitabine

Table 2. Selected Grade 3 or 4 Events While Receiving Randomized Antiretroviral Drugs

	ABC/3TC (n=54)	TDF/FTC (n=55)	Total (n=109)
Overall, n (%)	13 (24)	10 (18)	23 (21)
Laboratory, n (%)	12 (22)	7 (13)	19 (17)
Alanine aminotransferase, n	0	1	1
LDL-cholesterol, n	6	2	8
Triglycerides, n	0	3	3
Uric acid, n	1	0	1
Serum phosphate, n	2	0	2
Serum calcium, n	1	0	1
Serum creatinine, n	1	0	1
Platelets count, n	1	1	2
Symptoms, n (%)	1 (2)	3 (5)	4 (4)
Depression, n	0	2	2
Fever, n	1	1	2

More than one event occurred in 2 patients.
LDL: low-density lipoprotein

difference of 6.9% (95% CI, -4.2% to 18%) (Fig. 3B). The primary and secondary efficacy analyses did not show a significant difference in viral efficacy between the two arms.

Safety and tolerability results

10 (18.5%) and 7 (12.7%) patients in the ABC/3TC and TDF/FTC groups, respectively, experienced 23 grade 3 or 4 adverse events related to the study drugs while on the initial regimen. The time to the first adverse event was not significantly different between the two arms (HR 0.66; 95% CI, 0.25-1.75, p=0.407) (Fig. 4A). Table 2 shows a list of selected grade 3 or 4 safety events. Among the adverse events, 48% included elevation of lipid markers. The tolerability endpoint, the time to first ART modification, was not significantly different between the two arms (HR 1.03; 95% CI, 0.33-3.19, p=0.964), and only 6 (11.1%) and 6 (10.9%) patients in the ABC/3TC and TDF/FTC arms, respectively, discontinued the initially allocated regimen by 96 weeks (Fig. 4B). The most common reason for regimen modification was drug toxicity (n=10; 4 in ABC/3TC and 6 in TDF/FTC arm; suspected ABC hypersensitivity reactions based on the appearance of rash and fever in HLA-B*5701-negative patient; n=1, depression; n=3, jaundice; n=3, nausea; n=2, and lipodystrophy; n=1). One patient in the ABC/3TC group developed a cerebral infarction during week 39 but was able to continue the study drugs. No deaths were registered during the study period.

Changes in the CD4 cell count and other parameters of interest

The increase in the median CD4 count from baseline to 48 weeks was marginally larger in the ABC/3TC arm than in the TDF/FTC arm (median: ABC/3TC: 216, TDF/FTC: 192, p=0.107). This difference was significantly larger at 96

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Table 3. Median Values of Changes in Parameters of Interest from Baseline to 96 Weeks

	ABC/3TC (n=54)				TDF/FTC (n=55)				
	Number tested (baseline, week 96)	Baseline	Week 96	Median Δ	Number tested (baseline, week 96)	Baseline	Week 96	Median Δ	p value
CD4 cell count (/μL)	54, 43	236.5	545	328	55, 45	269	493	216	0.031
Lipids									
LDL-cholesterol (mg/dL)	54, 16	91.5	149	31.5	53, 16	94	97	2	0.026
Triglyceride (mg/dL)	54, 29	132	257	111	55, 26	114	202	40.5	0.037
Renal tubular markers									
Urinary β2 microglobulin (μg/g Cre)	49, 32	195.8	99.2	-94.9	52, 38	138.4	303.9	86.6	<0.001
Tubular resorption of phosphate (%)	49, 32	93	92	-1.4	50, 36	92	91	-2.6	0.930

LDL: low-density lipoprotein

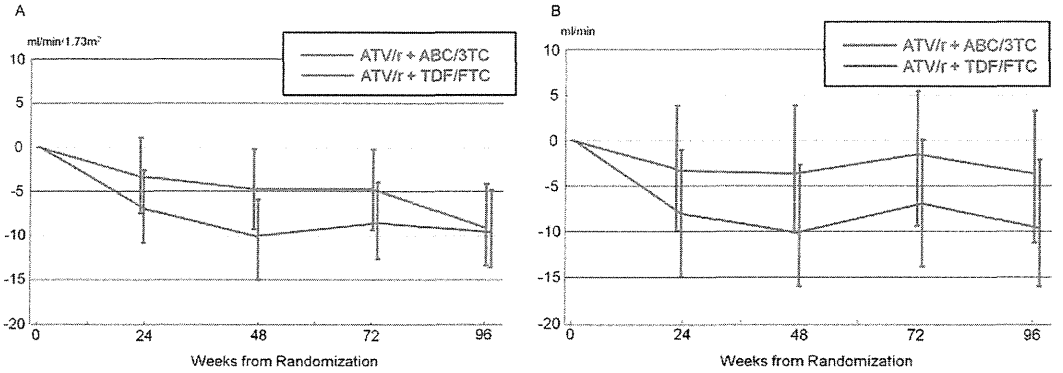

Figure 5. Changes in the renal function between baseline and 96 weeks. (A) Changes in the estimated glomerular filtration rate calculated with the Modification of Diet in Renal Disease study equation adjusted for the Japanese population. (B) Changes in creatinine clearance calculated with the Cockcroft-Gault equation. The data are presented as the mean±95% confidence interval. ATV/r: ritonavir-boosted atazanavir, ABC/3TC: abacavir/lamivudine, TDF/FTC: tenofovir/emtricitabine

weeks (ABC/3TC: 328, TDF/FTC: 236, p=0.031, Table 3). The increases in both LDL-cholesterol and triglycerides from baseline to 96 weeks were more significant in the ABC/3TC arm than in the TDF/FTC arm. One patient in the TDF/FTC arm had been treated with lipid-lowering medications prior to study enrollment. Furthermore, 7 patients and 1 patient in the ABC/3TC and TDF/FTC arms, respectively, started lipid-lowering agents during the study period. With regard to renal tubular markers, the levels of urinary β2 microglobulin increased in the TDF/FTC arm (median: 86.6 μg/g Cre), whereas it decreased in the ABC/3TC arm (median: -94.9 μg/g Cre). These changes were significantly different between the two arms (p<0.001). On the other hand, tubular resorption of phosphate did not show changes from baseline to 96 weeks in the two groups, and the levels were not different between the two arms (Table 3).

Changes in the renal function

A data analysis using repeated measures mixed models showed a significant decrease in the mean eGFR from baseline to 96 weeks in both groups (ABC/3TC: -8.7 mL/min/1.73 m^2, 95%CI -13.3 to -4.2, p<0.001; TDF/FTC: -9.2 mL/min/1.73 m^2, 95%CI -13.7 to -4.7, p<0.001) (Fig. 5A). There was no significant interaction between the trend of the two arms over time (p=0.202), thus indicating that the change in eGFR from baseline to 96 weeks was not significantly different between the two arms. A sensitivity analysis of creatinine clearance calculated using the Cockcroft-Gault equation showed that creatinine clearance decreased significantly from the baseline in the TDF/FTC arm (-9.6 mL/min, 95%CI -16.6 to -2.5, p<0.001) but not in the ABC/3TC arm (-4.1 mL/min, 95%CI -11.2 to 3.0, p=0.466)(Fig. 5B). No significant interaction between the trend of the two arms was observed with respect to creatinine clearance (p=0.403). Two patients in the ABC/3TC arm progressed to more advanced chronic kidney disease (CKD) stage by the last per protocol visit: one patient progressed to stage 4 CKD (eGFR <30 mL/min/1.73 m^2) and the other to stage 3 CKD (eGFR <60 mL/min/1.73 m^2). However, ABC/3TC did not appear to be the causative drug for renal dysfunction in these two cases because the deterioration in the renal function was associated with the development of malignant lymphoma in the former patient and with the commencement of fenofibrate treatment in the latter; renal function recovered rapidly in the latter patient after the discontinuation of fenofibrate.

Discussion

Although insufficiently powered to show the non-inferiority of the viral efficacy of ABC/3TC relative to TDF/

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FTC, this pilot study is the first randomized study conducted in Asia to elucidate the efficacy and safety of fixed doses of these two regimens each administered in combination with ATV/r for initial HIV-1 therapy. Viral efficacy, safety, and tolerability were not significantly different in the two arms of Japanese patients with a baseline HIV viral load <100,000 copies/mL over 96 weeks. Both regimens showed favorable viral efficacy, as in the ITT population, 72.2% and 78.2% of the patients in the ABC/3TC and TDF/FTC arms, respectively, had HIV-1 viral loads of <50 copies/mL at 96 weeks. Both regimens were also well-tolerated, as only 11.1% and 10.9% of the patients in the ABC/3TC and TDF/FTC arms, respectively, discontinued the allocated regimen by 96 weeks. Clinically suspected (not immunologically-confirmed) ABC-associated hypersensitivity reaction occurred in only one (1.9%) patient in the ABC/3TC arm, confirming that ABC hypersensitivity is rare in populations in which HLA-B*5701-positive patients are uncommon. Thus, this trial suggests that ABC/3TC may be an efficacious and safe regimen for use in HLA-B*5701-negative populations, such as the Japanese, with a baseline HIV viral load <100,000 copies/mL.

The usefulness of ABC/3TC has recently received higher recognition for two reasons. One, a meta-analysis by the FDA did not confirm the association between ABC use and myocardial infarction (9). Two, it became clear that TDF-induced renal tubulopathy results in decreased bone mineral density due to phosphate wasting and a decreased renal function, both of which might develop into serious complications with long-term TDF use (12-14, 29, 30). On the other hand, greater deteriorations in the levels of lipid markers were noted in ABC/3TC than in TDF/FTC in clinical trials comparing these two agents (16, 17). The present study also demonstrated that the increases in the LDL-cholesterol and triglyceride levels were higher in the ABC/3TC arm than in the TDF/FTC arm.

TDF-induced nephrotoxicity is of particular interest in this study because a low body weight is an important risk factor, and body stature was much smaller in this study population (median baseline body weight 64 kg), than in the ASSERT study (72 kg), which compared the renal function between patients receiving ABC/3TC and TDF/FTC with efavirenz in Europe (17, 18, 20). This study showed that changes in the renal function from baseline were not significantly different between the two arms, similar to the findings of the ASSERT study. None of the patients in the TDF/FTC arm exhibited progression of CKD stage. On the other hand, the levels of urinary β2 microglobulin deteriorated significantly from baseline in the TDF/FTC arm, whereas improvements were observed in the ABC/3TC arm. This is also similar to the findings reported by the ASSERT trial. This suggests that urinary β2 microglobulin is a more sensitive marker for evaluating TDF nephrotoxicity than the renal function calculated by serum creatinine, as also demonstrated in our previous work (31). Tubular resorption of phosphate, another marker examined to evaluate the renal tubular function, did not exhibit any changes from baseline or between the two arms, suggesting that urinary β2 microglobulin may be a better marker for evaluating TDF nephrotoxicity than tubular resorption of phosphate. Of note, in both arms, the renal function did significantly decrease from baseline. To our knowledge, this is the first randomized trial comparing ABC/3TC and TDF/FTC that observed deterioration of the renal function after the initiation of ART. This result highlights the importance of regular monitoring of renal function after initiation of ART, although it is difficult to draw a firm conclusion on the prognosis of the renal function from this study, due to the limited length of the observation period and the small number of enrolled patients.

Only one patient (1.9%) in the ABC/3TC arm developed a clinically suspected ABC-associated hypersensitivity reaction, which was diagnosed based on the appearance of a skin rash and fever six weeks after commencement of the study drug. The patient fully recovered after discontinuation of the drugs. The ASSERT trial of HLA-B*5701-negative patients reported a similar incidence (3%) of clinically suspected ABC hypersensitivity reactions (17). The one case observed in our trial could be a false positive, because ABC hypersensitivity reactions commonly occur 9-11 days after the initiation of therapy (32), and ABC hypersensitivity was not confirmed immunologically. Nonetheless, immediate discontinuation of ABC is highly recommended even in HLA-B*5701-negative patients suspected of ABC hypersensitivity, since ABC hypersensitivity can occur in such patients (33) and errors in genotyping for HLA or reporting a genotype might occur in practice (34).

Several limitations of this trial should be acknowledged. First, due to the shortage of enrolled patients, the trial was insufficiently powered to test non-inferiority of the viral efficacy of ABC/3TC against TDF/FTC, as initially planned. However, the safety and tolerability data of these regimens in Asia are a valuable asset for patients from this region, and efficacy data could be utilized as part of a meta-analysis in the future. Second, the enrolled subjects were mostly men (primarily men who had sex with men and very few injection drug users). Further studies are needed to examine the efficacy and safety of these regimens in women and patients with different routes of transmissions in Asia.

In summary, this randomized trial demonstrated high efficacy and safety of fixed-dose ABC/3TC and TDF/FTC in combination with ATV/r over 96 weeks for treatment-naïve Japanese patients with a baseline HIV-1 viral load <100,000 copies/mL, although it was insufficiently powered to show non-inferiority of the viral efficacy of ABC/3TC compared with TDF/FTC. ABC/3TC with ATV/r is a safe and efficacious initial regimen for treating HLA-B*5701-negative patients with a baseline HIV-1 viral load <100,000 copies/mL.

Author's disclosure of potential Conflicts of Interest (COI).
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Authors' contributions

SO, MT (Takano), MI, HG, YK and YT designed the study. TE, MH, SK, HU, TK, TN (Naito), MY (Yoshida), NT, MU, YY, TF, SH, KT, MY (Yamamoto), SM, MT (Tateyama) and YT collected the data. HM supervised the study and reviewed and approved study report. TN (Nishijima), HK, HG and SO analyzed and interpreted the data. TN (Nishijima), HK, HG and SO drafted the manuscript and all other authors revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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CASE REPORT

Combination of high-dose dexamethasone and antiretroviral therapy rapidly improved and induced long-term remission of HIV-related thrombocytopenic purpura

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Abstract We present a case of HIV-related thrombocytopenic purpura (HIV-ITP) successfully treated with high-dose dexamethasone and antiretroviral therapy (ART). Although high-dose dexamethasone is regarded as the first-line therapy in adult patients with non-HIV ITP, there is limited information on treatment of HIV-ITP and long-term prednisone therapy is considered the standard therapy. High-dose dexamethasone is preferable to conventional long-term prednisone therapy, because of fewer side effects mainly due to shorter steroid use. The ART helps achieve long-term remission for HIV-ITP, although this therapy lacks an immediate effect. In our patient, administration of high-dose dexamethasone resulted in rapid rise in platelet count and ART maintained long-term remission of HIV-ITP. The combination therapy is potentially suitable strategy for the treatment of patients with HIV-ITP and severe thrombocytopenia or bleeding.

Keywords HIV-related immune thrombocytopenic purpura · High-dose dexamethasone · Antiretroviral therapy · HIV-1 infection

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Introduction

HIV-related thrombocytopenic purpura (HIV-ITP) is the most common cause of low platelet count encountered in patients with HIV-1 infection [1]. It is similar to classic immune thrombocytopenic purpura (ITP) in non-HIV patients, and long-term steroid therapy is regarded the standard treatment [2]. High-dose dexamethasone (HD-DXM) is effective in non-HIV ITP [3–5], however, little is known about its effectiveness in HIV-ITP [6, 7]. We describe a 72-year-old man who presented with HIV-ITP and was effectively treated with HD-DXM combined with antiretroviral therapy (ART).

Case report

A 72-year-old Japanese man was admitted to our hospital with thrombocytopenia. The patient had been diagnosed with HIV-1 infection 10 years earlier and ART was initiated 3 months after the diagnosis. However, adherence to therapy was poor, and the platelet count tended to decrease at times of high HIV-1 RNA viral load during poor adherence. Three months before admission, ART was changed to once-daily ritonavir-boosted darunavir (DRV/r) plus tenofovir/emtricitabine (TDF/FTC) to enhance adherence to therapy. Although repeated HIV-1 resistance testing showed no major mutation, HIV-1 RNA viral load was >1,000 copies/ml over several months. Apart from ART, there was no change in his medications and he had not had any infections during 6 months before admission. On admission, platelet count was 20,000/μl and CD4 count was 168/μl. The patient was alert and oriented with body temperature of 36.2 °C. Physical examination showed no signs of bleeding (e.g., no petechiae, purpura, or mucosal