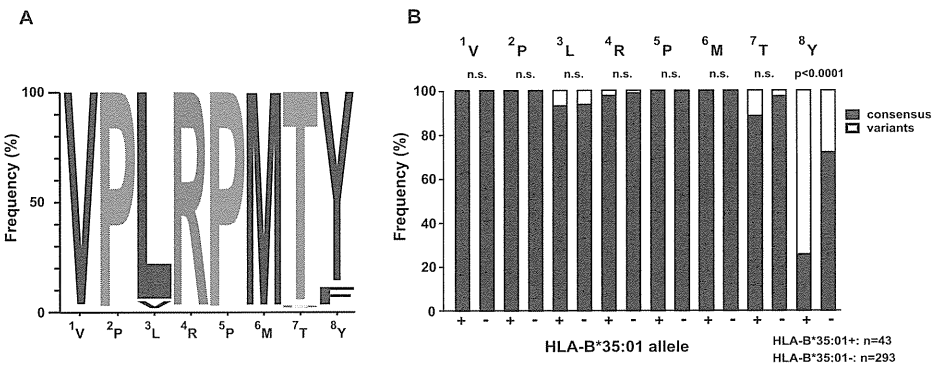

Figure 2. Naturally arising antigenic variations in the VY8 epitope. (**A**) Graphical representation showing the frequency of amino acid residues within the VY8 epitope in subtype B Nef sequences retrieved from the Los Alamos database (n = 1191). WebLogo 3 was used to generate the graphic. (**B**) The frequency of consensus (subtype B) and variant amino acid residues at each position of the VY8 epitope is shown for autologous plasma viral sequences derived from a total of 336 HIV-1-infected individuals, segregated according to *HLA-B*35:01* status. Statistical analysis was performed using Fisher's exact test. *n.s.*, not significant.
doi:10.1371/journal.pone.0066152.g002

Cross-reactivity Analysis of VY8-specific T cells

The cross-reactivity profiles of VY8-specific T cell lines and clones were analyzed using a CPL containing a total of 2.4×10^{10} different octamer peptides, which allowed qualitative mapping of preferred T cell recognition residues at each position along the peptide backbone [4,18]. Different VY8-specific T cell lines and clones preferentially recognized different amino acid residues across the octamer peptide backbone (Figure S1). We employed a graphical representation of these preferential recognition residues by the VY8-specific T cells (Figure 1). Despite these unique cross-reactivity patterns, all T cells tested recognized the index VY8 residues efficiently (Figure 1). This finding contrasts with previous observations using tumor-specific and autoreactive T cell clones [5,21–23], which typically prefer non-index amino acid residues. Across all clones, more stringent recognition was observed at position 2 (P2) and P8 (Figure 1). This most likely reflects the anchor role of these positions in peptide binding to HLA-B*35:01 [12,24]. The VY8-specific T cell clones, K51, K105 and K810, showed inherently unique cross-reactivity footprints but less flexible cross-recognition compared to the parental T cell line (Figure 1), suggesting increased coverage of viral antigenic variation through polyclonal TCR cross-reactivity.

Naturally Occurring Antigenic variations within the VY8 Epitope

To investigate the correlation between T cell cross-reactivity and naturally occurring antigenic variation, we analyzed sequence polymorphisms within the VY8 epitope. Despite the remarkable variability of HIV-1 Nef, VY8 is highly conserved, most likely due to its location partially within a Src homology 3 binding motif that

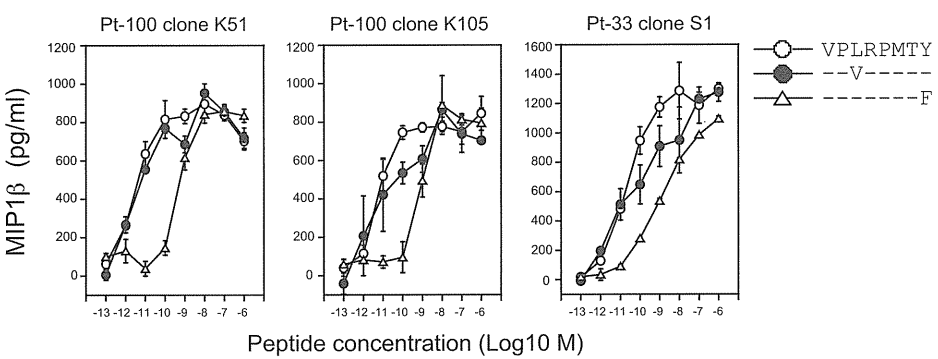

Figure 3. VY8-specific CD8⁺ T cell sensitivity towards peptide variants. The sensitivity of T cell clones towards the VY8, VY8-3V and VY8-8F peptides was quantified by measuring the amount of MIP-1β secreted in response to antigen stimulation. Data are representative of duplicate assays and standard deviation from the mean of two replicates is shown.
doi:10.1371/journal.pone.0066152.g003

Table 3. Sensitivity of VY8-specific CD8⁺ T cells.

CD8⁺ T cells		EC_{50} (M)		
		VY8	VY8-3V	VY8-8F
lines	Pt-100	5.9×10^{-12} (x 1)	nd	3.9×10^{-10} (x 66)
	Pt-168	4.0×10^{-12} (x 1)	nd	4.3×10^{-10} (x 105)
clones	33-S1	2.3×10^{-11} (x 1)	3.9×10^{-12} (x 0.17)	1.2×10^{-9} (x 52)
	100-K51	3.1×10^{-12} (x 1)	5.8×10^{-12} (x 1.8)	4.2×10^{-10} (x 135)
	100-K105	5.1×10^{-12} (x 1)	3.9×10^{-12} (x 0.76)	6.7×10^{-10} (x 131)

EC_{50}, determined by duplicate assays; nd, not done; in parenthesis, fold changes in sensitivity relative to index.
doi:10.1371/journal.pone.0066152.t003

is critical for several Nef functions [25], including HLA-I down-regulation [13,26]. Nevertheless, in the Los Alamos HIV Sequence database (http://www.hiv.lanl.gov/content/index), some variability within HIV-1 subtype B has been reported at P3 Leu and P8 Tyr of the VY8 epitope, with 2.4% and 8.2% of viral clones showing polymorphisms in these positions, respectively (Figure 2A). Given that approximately 40% of Nef sequence polymorphisms are associated with host HLA-I alleles [1], we examined these particular variants for HLA-I association. Our previous smaller study of 69 HIV-1-infected patients indicated that Phe at P8 might be associated with the *HLA-B*35:01* allele [13]. To confirm this association and examine polymorphisms at P3, we recruited a larger cohort comprising 336 treatment-naïve individuals with chronic HIV-1 infection and determined autologous *nef* sequences from plasma viral RNA. Although we found some variability at P3 (3%), there were no statistically significant amino acid differences at P1–P7 between individuals with or without *HLA-B*35:01* (Figure 2B). In fact, CPL scanning showed that, at P3, hydrophobic residues including both the index Leu and mutant Val were preferentially recognized by all VY8-specific T cells tested (Figure 1). Such flexible TCR recognition at P3 helps to explain why the Val mutant is not selected in *HLA-B*35:01*⁺ individuals. Conversely, we found a statistically significant difference in the frequency of polymorphisms at P8 between individuals with or without *HLA-B*35:01* (Figure 2B); indeed, the vast majority (74%) of *HLA-B*35:01*⁺ donors harboured viral sequences with Phe at P8. However, CPL scanning showed that Phe was a favoured amino acid residue recognized by T cell lines and some clones, such as K105 (Figure 1 and Figure S1). In these instances, CPL data alone do not simply explain the emergence of this viral mutation in *HLA-B*35:01*⁺ individuals.

VY8-specific T cell Sensitivity Towards Peptide Variants

To verify the effect of single mutations within the VY8 peptide on TCR sensitivity, we performed competitive titration assays across our panel of VY8-specific T cells (Figure 3). Consistent with the CPL scan data, all T cells tested recognized the VY8 and VY8-3V peptides comparably (<2 fold difference in EC_{50} values; Table 3). In contrast, the EC_{50} values for VY8-8F were >50 fold higher than index for all T cells tested (Table 3). These observations are consistent with previous reports showing that VY8-specific T cells could not recognize CD4⁺ T cells or macrophages infected with HIV-1 carrying this Nef variant at P8 [13,26].

Although P8 is an anchor residue for VY8, our previous HLA-I stabilization studies showed comparable binding activity between HLA-B*35:01 and either VY8 or VY8-8F [13]. The crystal structure of the VY8/HLA-B*35:01 complex shows that P8 Tyr lies deep inside the F pocket of the HLA-I molecule [24]. Substitution at this position with the aromatic residue Phe may not induce substantial structural changes. Consequently, impaired T cell recognition of P8 Phe may be mediated by indirect conformational changes imposed by the peptide upon TCR binding [17]. In the context of HLA-A*02:01, however, a Tyr to Phe substitution at the secondary anchor P3 of an antigenic peptide (SLFNTVATL) leads to unexpectedly large conformational changes in the peptide backbone [27]. Accordingly, further structural studies are needed to elucidate the precise mechanism through which anchor residue substitution leads to impaired T cell recognition of the VY8 epitope.

Previous studies have shown that the double substitution of Arg-71 to Thr and Tyr-81 to Phe (P8 at VY8) [13], or Pro-75 to Ala (P2 at VY8) as a single mutation, impair Nef-mediated down-regulation of HLA-I and thereby increase the susceptibility of HIV-1-infected cells to killing by CD8⁺ T cells targeting other epitopes [26,28]. In contrast, the Tyr-81 to Phe (P8 at VY8) mutation alone exerts virtually no effect on Nef-mediated activities [13,26]. Collectively, these data suggest that the P8 Phe mutation does not compromise viral fitness.

Concluding Remarks

CD8⁺ T cell responses against the immunominant HIV-1 subtype B-derived Nef epitope VY8 presented by HLA-B*35:01 are highly polyclonal, broadly cross-reactive and capable of tolerating natural viral variation with one notable exception. Specifically, the observed Phe substitution at P8, which is neutral in terms of Nef-mediated function [13,26], was found to reduce CD8⁺ T cell recognition by >50 fold. The association of this mutation with *HLA-B*35:01*⁺ strongly suggests that evasion of VY8-specific CD8⁺ T cell activity confers a selection advantage *in vivo*. Thus, even CD8⁺ T cell responses with extensive cross-reactivity profiles can succumb to immune escape at a single position.

Supporting Information

Figure S1 CPL scanning of VY8-specific CD8⁺ T cells. The cross-reactivity profiles of T cell lines and clones specific for VY8 were tested by using 160 CPL sub-mixtures (100 μg/ml) comprising a total of 2.4×10^{10} different octamer peptides. In every peptide mixture, one position has a fixed amino acid residue and all other positions are degenerate, with the possibility of any one of 19 natural amino acids being incorporated in each individual position (cysteine is excluded). The amount of MIP-1β secreted in response to antigen was quantified by ELISA. Data are background-subtracted and the relative T cell response is shown as a ratio of MIP-1β production with respect to the index residue at each position. Responses >20% were considered positive and used to construct Figure 1. A representative set of duplicate assays is shown. Red bars depict residues corresponding to the VY8 index sequence.
(EPS)

Acknowledgments

We thank Dr. L. Wooldridge for providing reagents and assistance for this study.

Author Contributions

Conceived and designed the experiments: CM JJM AKS TU. Performed the experiments: CM JJM ZH SCM TU. Analyzed the data: CM JJM ZH

SCM DAP AKS TU. Contributed reagents/materials/analysis tools: HG SO. Wrote the paper: CM JJM DAP AKS TU.

References

1. Brumme ZL, John M, Carlson JM, Brumme CJ, Chan D, et al. (2009) HLA-associated immune escape pathways in HIV-1 subtype B Gag, Pol and Nef proteins. PLoS One 4: e6687.
2. Kawashima Y, Pfafferott K, Frater J, Matthews P, Payne R, et al. (2009) Adaptation of HIV-1 to human leukocyte antigen class I. Nature 458: 641–645.
3. Mason D (1998) A very high level of crossreactivity is an essential feature of the T-cell receptor. Immunol Today 19: 395–404.
4. Sewell AK (2012) Why must T cells be cross-reactive? Nat Rev Immunol 12: 669–677.
5. Wooldridge L, Ekeruche-Makinde J, van den Berg HA, Skowera A, Miles JJ, et al. (2012) A single autoimmune T cell receptor recognizes more than a million different peptides. J Biol Chem 287: 1168–1177.
6. Dong T, Stewart-Jones G, Chen N, Easterbrook P, Xu X, et al. (2004) HIV-specific cytotoxic T cells from long-term survivors select a unique T cell receptor. J Exp Med 200: 1547–1557.
7. Kosmrlj A, Read EL, Qi Y, Allen TM, Altfeld M, et al. (2010) Effects of thymic selection of the T-cell repertoire on HLA class I-associated control of HIV infection. Nature 465: 350–354.
8. Iglesias MC, Almeida JR, Fastenackels S, van Bockel DJ, Hashimoto M, et al. (2011) Escape from highly effective public CD8+ T-cell clonotypes by HIV. Blood 118: 2138–2149.
9. Chen H, Ndhlovu ZM, Liu D, Porter LC, Fang JW, et al. (2012) TCR clonotypes modulate the protective effect of HLA class I molecules in HIV-1 infection. Nat Immunol 13: 691–700.
10. Hoof I, Perez CL, Buggert M, Gustafsson RK, Nielsen M, et al. (2010) Interdisciplinary analysis of HIV-specific CD8+ T cell responses against variant epitopes reveals restricted TCR promiscuity. J Immunol 184: 5383–5391.
11. Ladell K, Hashimoto M, Iglesias MC, Wilmann PG, McLaren JE, et al. (2013) A Molecular Basis for the Control of Preimmune Escape Variants by HIV-Specific CD8(+) T Cells. Immunity 38: 425–436.
12. Motozono C, Yanaka S, Tsumoto K, Takiguchi M, Ueno T (2009) Impact of intrinsic cooperative thermodynamics of peptide-MHC complexes on antiviral activity of HIV-specific CTL. J Immunol 182: 5528–5536.
13. Ueno T, Motozono C, Dohki S, Mwimanzi P, Rauch S, et al. (2008) CTL-mediated selective pressure influences dynamic evolution and pathogenic functions of HIV-1 Nef. J Immunol 180: 1107–1116.
14. Ueno T, Tomiyama H, Takiguchi M (2002) Single T cell receptor-mediated recognition of an identical HIV-derived peptide presented by multiple HLA class I molecules. J Immunol 169: 4961–4969.
15. De Rosa SC, Lu FX, Yu J, Perfetto SP, Falloon J, et al. (2004) Vaccination in humans generates broad T cell cytokine responses. J Immunol 173: 5372–5380.
16. Betts MR, Nason MC, West SM, De Rosa SC, Migueles SA, et al. (2006) HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood 107: 4781–4789.
17. Cole DK, Edwards ES, Wynn KK, Clement M, Miles JJ, et al. (2010) Modification of MHC anchor residues generates heteroclitic peptides that alter TCR binding and T cell recognition. J Immunol 185: 2600–2610.
18. Ekeruche-Makinde J, Miles JJ, van den Berg HA, Skowera A, Cole DK, et al. (2013) Peptide length determines the outcome of TCR/peptide-MHCI engagement. Blood 121: 1112–1123.
19. Douek DC, Betts MR, Brenchley JM, Hill BJ, Ambrozak DR, et al. (2002) A novel approach to the analysis of specificity, clonality, and frequency of HIV-specific T cell responses reveals a potential mechanism for control of viral escape. J Immunol 168: 3099–3104.
20. Meyer-Olson D, Brady KW, Bartman MT, O'Sullivan KM, Simons BC, et al. (2006) Fluctuations of functionally distinct CD8+ T-cell clonotypes demonstrate flexibility of the HIV-specific TCR repertoire. Blood 107: 2373–2383.
21. Wooldridge L, Laugel B, Ekeruche J, Clement M, van den Berg HA, et al. (2010) CD8 controls T cell cross-reactivity. J Immunol 185: 4625–4632.
22. Ekeruche-Makinde J, Clement M, Cole DK, Edwards ES, Ladell K, et al. (2012) T-cell receptor-optimized peptide skewing of the T-cell repertoire can enhance antigen targeting. J Biol Chem 287: 37269–37281.
23. Bulek AM, Cole DK, Skowera A, Dolton G, Gras S, et al. (2012) Structural basis for the killing of human beta cells by CD8(+) T cells in type 1 diabetes. Nat Immunol 13: 283–289.
24. Smith KJ, Reid SW, Stuart DI, McMichael AJ, Jones EY, et al. (1996) An altered position of the alpha 2 helix of MHC class I is revealed by the crystal structure of HLA-B*3501. Immunity 4: 203–213.
25. Saksela K, Cheng G, Baltimore D (1995) Proline-rich (PxxP) motifs in HIV-1 Nef bind to SH3 domains of a subset of Src kinases and are required for the enhanced growth of Nef+ viruses but not for down-regulation of CD4. EMBO J 14: 484–491.
26. Mwimanzi P, Hasan Z, Hassan R, Suzu S, Takiguchi M, et al. (2011) Effects of naturally-arising HIV Nef mutations on cytotoxic T lymphocyte recognition and Nef's functionality in primary macrophages. Retrovirology 8: 50.
27. Lee JK, Stewart-Jones G, Dong T, Harlos K, Di Gleria K, et al. (2004) T cell cross-reactivity and conformational changes during TCR engagement. J Exp Med 200: 1455–1466.
28. Yamada T, Kaji N, Odawara T, Chiba J, Iwamoto A, et al. (2003) Proline 78 is crucial for human immunodeficiency virus type 1 Nef to down-regulate class I human leukocyte antigen. J Virol 77: 1589–1594.

Naturally Selected Rilpivirine-Resistant HIV-1 Variants by Host Cellular Immunity

Hiroyuki Gatanaga,[1,2] Hayato Murakoshi,[2] Atsuko Hachiya,[1,3] Tsunefusa Hayashida,[1,4] Takayuki Chikata,[2] Hirotaka Ode,[3,4] Kiyoto Tsuchiya,[1] Wataru Sugiura,[3] Masafumi Takiguchi,[2] and Shinichi Oka[1,2]

[1]AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo; [2]Center for AIDS Research, Kumamoto University; [3]National Hospital Organization, Nagoya Medical Center; and [4]Japan Foundation for AIDS Prevention, Tokyo, Japan

Background. Rilpivirine is listed as an alternative key drug in current antiretroviral therapy (ART) guidelines. E138G/A/K in human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) are rilpivirine resistance-associated mutations and can be identified in a few ART-naive patients, although at low frequency. The 138th position in HIV-1 RT is located in one of the putative epitopes of human leukocyte antigen (HLA)–B*18–restricted cytotoxic T lymphocytes (CTLs). CTL-mediated immune pressure selects escape mutations within the CTL epitope. Here we tested whether E138G/A/K could be selected by HLA-B*18-restricted CTLs.

Methods. The amino acid variation at the 138th position was compared between ART-naive HIV-1–infected patients with and without HLA-B*18. The optimal epitope containing the 138th position was determined and the impact of E138G/A/K on CTL response was analyzed by epitope-specific CTLs. The effect of E138G/A/K on drug susceptibility was determined by constructing recombinant HIV-1 variants.

Results. The prevalence of E138G/A/K was 21% and 0.37% in 19 and 1088 patients with and without HLA-B*18, respectively (odds ratio, 72.3; $P = 4.9 \times 10^{-25}$). The CTL response was completely abolished by the substitution of E138G/A/K in the epitope peptide. E138G/A/K conferred 5.1-, 7.1-, and 2.7-fold resistance to rilpivirine, respectively.

Conclusions. E138G/A/K can be selected by HLA-B*18-restricted CTLs and confer significant rilpivirine resistance. We recommend drug resistance testing before the introduction of rilpivirine-based ART in HLA-B*18-positive patients.

Keywords. rilpivirine; E138G/A/K; HLA-B*18; CTL.

Rilpivirine is a new-generation nonnucleoside reverse transcriptase inhibitor (NNRTI), with noninferior clinical efficacy demonstrated in large clinical trials, compared with efavirenz [1, 2], and is listed as an alternative key drug in current antiretroviral therapy (ART) guidelines [3, 4]. In those clinical trials, rilpivirine showed more-favorable safety and tolerance profiles compared with efavirenz, although it was also associated with a higher virological failure rate. The most commonly observed NNRTI resistance-associated mutation in rilpivirine-treated patients with virological failure has so far been E138 K [1, 2]. Not only E138 K, but also other substitutions at the 138th position in human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT), might confer significant rilpivirine resistance [5–7]. The glutamic acid at the 138th position (E138) is well conserved among HIV-1 strains and clinical isolates throughout clades [8]. However, some ART-naive patients are infected with HIV-1 variants harboring other amino acids at the 138th position (E138X), although the proportion of such patients is low [9]. The 138th position is located in one of the putative epitopes of human leukocyte antigen (HLA)–B*18-restricted cytotoxic T lymphocytes (CTLs) [10, 11]. Because CTL immune pressure often selects escape mutations within the epitope [11], E138X may be selected by HLA-B*18-restricted CTLs. In this study, we analyzed the frequency of amino acid variations at the 138th position in ART-naive patients with or without

Received 29 April 2013; accepted 13 June 2013; electronically published 23 June 2013.

Correspondence: Hiroyuki Gatanaga, MD, AIDS Clinical Center, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan (higatana@acc.ncgm.go.jp).

Clinical Infectious Diseases 2013;57(7):1051–5

DOI: 10.1093/cid/cit430

Table 1. Amino Acid Variations at the 138th Position of HIV-1 Reverse Transcriptase and Human Leukocyte Antigen–B*18

Amino Acid	HLA-B*18(+)	HLA-B*18(–)
E138 (wild-type)	15	1084
E138G	2	1
E138A	1	2
E138K	1	1

Abbreviation: HLA, human leukocyte antigen.

HLA-B*18, determined the impact of E138X on CTL response, and analyzed the drug susceptibility of recombinant HIV-1 variants harboring E138X.

METHODS

Sequences of HIV-1 Reverse Transcriptase

HIV-1 RT sequences were analyzed using viral RNA extracted from plasma samples [12], and HLA type was determined by standard sequence-based genotyping in 1107 ART-naive infected individuals who visited the Outpatient Clinic of the AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, between 2003 and 2012. The amino acid variation at the 138th position of HIV-1 RT was compared between individuals with and those without HLA-B*18, and the statistical significance of the difference was analyzed by Fisher exact test using the Statistical Package for Social Sciences, version 17.0 (SPSS, Chicago, Illinois). This study was approved by the institutional ethical committee of the National Center for Global Health and Medicine, and written informed consent was obtained from all the participants according to the Declaration of Helsinki.

Intracellular Cytokine Staining Assay

HIV-1-derived peptides and mutant peptides were synthesized using an automated multiple peptide synthesizer and purified by high-performance liquid chromatography. Peripheral blood mononuclear cells (PBMCs) from chronically HIV-1–infected HLA-B*18-positive patients were stimulated with the peptide (100 nM) in culture medium (RPMI 1640 medium supplemented with 10% fetal calf serum and 200 U/mL recombinant human interleukin 2). After 14 days in culture, the cells were assessed for interferon (IFN)–γ production activity using a FACSCanto II (BD Biosciences, San Jose, California) [13, 14].

Drug Susceptibility Assay

The desired mutations were introduced into the *XmaI-NheI* region of pTZNX, which encodes the 15th–267th positions of HIV-1 RT (strain BH10) [15, 16]. The *XmaI-NheI* fragment was inserted into pNL_{H219Q}, which was modified from pNL101 and encoded the full genome of HIV-1. Each molecular clone was transfected into COS-7 cells, and the obtained virions were harvested 48 hours after transfection and stored at −80°C until use. Efavirenz and nevirapine were generously provided by Merck Co, Inc (Rahway, New Jersey) and Boehringer Ingelheim Pharmaceutics Inc (Ridgefield, Connecticut), respectively. Etravirine and rilpivirine were purchased from Toronto Research Chemicals Inc (North York, Ontario, Canada). The susceptibility of recombinant HIV-1 variants to efavirenz, nevirapine, etravirine, and rilpivirine was determined in triplicate and repeated 3 times [16]. Fold resistance was calculated by comparing the viral 50% inhibitory concentration (IC_{50}) with that of monoclonal wild-type HIV-1.

Structural Modeling

We constructed structural models of the HIV-1 RT and rilpivirine complex by computational analysis, as described in our previous reports [15, 16]. In brief, the initial models of wild-type RT with rilpivirine were first constructed by homology modelling. The crystal structures of RT with NNRTI (PDB code: 2ZD1 [17]) was used for template structure. We also constructed the respective mutant RTs with rilpivirine by considering every possible conformer of the respective mutant models. The possible conformers were generated from the wild-type homology models using PyMOL software (http://www.pymol.org). Among the conformers, we selected those with the lowest energy as each mutant model.

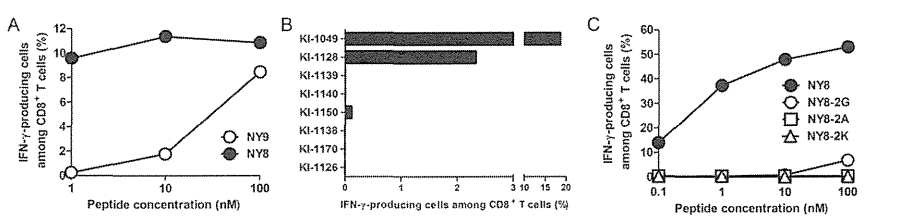

Figure 1. Recognition of human leukocyte antigen (HLA)–B*18-restricted $CD8^+$ T cells. *A*, Identification of the optimal epitope of HLA-B*18-restricted $CD8^+$ T cells. Peripheral blood mononuclear cells (PBMCs) from an HLA-B*18-positive individual chronically infected with human immunodeficiency virus type 1 (HIV-1) were stimulated with NY9 peptide and cultured for 2 weeks. Recognition of the bulk $CD8^+$ T cells toward each peptide was measured by the intracellular cytokine staining (ICS) assay. *B*, Induction of NY8-specific $CD8^+$ T cells in HLA-B*18-positive individuals chronically infected with HIV-1. PBMCs from 8 chronically HIV-1-infected HLA-B*18-positive individuals were stimulated with NY9 peptide and cultured for 2 weeks. Recognition of the bulk $CD8^+$ T cells toward NY8 peptide were measured by the ICS assay. *C*, Effects of E138G/A/K substitutions on the recognition of HLA-B*18-restricted $CD8^+$ T cells. Recognition of the bulk $CD8^+$ T cells toward each wild-type or mutant peptide was measured by the ICS assay. Abbreviations: IFN-γ, interferon gamma; NY8, NETPGIRY; NY8-2G, NGTPGIRY; NY8-2A, NATPGIRY; NY8-2 K, NKTPGIRY; NY9, NNETPGIRY.

Table 2. Susceptibility of Recombinant HIV-1 Variants to 4 Nonnucleoside Reverse Transcriptase Inhibitors

	IC_{50} (nM), Fold Resistance[a]			
Amino Acid	EFV	NVP	ETR	RPV
E138 (wild-type)	1.2 ± 0.2 (1)	31 ± 3 (1)	1.1 ± 0.1 (1)	0.16 ± 0.04 (1)
E138G	1.6 ± 0.2 (1.3)	30 ± 10 (0.97)	2.4 ± 0.3 (2.2)	0.82 ± 0.09 (5.1)
E138A	2.1 ± 0.3 (1.8)	30 ± 2 (0.97)	2.6 ± 0.2 (2.4)	1.13 ± 0.20 (7.1)
E138K	2.4 ± 0.4 (2.0)	50 ± 10 (1.6)	2.4 ± 0.1 (2.2)	0.43 ± 0.10 (2.7)

Data are presented as mean ± standard deviation.

Abbreviations: EFV, efavirenz; ETR, etravirine; IC_{50}, viral 50% inhibitory concentration; HIV-1, human immunodeficiency virus type 1; NVP, nevirapine; RPV, rilpivirine.

[a] Fold resistance was calculated by comparing viral IC_{50} with that of monoclonal wild-type HIV-1.

RESULTS

First, we analyzed the frequency of amino acid variations at the 138th position of HIV-1 RT in 1107 ART-naive individuals. As expected, E138 was found in the majority (1099 cases [99%]) of the analyzed patients. However, 8 cases showed amino acid substitutions, including 3 cases of substitution with glycine (E138G), 3 cases with alanine (E138A), and 2 cases with lysine (E138 K). The frequency of E138G/A/K substitutions was 21% and 0.37% in 19 and 1088 individuals with and without HLA-B*18, respectively (Table 1). There was a significant difference in the frequency of the substitutions (odds ratio, 72.3; $P = 4.9 \times 10^{-25}$), suggesting that E138G/A/K could be selected by HLA-B*18-restricted CTLs.

Next, we delineated the impact of E138G/A/K on the response of HLA-B*18-restricted CTLs. The putative HLA-B*18-restricted CTL epitopes containing the 138th position of HIV-1 RT were NETPGIRYQY (NY10; position 137–146), NETPGIRYQ (NQ9; position 137–145), and NNETPGIRY (NY9; position 136–144) [10, 11]. These 3 peptides were used to stimulate PBMCs of 8 ART-treated HLA-B*18-positive patients chronically infected with HIV-1. IFN-γ production activity was detected in PBMCs from 1 of the 8 patients when stimulated with NY9. To determine the optimal epitope, the bulk $CD8^+$ T cells were further analyzed for NY9 and NETPGIRY (NY8; position 137–144). The bulk $CD8^+$ T cells more efficiently recognized NY8 than NY9 at 1-nM, 10-nM, and 100-nM concentrations (Figure 1*A*). These findings indicate that NY8 was the optimal epitope of HLA-B*18-restricted CTLs. Indeed, NY8-specific $CD8^+$ T cells were induced in 3 of the 8 patients (Figure 1*B*). A

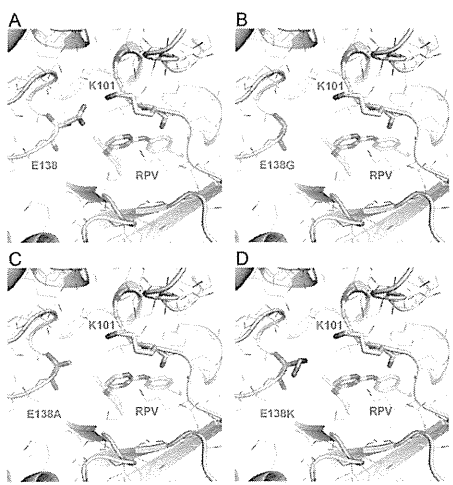

Figure 2. Structural models of human immunodeficiency virus type 1 reverse transcriptase (RT) and rilpivirine. The binding clefts of 4 complexes are shown: $RT_{E138(wild\text{-}type)}$ (*A*), RT_{E138G} (*B*), RT_{E138A} (*C*), and RT_{E138K} (*D*). Sticks indicate the amino acids at positions 101 and 138 of RT, and the atoms of rilpivirine. The mutated residues (E138G, E138A, and E138 K) and rilpivirine atoms are represented by orange and greenish-blue sticks, respectively. Abbreviation: RPV, rilpivirine.

previous study showed that HLA-B*18-binding peptides have 2 anchor residues, E at position 2 and Y/F at the C-terminus [18]. NY8 also had these 2 anchor residues, supporting that this peptide is a HLA-B*18-restricted CTL epitope. To analyze the effect of E138G/A/K on the CTL response, 3 mutant peptides, NGTPGIRY (NY8-2G), NATPGIRY (NY8-2A), and NKTPGIRY (NY8-2 K), were synthesized, and the recognition of the bulk CTLs for these mutant peptides was compared with that for NY8. The bulk CTLs failed to recognize these peptides at 0.1-nM, 1-nM, 10-nM, and 100-nM concentrations, although it effectively recognized NY8 (Figure 1*C*). These substitutions at the 138th position may affect peptide binding to the HLA-B*18 molecule because the second position of HLA-B*18-binding peptides is an anchor for HLA-B*18 [18]. These findings indicate that each of the E138G/A/K affected CTL recognition and allow escape from the HLA-B*18-restricted CTLs.

Finally, we analyzed the effect of E138G/A/K on viral susceptibility to NNRTIs by constructing recombinant HIV-1 variants. Each HIV-1 variant harboring one of E138G/A/K showed comparable replication fitness with wild-type HIV-1. Although the substitutions of E138G/A/K did not confer >2-fold resistance to efavirenz and nevirapine, they conferred mild resistance (2.2- to 2.4-fold) to etravirine. With regard to rilpivirine, E138 K, which was commonly observed in patients with virological failure under rilpivirine-based ART [1, 2], conferred mild resistance, whereas E138G and E138A conferred >5-fold resistance (Table 2). These findings indicate that in addition to E138 K, E138G and E138A can also reduce the clinical response to rilpivirine. The structural modeling suggests that substitution of E138 changes interactions around the rilpivirine-binding cleft (Figure 2). The side chain of E138 in the wild-type RT forms a salt bridge with the lysine at the 101th position (K101) at the edge of the cleft and establishes direct interactions with the pyrimidine moiety of rilpivirine, as seen in the crystal structure of RT with rilpivirine [17]. Meanwhile, mutant RTs with E138G/A/K substitutions could not create such a salt bridge, resulting in changes in the morphology of the binding cleft. In particular, RTs with E138G or E138A can reduce interactions with rilpivirine by creating large gaps between rilpivirine and the substituted 138th residues with small side chains, which seems to cause significant resistance to rilpivirine.

DISCUSSION

The major findings of the present study were as follows: (1) E138G/A/K substitutions were escape mutations of HLA-B*18-restricted CTLs and they were observed more frequently in HLA-B*18-positive patients than HLA-B*18-negative patients; and (2) we confirmed that these substitutions conferred significant resistance to rilpivirine, demonstrating that drug resistance–associated mutations can be selected naturally by CTL when its epitope is located in the viral protein of antiretroviral targets.

Studies of cellular immunology in HIV-1 have focused mainly on Gag [19, 20]. However, considering that many of the recently identified CTL epitopes are located in Pol [13, 14, 21], analysis of the interaction between CTL and drug susceptibility is warranted. Some escape mutations can persist after viral transmission to other hosts even if the new hosts do not have the corresponding HLAs [22]. Therefore, HIV-1 can adapt to HLA at a population level [23]. In fact, we identified E138G/A/K in ART-naive HLA-B*18-negative patients, although the frequency of such variations was extremely low. However, the same analysis performed in areas with higher prevalence of HLA-B*18, such as Eastern Europe [24], would probably detect higher frequency of E138G/A/K.

HIV drug resistance testing is recommended not only after treatment failure but also before the introduction of the initial treatment, considering the risk that the patient may have acquired drug-resistant viruses from those with treatment failure [3, 25]. The present study may add another reason for drug resistance testing of ART-naive patients: drug resistance–associated mutations may have evolved in the patients selected by their own immunity even if the original transmitted viruses were drug sensitive. At the very least, drug resistance testing should be performed before the introduction of rilpivirine-based ART in HLA-B*18-positive patients.

Notes

Acknowledgments. We thank all physicians and nurses at the AIDS Clinical Center, National Center for Global Health and Medicine, for the clinical practice and patient care. We also thank A. Nakano for the excellent project coordination.

Financial support. This work was supported in part by Grants-in Aid for AIDS research from the Ministry of Health, Labour, and Welfare, Japan; the Global COE Program (Global Education and Research center Aiming at the control of AIDS); MEXT, Japan; and Japan Foundation for AIDS Prevention.

Potential conflicts of interest. H. G. has received honoraria from ViiV Healthcare, MSD K.K., Abbott Japan, Janssen Pharmaceutical K.K., and Torii Pharmaceutical. S. O. has received honoraria and research grants from MSD K.K., Abbott Japan, Janssen Pharmaceutical K.K., Pfizer, ViiV Healthcare, and Roche Diagnostics K.K., and has received honoraria from Astellas Pharmaceutical K.K., Bristol-Myers K.K., Daiichisankyo, Dainippon Sumitomo Pharma, GlaxoSmithKline, K.K., Taisho Toyama Pharmaceutical, and Torii Pharmaceutical. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Molina JM, Cahn P, Grinsztejn B, et al. Rilpivirine versus efavirenz with tenofovir and emtricitabine in treatment-naïve adults infected with HIV-1 (ECHO): a phase 3 randomised double-blind active-controlled trial. Lancet **2011**; 378:238–46.
2. Cohen CJ, Andrade-Vilanueva J, Clotet B, et al. Rilpivirine versus efavirenz with two background nucleoside or nucleotide reverse transcriptase inhibitors in treatment-naïve adults infected with HIV-1 (THRIVE): a phase 3, randomised, non-inferiority trial. Lancet **2011**; 378:229–37.
3. Department of Health and Human Services Panel on Antiretroviral Guidelines for Adult and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf. Accessed 26 March 2013.
4. Thompson MA, Aberg JA, Hoy JF, et al. Antiretroviral treatment of adult HIV infection: 2012 recommendations of the International Antiviral Society–USA panel. JAMA **2012**; 308:387–402.
5. Johnson VA, Calvez V, Gunthard HF, et al. Update of the drug resistance mutations in HIV-1: March 2013. Top Antivir Med **2013**; 21:6–14.
6. Azunj H, Tirry I, Vingerhoets J, et al. TMC278, a next-generation nonnucleoside reverse transcriptase inhibitor (NNRTI), active against wild-type and NNRTI-resistant HIV-1. Antimicrob Agents Chemother **2010**; 54:718–27.
7. Asahchop EL, Wainberg MA, Oliveira M, et al. Distinct resistance patterns to etravirine and rilpivirine in viruses containing nonnucleoside reverse transcriptase inhibitor mutations at baseline. AIDS **2013**; 27:879–87.
8. Lambert-Niclot S, Charpentier C, Storto A, et al. Prevalence of pre-existing resistance-associated mutations to rilpivirine, emtricitabine and tenofovir in antiretroviral-naive patients infected with B and non-B subtype HIV-1 viruses. J Antimicrob Chemother **2013**; 68:1237–42.
9. Siegel MO, Swierzbinski M, Kan VL, Parenti DM. Baseline E138 reverse transcriptase resistance-associated mutations in antiretroviral-naive HIV-infected patients. AIDS **2012**; 26:1181–2.
10. Liu Y, McNevin J, Cao J, et al. Selection on the human immunodeficiency virus type 1 proteome following primary infection. J Virol **2006**; 80:9519–29.
11. Brumme ZL, John M, Carlson JM, et al. HLA-associated immune escape pathways in HIV-1 subtype B Gag, Pol and Nef proteins. PLoS One **2009**; 4:e6687.
12. Gatanaga H, Ibe S, Matsuda M, et al. Drug-resistant HIV-1 prevalence in patients newly diagnosed with HIV/AIDS in Japan. Antiviral Res **2007**; 75:75–82.
13. Honda K, Zheng N, Murakoshi H, et al. Selection of escape mutant by HLA-C-restricted HIV-1 Pol-specific T lymphocytes carrying strong ability to suppress HIV-1 replication. Eur J Immunol **2011**; 41:97–106.
14. Watanabe T, Murakoshi H, Gatanaga H, et al. Effective recognition of HIV-1-infected cells by HIV-1 integrase-specific HLA-B*4002-restricted T cells. Microbes Infect **2011**; 13:160–6.
15. Gatanaga H, Ode H, Hachiya A, Hayashida T, Sato H, Oka S. Impact of human leukocyte antigen-B*51-restricted cytotoxic T-lymphocyte pressure on mutation patterns of nonnucleoside reverse transcriptase inhibitor resistance. AIDS **2010**; 24:F15–22.
16. Gatanaga H, Ode H, Hachiya A, Hayashida T, Sato H, Oka S. Combination of V106I and V179D polymorphic mutations in human immunodeficiency virus type 1 reverse transcriptase confers resistance to efavirenz and nevirapine but not etravirine. Antimicrob Agents Chemother **2010**; 54:1596–602.
17. Das K, Bauman JD, Clark AD Jr, et al. High-resolution structures of HIV-1 reverse transcriptase/TMC278 complexes: strategic flexibility explains potency against resistance mutations. Proc Natl Acad Sci U S A **2008**; 105:1466–71.
18. Hickman HD, Luis AD, Buchli R, et al. Toward a definition of self: proteomic evaluation of the class I peptide repertoire. J Immunol **2004**; 172:2944–52.
19. Brumme ZL, Tao I, Szeto S, et al. Human leukocyte antigen-specific polymorphisms in HIV-1 Gag and their association with viral load in chronic untreated infection. AIDS **2008**; 22:1277–86.
20. Martinez-Picado J, Prado JG, Fry EE, et al. Fitness const of escape mutations in p24 Gag in association with control of human immunodeficiency virus type 1. J Virol **2006**; 80:3617–23.
21. Brumme ZL, Brumme CJ, Carlson J, et al. Marked epitope- and allele-specific differences in rates of mutation in human immunodeficiency virus type 1 (HIV-1) Gag, Pol, and Nef cytotoxic T-lymphocyte epitopes in acute/early HIV-1 infection. J Virol **2008**; 82:9216–27.
22. Goulder PJ, Brander C, Tang Y, et al. Evolution and transmission of stable CTL escape mutations in HIV infection. Nature **2001**; 412:334–8.
23. Kawashima Y, Pfafferott K, Frater J, et al. Adaptation of HIV-1 to human leukocyte antigen class I. Nature **2009**; 458:641–5.
24. Solberg OD, Mack SJ, Lancaster AK, et al. Balancing selection and heterogeneity across the classic human leukocyte antigen loci: a meta-analytic review of 497 population studies. Hum Immunol **2008**; 69: 443–64.
25. Hirsh MS, Gunthard HF, Schapiro JM, et al. Antiretroviral drug resistance testing in adult HIV-1 infection. Clin Infect Dis **2008**; 47: 266–85.

OPEN ACCESS Freely available online

PLOS ONE

Preemptive Therapy Prevents Cytomegalovirus End-Organ Disease in Treatment-Naïve Patients with Advanced HIV-1 Infection in the HAART Era

Daisuke Mizushima[1,2], Takeshi Nishijima[1,2], Hiroyuki Gatanaga[1,2]*, Kunihisa Tsukada[1], Katsuji Teruya[1], Yoshimi Kikuchi[1], Shinichi Oka[1,2]

1 AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan, **2** Center for AIDS Research, Kumamoto University, Kumamoto, Japan

Abstract

Background: The efficacy of preemptive therapy against cytomegalovirus (CMV) infection remains unknown in treatment-naïve patients with advanced HIV-1 infection in the HAART era.

Methods: The subjects of this single-center observation study were126 treatment-naïve HIV-1 infected patients with positive CMV viremia between January 1, 2000 and December 31, 2006. Inclusion criteria were age more than 17 years, CD4 count less than 100/μl, plasma CMV DNA positive, never having received antiretroviral therapy (ART) and no CMV end-organ disease (EOD) at first visit. The incidence of CMV-EOD was compared in patients with and without preemptive therapy against CMV-EOD. The effects of the CMV preemptive therapy were estimated in uni- and multivariate Cox hazards models.

Results: CMV-EOD was diagnosed in 30 of the 96 patients of the non-preemptive therapy group (31%, 230.3 per 1000 person-years), compared with 3 of the 30 patients of the preemptive therapy group (10%, 60.9 per 1000 person-years). Univariate (HR = 0.286; 95%CI, 0.087–0.939; p = 0.039) and multivariate (adjusted HR = 0.170; 95%CI, 0.049–0.602; p = 0.005) analyses confirmed that CMV-EOD is significantly prevented by CMV preemptive therapy. Multivariate analysis showed that plasma CMV DNA level correlated significantly with CMV-EOD (per log10/ml, adjusted HR = 1.941; 95%CI, 1.266–2.975; p = 0.002). Among the 30 patients on preemptive therapy, 7 (23.3%) developed grade 3–4 leukopenia. The mortality rate was not significantly different between the two groups (p = 0.193, Log-rank test).

Conclusions: The results indicate that preemptive therapy lowers the incidence of CMV-EOD by almost 25%. Preemptive therapy for treatment-naïve patients with CMV viremia is effective, although monitoring of potential treatment-related side effects is required.

Citation: Mizushima D, Nishijima T, Gatanaga H, Tsukada K, Teruya K, et al. (2013) Preemptive Therapy Prevents Cytomegalovirus End-Organ Disease in Treatment-Naïve Patients with Advanced HIV-1 Infection in the HAART Era. PLoS ONE 8(5): e65348. doi:10.1371/journal.pone.0065348

Editor: Michael Nevels, University of Regensburg, Germany

Received January 7, 2013; **Accepted** April 24, 2013; **Published** May 28, 2013

Funding: This work was supported by a Grant for National Center for Global Health and Medicine (23-114). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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

* E-mail: higatana@acc.ncgm.go.jp

Introduction

Although the incidence of new cases of cytomegalovirus (CMV) end-organ disease (EOD) has decreased by 75%–80% with the advent of antiretroviral therapy (ART) and is currently estimated to be <6 cases per 100 person-years [1], CMV-EOD is still one of the major debilitating diseases among patients with advanced HIV infection.

CMV preemptive therapy is commonly used for patients scheduled for hematopoietic cell transplantation and solid organ transplantation, with clinical evidence of efficacy[2–6], however, it is not generally recommended in HIV patients [7] because of concerns regarding cost-effectiveness, risk of developing CMV resistance, side effect and the lack of a proven survival benefit [8]. A prospective trial in cooperation with Roche company to evaluate the efficacy of preemptive therapy in the pre-HAART (highly active ART) era showed significant preventive effect of oral ganciclovir (GCV) [9]. However; other studies conducted in both pre-HAART and HAART eras showed no significant effect [10,11]. However, the above studies included patients who had previously received ART. Therefore, the efficacy of preemptive therapy against CMV infection remains unknown in treatment-naïve patients with advanced HIV-1 infection in the HAART era.

We retrospectively compared the incidence of CMV-EOD in a cohort of ART-naïve adult patients with advanced HIV infection (low CD4 count and plasma CMV-DNA-positive). One group of these patients had received CMV preemptive therapy, while the other had not received such therapy.

Methods

Ethics Statement

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

Study design

We performed a retrospective, single-center cohort study to elucidate the effectiveness of preemptive CMV treatment in HIV-infected patients with positive CMV viral load in the prevention of CMV-EOD. The study was conducted at the National Center for Global Health and Medicine, Tokyo, one of the largest clinics for patients with HIV infection in Japan, with more than 2,700 registered patients as of December 2006. The study population comprised treatment-naïve HIV infected patients aged more than 17 years, with CD4 count less than 100/μl and positive plasma CMV DNA viral load, who presented for the first time at our hospital between January 1, 2000 and December 31, 2006. Those with CMV-EOD at presentation and those with <3 months of follow-up were excluded. The follow-up period was 2 years from the initial visit.

Definition of CMV-EOD and CMV preemptive therapy

CMV-EOD was diagnosed according to standardized ACTG criteria (see Table S1) [11]. CMV retinitis was routinely screened for by dilated indirect ophthalmoscopy at both the first visit to the hospital and a few months after the commencement of ART. Other evaluations, such as endoscopy and bronchoscopy, were carried out in response to the symptoms and clinical condition. The diagnosis of CMV-EOD was established by at least two experts from our hospital.

CMV preemptive therapy was prescribed based on the clinician's assessment. CMV preemptive therapy was provided at our institution for patients with plasma CMV DNA of >5000 copies/ml. For patients with plasma CMV DNA of >3000 but less than 5000 copies/ml, the decision to initiate preemptive therapy was left to the attending physician, taking into consideration the overall clinical condition, such as subsequent rise in plasma CMV DNA and/or use of immunosuppressants, such as steroids and chemotherapeutic agents. Ganciclovir (GCV) and valganciclovir (VGCV) were the most commonly used agents, followed by foscarnet (FOS). The choice of induction (intravenous GCV 5 mg/kg every 12 hours, oral VGCV900 mg twice a day or intravenous FOS 90 mg/kg every 12 hours) or maintenance dose (intravenous GCV 5 mg/kg every 24 hours, oral VGCV 900 mg a day or intravenous FOS 90 mg/kg every 24 hours) was based on the clinical condition, such as the level of plasma CMV DNA or state of immunosuppression. The duration of therapy varied across individuals. CMV preemptive therapy was defined as at least a 7-day treatment with agents effective against CMV. The normal course of CMV preemptive therapy was 2 weeks of GCV induction dose followed by VGCV or GCV maintenance dose until plasma CMV DNA became negative. Patients were retreated based on clinicians' decision under some conditions with high risks for CMV-EOD as described above, if plasma CMV DNA became positive again after preemptive therapy.

Measurements

Plasma CMV DNA was measured using real-time PCR with a lower limit of detection of 200 copies/mL(CMV geniQ, Bio Medical Laboratory, Inc., Tokyo, Japan). Plasma CMV DNA was measured routinely at the first visit in patients with CD4 count of <100/μl, and re-examined every week or monthly, according to the level of plasma CMV DNA viral load or immune status and at the discretion of the attending physician.

In this study, the primary exposure variable was CMV preemptive therapy over no CMV preemptive therapy. The potential risk factors for CMV-EOD were determined based on previous studies [12–18], and included basic demographics and laboratory data, including age, sex, CD4 cell count, HIV viral load, plasma CMV DNA, and presence or absence of other medical conditions (concurrent use of steroids, concurrent chemotherapy and concurrent AIDS-defining diseases). For each patient, data on or closest to the day of the first visit to our hospital were retrieved for analysis.

Statistical analysis

Categorical and continuous baseline demographics and laboratory data were analyzed using Pearson's chi-square test and Student's t-test, respectively. The time from the first visit to our hospital to the development of CMV-EOD was analyzed by the Kaplan Meier method for patients on CMV preemptive therapy and no CMV preemptive therapy, and the log-rank test was used to determine the statistical significance. Censored cases represented those who died, dropped out, or were referred to other facilities before the end of follow-up period. The Cox proportional hazards regression analysis was used to estimate the impact of CMV preemptive therapy on the incidence of CMV-EOD. The impact of basic demographics, baseline laboratory data, and other medical conditions was also estimated with univariate Cox proportional hazards regression.

To estimate the unbiased prognostic impact of CMV preemptive therapy, we used three models based on multivariate Cox proportional hazards regression analysis. Model 1 was the aforementioned univariate analysis for CMV preemptive therapy. Model 2 included age and sex, plus Model 1, in order to adjust for basic characteristics. In Model 3, we added variables with significant relation to CMV-EOD by univariate analysis or assumed as risk factor(s) for CMV-EOD in the literature[12–20] (e.g., CD4 count per 1/μl decrement, HIV viral load per log10/ml, CMVDNA viral load per log10/ml, concurrent steroid use, concurrent chemotherapy and concurrent AIDS defining disease). Statistical significance was set at two-sided p values <0.05. We used hazard ratios (HRs) and 95% confidence intervals (95%CIs) to estimate the impact of each variable on CMV-EOD. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, IL).

Results

Of the 199 HIV-infected patients with CD4 count <100/μl and positive plasma CMV DNA viral load referred to our hospital between January 1, 2000 and December 31, 2006, 126 patients were recruited in the study. Of these, 96 patients received CMV preemptive therapy while 30 did not (Figure 1). Table 1 lists the demographics, laboratory data, and medical conditions of the study population at baseline. The majority of the study population were males, East Asians, and relatively young (median: 42 years). There were no differences in baseline CD4 count (p = 0.595) and HIV viral load (p = 0.628) between the two groups. Patients of the CMV preemptive therapy group had higher plasma CMV DNA viral load (p<0.001), more likely to have developed AIDS defining diseases (p = 0.042), and tended to have been treated concurrently with steroids (p = 0.009), compared with the non-CMV preemptive group. There were no significant differences in the use of chemotherapy (p = 1.000) and in time to initiation of ART since study entry (p = 0.393, Table 1) between the two groups.

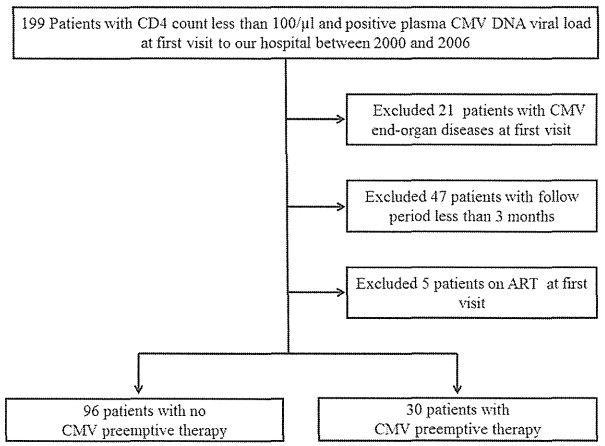

Figure 1. Flow chart of inclusion and exclusion criteria. Of the 199 subjects, 73 were excluded and the remaining 126 were included in the study. The latter group was divided into the preemptive therapy group (n = 30) and the non-therapy group (n = 96).
doi:10.1371/journal.pone.0065348.g001

During the follow-up period, CMV-EOD occurred in 3 (10.0%) patients of the preemptive therapy group and 30 (31.3%) of the non-preemptive therapy group, with an estimated incidence of 60.9 and 230.3 per 1000 person-years, respectively. Figure 2 depicts the time from the first visit to our hospital to the development of CMV-EOD by Kaplan Meier method in the two groups. The incidence of new cases of CMV-EOD was significantly higher in the non-preemptive therapy group, compared with the preemptive therapy group (p = 0.027, Log-rank test). The median time from the first visit to the diagnosis of CMV-EOD was 67 days (range, 25–67) for the preemptive therapy group, and 54 days (range, 14–326 days) for the non-preemptive therapy group.

Univariate analysis showed a significant relationship between CMV preemptive therapy and low incidence of CMV-EOD (HR = 0.286; 95%CI, 0.087–0.939; p = 0.039) (Table 2). On the other hand, high CMV viral load and HIV viral load tended to be associated with CMV-EOD, while old age, low baseline CD4 count, use of steroids, chemotherapy, and concurrent AIDS defining diseases were not associated with CMV-EOD. Multivariate analysis identified CMV preemptive therapy as a significant preventive factor against CMV-EOD after adjustment for age and sex (Model 2; adjusted HR = 0.289; 95%CI, 0.088–0.949; p = 0.041, Table 3), and after adjustment for other risk factors (Model 3; adjusted HR = 0.172; 95%CI, 0.049–0.602; p = 0.005, Table 3). In addition, multivariate analysis showed that high CMV viral load correlated significantly with CMV-EOD (Model 3; adjusted HR = 1.941; 95%CI, 1.266–2.975; p = 0.002, Table 3).

Of the 33 patients with CMV-EOD, 22 (66.7%) developed CMV retinitis, 4 (12.1%) developed esophagitis, 3 (9.1%) developed gastroduodenitis, 6 (18.2%) developed colitis and 1 (3.0%) developed pneumonitis. All 3 patients with CMV-EOD of the preemptive therapy group developed retinitis (Table 4).

Table 1. Baseline demographics and laboratory data of patients who did and did not receive CMV preemptive therapy.

	Non-preemptive therapy (n = 96)	Preemptive therapy (n = 30)	P value
Sex (male), n (%)	88 (91.7)	29 (96.7)	0.685
Median (range) age	41 (24–76)	44 (25–66)	0.729
Ethnicity, n (%)			
East Asians	86 (89.5)	29 (96.7)	
Southeast Asian	5 (5.2)	0 (0.0)	
Black	3 (3.1)	0 (0.0)	
White	2 (2.1)	1 (3.3)	
Median (range) CD4 count (/μl)	28.0 (0–97)	35.5 (3–87)	0.595
Median (range) HIV RNA viral load (log10/ml)	5.3 (3–6)	5.35 (4–7)	0.628
Median (range) CMVDNA viral load (log10/ml)	3.0 (2–5)	4.3 (2–5)	<0.001
Concurrent AIDS, n (%)	78 (81.3)	29 (96.7)	0.042
Steroid use, n (%)	38 (39.6)	20 (66.7)	0.009
Chemotherapy, n (%)	9 (9.4)	2 (6.7)	1.000
Median (range) time (days) to ART*	66 (2–399)	59 (13–158)	0.393
Median (range) follow-up (days)	730 (14–730)	730 (25–730)	0.064

*11 missing values.
Categorical and continuous variables were analyzed using Pearson's chi-square test and Student's t-test, respectively.
doi:10.1371/journal.pone.0065348.t001

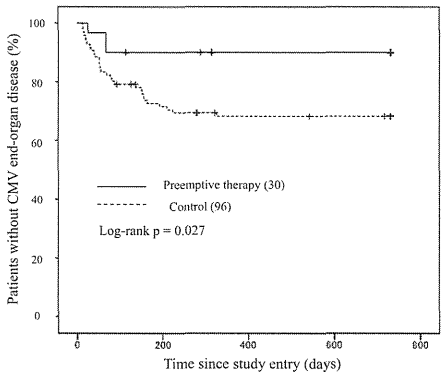

Figure 2. Kaplan-Meier curve showing the time to development of cytomegalovirus (CMV)- end-organ disease (EOD) in the preemptive and non-preemptive therapy groups. Compared to patients on CMV preemptive therapy, those who did not receive preemptive therapy were more likely to develop CMV-EOD (p = 0.027, Log-rank test).
doi:10.1371/journal.pone.0065348.g002

Table 2. Results of univariate analysis to estimate the risk of various factors in inducing CMV end-organ disease.

	Hazard ratio	95% CI	P value
CMV preemptive therapy	0.286	0.087–0.939	0.039
Female	1.284	0.392–4.209	0.680
Age per 1 year	0.982	0.951–1.013	0.240
CD4 count per 1/μl decrement	1.001	0.989–1.013	0.867
HIV viral load per log10/ml	1.875	0.905–3.884	0.091
CMV viral load per log10/ml	1.450	0.984–2.136	0.060
Use of steroid	0.716	0.356–1.439	0.348
Chemotherapy	1.390	0.488–3.955	0.537
Concurrent AIDS	0.703	0.290–1.704	0.436

CI: confidence interval
The Cox proportional hazards regression analysis was used.
doi:10.1371/journal.pone.0065348.t002

Of 30 patients who received preemptive therapy, 20 (66.7%) received an induction dose of GCV, and 7 patients (23.3%) received maintenance dose. The remaining agents used for preemptive therapy were an induction dose of VGCV, a maintenance dose of FOS and an induction dose of cidofovir. The duration of the preemptive therapy varied between 7 days and 2 months. The following side effects were noted in patients on CMV preemptive therapy: grade 3/4 leukopenia (n = 7, 23.3%) and grade 2 hypercreatininemia (n = 1, 3.3%). Both side effects developed during the use of GCV. Five patients (5.2%) of the non-preemptive therapy group and 4 patients (13.3%) of the preemptive therapy group died during the study period. Of the former group, 3 deaths were due to opportunistic infections (cryptococcus meningitis, non-tuberculous mycobacterial infection and *Pneumocystis jiroveci* pneumonia), 1 due to bacterial infection (sepsis), and 1 due to suicide. Of the latter group, 2 deaths were due to opportunistic infections (malignant lymphoma and *P. jiroveci* pneumonia) and 2 due to bacterial infection (bacterial pneumonias). Deaths and bacterial infections related to preemptive therapy were not observed in our study. The mortality rate was not significantly different between the two groups (p = 0.193, Log-rank test, Figure 3).

Discussion

The results of this observational cohort of treatment-naïve HIV-infected patients with positive plasma CMV DNA showed a significantly lower incidence of CMV-EOD by one-fourths in the CMV preemptive therapy group than in the non-preemptive therapy group, over the 2-year observation period. This finding was significant, despite higher risk for CMV-EOD in the preemptive therapy group, such as higher plasma CMV DNA, higher prevalence of concurrent AIDS defining diseases and more concurrent steroid use, compared with the other group. Univariate and multivariate analyses identified anti-CMV preemptive therapy as a significant preventive factor against CMV-EOD.

Our study is the first to illustrate the significance of anti-CMV preemptive therapy in treatment- naïve HIV-infected patients with CMV viremia and CD4 count less than 100/μl in the HAART era. The hazard ratio of development of CMV-EOD decreased by 82.8% following preemptive therapy, compared with no preemptive therapy, even after adjustment for plasma CMV DNA viral load and other factors. The current guidelines do not generally recommend anti-CMV preemptive therapy although this is based on sparse evidence, such as cost effectiveness, CMV resistance, and drug side effects [7]. However, our study suggests that preemptive therapy is a feasible option, if the effective target of preemptive therapy could be selected. Furthermore, the study confirmed that plasma CMV DNA, a known risk factor for CMV-EOD [12–18], was a significant independent risk factor.

A few prospective clinical trials investigated the efficacy of preemptive therapy in both the pre-HAART era and HAART era. In these studies, oral GCV at 1000 mg thrice daily was used in the pre-HAART era regimen [9,10] while VGCV at 900 mg twice daily was the regimen used in the HAART era [11]. The patients investigated in the above three studies were HIV-treatment-experienced patients. One study in the pre-HAART era reported the efficacy of preemptive therapy in patients with CD4 count<50 μl [9], while the other studies showed no significant preventive effect [10,11]. In the ACTG A5030 study, the prospective clinical trial in the HAART era, which evaluated the efficacy of oral VGCV 900 mg twice a day for 3 weeks among HIV-infected patients with CD4 count <100 cells/mm^3, plasma HIV RNA >400 copies/mL, plasma CMV viremia and on stable

Table 3. Results of multivariate analysis to estimate the preventive effect of CMV preemptive therapy against CMV end-organ disease.

	Model 1 Crude		*Model 2 Adjusted*		*Model 3 Adjusted*	
	HR	**95% CI**	**HR**	**95%CI**	**HR**	**95%CI**
CMV preemptive therapy*	0.286	0.087–0.939	0.289	0.088–0.949	0.172	0.049–0.602
Age			0.982	0.952–1.014	0.990	0.958–1.022
Female			1.033	0.310–3.441	0.988	0.267–3.653
CD4 count per 1/µl decrement					0.995	0.983–1.008
HIV viral load per log10/ml					2.217	0.912–5.393
CMV viral load per log10/ml*					1.941	1.266–2.975
Use of steroid					0.664	0.288–1.534
Chemotherapy					1.668	0.540–5.151
Concurrent AIDS					0.930	0.337–2.569

*P<0.05 in Model 3
HR: hazard ratio, CI: confidence interval
The Cox proportional hazards regression analysis was used.
Variables with significant difference by univariate analysis or assumed as risk factors for CMV-EOD in the literature were included in model 3.
doi:10.1371/journal.pone.0065348.t003

or no HAART, the authors reported a low incidence of CMV-EOD among subjects both with and without preemptive therapy [11]. The authors attributed the low incidence to improvement of immune function induced by potent ART. Actually, in that study [11], the number of patients who had received ART at study entry was about 80% of the total. In contrast, the subjects of our study were all treatment-naïve patients and possibly at higher risk for CMV-EOD compared to those enrolled in the ACTG A5030. Thus, the use of CMV preemptive therapy reported in our study under the clinical scenario of poor immune status without ART at study entry resulted in better outcome than in previous studies. In our study, there was no significant difference in the timing of ART between the two treatment groups. Although our study did not include the time to the initiation of ART as a variable in uni- and multivariate analysis because the values for 11 cases were missing, multivariate analysis with the time to the initiation of ART together with other variables similarly identified preemptive therapy as a significant preventive factor (adjusted HR = 0.235; 95%CI, 0.064–0.868; p = 0.030).

The survival benefits of CMV preemptive therapy were controversial in previous prospective clinical trials. One study suggested the survival benefits of 3 g/day oral GCV preemptive therapy [9], while other studies showed no evidence of the survival benefit [10]. On the other hand, two prospective cohort studies in the HAART era showed the relation between CMV viremia and high mortality [21] and suggested the benefit of CMV therapy [22], whereas our results showed no significant difference in mortality rate between the two groups. The reason for this discrepancy could be attributed to low mortality rate, small sample size and the disproportionally high risk of the therapy group in our study. The mortality rate (5.0 deaths per 100 person-years) in our study was similar to that in a study conducted in the HAART era (5.7 deaths per 100 person-years)[19] and was considerably lower than in studies from the pre-HAART era. Since the mortality rate has markedly decreased in advanced HIV infected patients following the introduction of potent ART in the HAART era [23,24], not only the survival benefit but also quality of life, such as improvement of eye function, should be emphasized in the future.

The side effects of preemptive therapy have also been of concern [25]. Our findings showed the development of grade 3 to 4 leukocytopenia in 23.3% of the patients who received intravenous GCV, and was the major side effect of preemptive therapy. Some patients who developed leukocytopenia required treatment with granulocyte colony-stimulating factor (G-CSF) and showed complete recovery. Thus; careful follow-up of patients on preemptive therapy is necessary. For these reasons, preemptive

Table 4. Details of CMV end-organ disease.

CMV-EOD	n (%)	Time to development (days)	Non-preemptive therapy group	Preemptive therapy group
Retinitis	22* (61.1%)	72 (14–326)	19* (57.6%)	3 (100%)
Esophagitis	4* (11.1%)	116.5(69–164)	4* (12.1%)	0
Gastroenteritis	3* (8.3%)	19 (14–40)	3* (9.1%)	0
Colitis	6* (16.7%)	40.5 (15–55)	6* (18.2%)	0
Pneumonitis	1 (2.8%)	31 (31–31)	1 (3.0%)	0
Total	36* (100%)	55 (14–326)	33* (100%)	3 (100%)

*Three patients of the non-preemptive therapy group had multiple CMV-EOD; one with retinitis plus esophagitis, one with retinitis plus gastroenteritis and the other with retinitis plus colitis.
doi:10.1371/journal.pone.0065348.t004

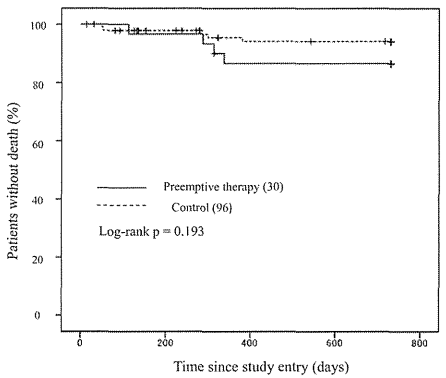

Figure 3. Kaplan-Meier curve showing the time to death in the preemptive and non-preemptive therapy groups. There was no significant difference in the survival rate between the two groups (p = 0.193, Log-rank test).
doi:10.1371/journal.pone.0065348.g003

therapy might place patients at greater risk in resource-limited setting, where close monitoring is difficult and the risk of bacterial infection is high. It is noteworthy, however, that death and bacterial infection related to preemptive therapy were not observed in our study.

The present study has several limitations. Due to its retrospective nature, it was not possible to control the baseline characteristics of the enrolled patients. However, patients with potential risk for CMV-EOD, such as those with high plasma CMV DNA, high concurrent AIDS and high steroid use, were more likely prescribed the preemptive therapy. It is noteworthy that the incidence of CMV-EOD was significantly lower in the preemptive therapy group despite this adverse environment.

Second, the criteria for treatment, choice of drugs and duration of CMV preemptive therapy were not rigidly controlled in the present study. Thus, it was difficult to determine which anti-CMV agent with what dosage is optimal for preemptive therapy. In the present study, about 90% of patients received induction dose or maintenance dose of GCV since the majority of patients of the preemptive therapy group were in-patients. Further prospective study is required to optimize effective preemptive therapy, including oral VGCV.

Third, CMV-EOD, especially enteritis, could have been overlooked at study entry since routine endoscopic screening was *not performed, compared with screening for retinitis* at the first visit. However, patients with abdominal pain were subjected to stool examination for occult blood, since the definition of CMV enteritis includes abdominal pain, and those with positive tests were subsequently considered for endoscopy. Thus, the possibility of latent CMV enteritis at study entry does not seem to have affected the results of the present study.

In conclusion, the present study demonstrated a lower incidence of CMV-EOD following CMV preemptive therapy by one-fourth, compared with no preemptive therapy, in treatment-naïve patients with CMV viremia. High plasma CMV DNA was identified as an independent risk for CMV-EOD. Further studies are warranted to elucidate the efficacy, safety and cost-effectiveness of anti-CMV preemptive therapy in HIV infected patients at high risk for EOD.

Supporting Information

Table S1 Definitions of CMV end-organ diseases used in this study.
(DOCX)

Acknowledgments

The authors thank all the clinical staff at the AIDS Clinical Center for their help in completion of this study.

Author Contributions

Conceived and designed the experiments: DM K. Tsukada K. Teruya. Performed the experiments: DM TN K. Teruya. Analyzed the data: DM *HG* YK SO. Contributed reagents/materials/analysis tools: YK K. Tsukada. Wrote the paper: DM TN HG SO.

References

1. Jabs DA, Van Natta ML, Holbrook JT, Kempen JH, Meinert CL, et al. (2007) Longitudinal study of the ocular complications of AIDS: 1. Ocular diagnoses at enrollment. Ophthalmology 114: 780–786.
2. Goodrich JM, Mori M, Gleaves CA, Du Mond C, Cays M, et al. (1991) Early treatment with ganciclovir to prevent cytomegalovirus disease after allogeneic bone marrow transplantation. N Engl J Med 325: 1601–1607.
3. Kalil AC, Levitsky J, Lyden E, Stoner J, Freifeld AG (2005) Meta-analysis: the efficacy of strategies to prevent organ disease by cytomegalovirus in solid organ transplant recipients. Ann Intern Med 143: 870–880.
4. Kalil AC, Freifeld AG, Lyden ER, Stoner JA (2009) Valganciclovir for cytomegalovirus prevention in solid organ transplant patients: an evidence-based reassessment of safety and efficacy. PLoS One 4: e5512.
5. Park JM, Lake KD, Arenas JD, Fontana RJ (2006) Efficacy and safety of low-dose valganciclovir in the prevention of cytomegalovirus disease in adult liver transplant recipients. Liver Transpl 12: 112–116.
6. Humar A, Kumar D, Preiksaitis J, Boivin G, Siegal D, et al. (2005) A trial of valganciclovir prophylaxis for cytomegalovirus prevention in lung transplant recipients. Am J Transplant 5: 1462–1468.
7. Kaplan JE, Benson C, Holmes KH, Brooks JT, Pau A, et al. (2009) Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. MMWR Recomm Rep 58: 1–207; quiz CE201-204.
8. Rose DN, Sacks HS (1997) Cost-effectiveness of cytomegalovirus (CMV) disease prevention in patients with AIDS: oral ganciclovir and CMV polymerase chain reaction testing. AIDS 11: 883–887.
9. Spector SA, McKinley GF, Lalezari JP, Samo T, Andruczk R, et al. (1996) Oral ganciclovir for the prevention of cytomegalovirus disease in persons with AIDS. Roche Cooperative Oral Ganciclovir Study Group. N Engl J Med 334: 1491–1497.
10. Brosgart CL, Louis TA, Hillman DW, Craig CP, Alston B, et al. (1998) A randomized, placebo-controlled trial of the safety and efficacy of oral ganciclovir for prophylaxis of cytomegalovirus disease in HIV-infected individuals. Terry Beirn Community Programs for Clinical Research on AIDS. AIDS 12: 269–277.
11. Wohl DA, Kendall MA, Andersen J, Crumpacker C, Spector SA, et al. (2009) Low rate of CMV end-organ disease in HIV-infected patients despite low CD4+ cell counts and CMV viremia: results of ACTG protocol A5030. HIV Clin Trials 10: 143–152.
12. Spector SA, Wong R, Hsia K, Pilcher M, Stempien MJ (1998) Plasma cytomegalovirus (CMV) DNA load predicts CMV disease and survival in AIDS patients. J Clin Invest 101: 497–502.
13. Spector SA, Hsia K, Crager M, Pilcher M, Cabral S, et al. (1999) Cytomegalovirus (CMV) DNA load is an independent predictor of CMV disease and survival in advanced AIDS. J Virol 73: 7027–7030.
14. Pergam SA, Xie H, Sandhu R, Pollack M, Smith J, et al. (2012) Efficiency and Risk Factors for CMV Transmission in Seronegative Hematopoietic Stem Cell Recipients. Biol Blood Marrow Transplant.
15. Kute VB, Vanikar AV, Shah PR, Gumber MR, Patel HV, et al. (2012) Post-renal transplant cytomegalovirus infection: study of risk factors. Transplant Proc 44: 706–709.
16. Fielding K, Koba A, Grant AD, Charalambous S, Day J, et al. (2011) Cytomegalovirus viremia as a risk factor for mortality prior to antiretroviral therapy among HIV-infected gold miners in South Africa. PLoS One 6: e25571.

17. Micol R, Buchy P, Guerrier G, Duong V, Ferradini L, et al. (2009) Prevalence, risk factors, and impact on outcome of cytomegalovirus replication in serum of Cambodian HIV-infected patients (2004-2007). J Acquir Immune Defic Syndr 51: 486–491.
18. Yoshida A, Hitomi S, Fukui T, Endo H, Morisawa Y, et al. (2001) Diagnosis and monitoring of human cytomegalovirus diseases in patients with human immunodeficiency virus infection by use of a real-time PCR assay. Clin Infect Dis 33: 1756–1761.
19. Erice A, Tierney C, Hirsch M, Caliendo AM, Weinberg A, et al. (2003) Cytomegalovirus (CMV) and human immunodeficiency virus (HIV) burden, CMV end-organ disease, and survival in subjects with advanced HIV infection (AIDS Clinical Trials Group Protocol 360). Clin Infect Dis 37: 567–578.
20. Hodge WG, Boivin JF, Shapiro SH, Shah KC, Dionne MA (2005) Iatrogenic risk factors for cytomegalovirus retinitis. Can J Ophthalmol 40: 701–710.
21. Deayton JR, Prof Sabin CA, Johnson MA, Emery VC, Wilson P, et al. (2004) Importance of cytomegalovirus viraemia in risk of disease progression and death in HIV-infected patients receiving highly active antiretroviral therapy. Lancet 363: 2116–2121.
22. Kempen JH, Martin BK, Wu AW, Barron B, Thorne JE, et al. (2003) The effect of cytomegalovirus retinitis on the quality of life of patients with AIDS in the era of highly active antiretroviral therapy. Ophthalmology 110: 987–995.
23. Ray M, Logan R, Sterne JA, Hernandez-Diaz S, Robins JM, et al. (2010) The effect of combined antiretroviral therapy on the overall mortality of HIV-infected individuals. AIDS 24: 123–137.
24. Sterne JA, May M, Costagliola D, de Wolf F, Phillips AN, et al. (2009) Timing of initiation of antiretroviral therapy in AIDS-free HIV-1-infected patients: a collaborative analysis of 18 HIV cohort studies. Lancet 373: 1352–1363.
25. Biron KK (2006) Antiviral drugs for cytomegalovirus diseases. Antiviral Res 71: 154–163.

Research Letters

AIDS 2013, **27**:839–848

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

Takeshi Nishijima[a,b], Hirokazu Komatsu[c], Katsuji Teruya[a], Junko Tanuma[a], Kunihisa Tsukada[a], Hiroyuki Gatanaga[a,b], Yoshimi Kikuchi[a] and Shinichi Oka[a,b]

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

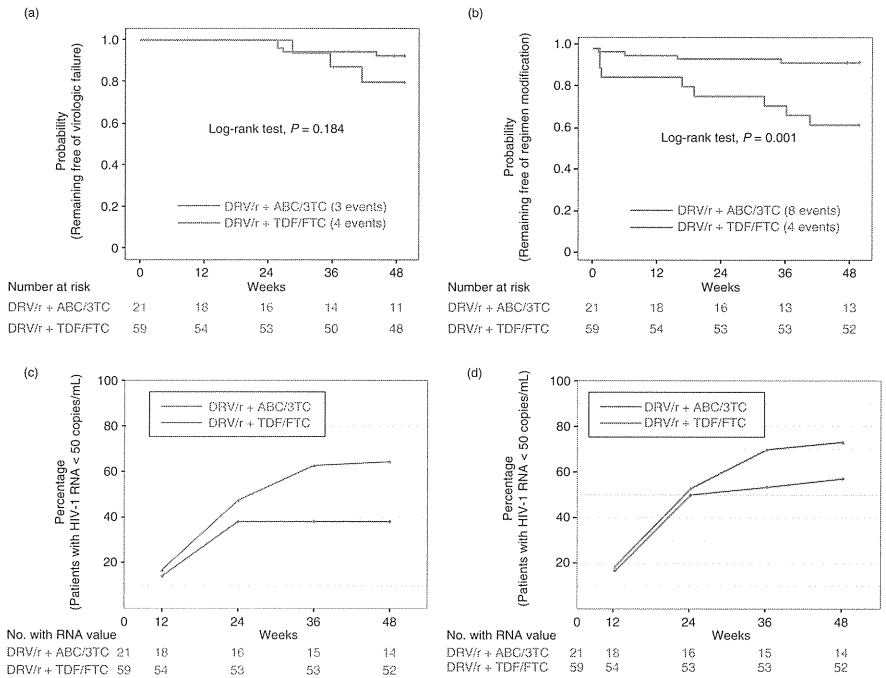

Fig. 1. Efficacy and tolerability results over 48 weeks. (a) Time to protocol-defined virologic failure. (b) Time to tolerability endpoint, defined as first change in treatment regimen. Percentage of patients with HIV-1 RNA less than 50 copies/ml at weeks 12, 24, 36, and 48, regardless of previous virologic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

Acknowledgements

The authors thank the patients and all the clinical staff at the AIDS Clinical Center.

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.

This work was supported by Grants-in Aid for AIDS research from the Japanese Ministry of Health, Labour, and Welfare (H23-AIDS-001), and the Global Center of Excellence Program (Global Education and Research Center Aiming at the Control of AIDS) from the Japanese Ministry of Education, Science, Sports and Culture.

[a]AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, [b]Center for AIDS Research, Kumamoto University, Kumamoto, and [c]Department of Community Care, Saku Central Hospital, Nagano, Japan.

Correspondence to Hiroyuki Gatanaga, MD, PhD, AIDS Clinical Center, National Center for Global Health and Medicine, 1-21-1, Toyama, Shinjuku, Tokyo 162-0052, Japan.
Tel: +81 3 3202 7181; fax: +81 3 5273 6483;
e-mail: higatana@acc.ncgm.go.jp

Received: 23 July 2012; revised: 31 October 2012; accepted: 15 November 2012.

References

1. Trottier B, Machouf N, Thomas R, Longpré D, Vézina S, Boissonnault M, *et al.* . **Effective and safe use of abacavir/lamivudine fixed-dose combination with ritonavir-boosted Darunavir, a novel regimen for HIV therapy [abstract CDB333].** Sixth *IAS Conference on HIV Pathogenesis, Treatment and Prevention*; 2011. Rome, Italy.
2. Nishijima T, Tsukada K, Teruya K, Gatanaga H, Kikuchi Y, Oka S. **Efficacy and safety of once-daily ritonavir-boosted darunavir and abacavir/lamivudine for treatment-naive patients: a pilot study.** *AIDS* 2012; **26**:649–651.
3. Clotet B, Bellos N, Molina JM, Cooper D, Goffard JC, Lazzarin A, *et al.* **Efficacy and safety of darunavir-ritonavir at week 48 in treatment-experienced patients with HIV-1 infection in POWER 1 and 2: a pooled subgroup analysis of data from two randomised trials.** *Lancet* 2007; **369**:1169–1178.
4. Madruga JV, Berger D, McMurchie M, Suter F, Banhegyi D, Ruxrungtham K, *et al.* **Efficacy and safety of darunavir-ritonavir compared with that of lopinavir-ritonavir at 48 weeks in treatment-experienced, HIV-infected patients in TITAN: a randomised controlled phase III trial.** *Lancet* 2007; **370**:49–58.
5. **Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents.** *Department of Health and Human Services.* 1–239. http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf. [Accessed on 4 July 2012].
6. Sax PE, Tierney C, Collier AC, Fischl MA, Mollan K, Peeples L, *et al.* **Abacavir-lamivudine versus tenofovir-emtricitabine for initial HIV-1 therapy.** *N Engl J Med* 2009; **361**:2230–2240.
7. **The Guidelines for the Treatment of HIV Infection, March 2012 version.** *The Japanese Ministry of Health, Labour and Welfare.* 1–154. http://www.haart-support.jp/pdf/guideline2012.pdf [Accessed on 4 July 2012].
8. Nelson MR, Katlama C, Montaner JS, Cooper DA, Gazzard B, Clotet B, *et al.* **The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years.** *AIDS* 2007; **21**:1273–1281.
9. Nishijima T, Komatsu H, Gatanaga H, Aoki T, Watanabe K, Kinai E, *et al.* **Impact of small body weight on tenofovir-associated renal dysfunction in HIV-infected patients: a retrospective cohort study of Japanese patients.** *PLoS One* 2011; **6**:e22661.
10. Rodriguez-Novoa S, Alvarez E, Labarga P, Soriano V. **Renal toxicity associated with tenofovir use.** *Expert Opin Drug Saf* 2010; **9**:545–559.
11. Ding X, Andraca-Carrera E, Cooper C, Miele P, Kornegay C, Soukup M, *et al.* **No association of myocardial infarction with ABC use: an FDA meta-analysis. [abstract O-1004].** 18th *Conference on Retroviruses and Opportunistic Infections*; 2011. Boston, USA.
12. Peyriere H, Reynes J, Rouanet I, Daniel N, de Boever CM, Mauboussin JM, *et al.* **Renal tubular dysfunction associated with tenofovir therapy: report of 7 cases.** *J Acquir Immune Defic Syndr* 2004; **35**:269–273.
13. McComsey GA, Kitch D, Daar ES, Tierney C, Jahed NC, Tebas P, *et al.* **Bone mineral density and fractures in antiretroviral-naive persons randomized to receive abacavir-lamivudine or tenofovir disoproxil fumarate-emtricitabine along with efavirenz or atazanavir-ritonavir: Aids Clinical Trials Group A5224s, a substudy of ACTG A5202.** *J Infect Dis* 2011; **203**:1791–1801.
14. Fux CA, Rauch A, Simcock M, Bucher HC, Hirschel B, Opravil M, *et al.* **Tenofovir use is associated with an increase in serum alkaline phosphatase in the Swiss HIV Cohort Study.** *Antivir Ther* 2008; **13**:1077–1082.

15. Gallant JE, Winston JA, DeJesus E, Pozniak AL, Chen SS, Cheng AK, *et al.* **The 3-year renal safety of a tenofovir disoproxil fumarate vs. a thymidine analogue-containing regimen in antiretroviral-naive patients.** *AIDS* 2008; **22**:2155–2163.
16. Nishijima T, Gatanaga H, Komatsu H, Tsukada K, Shimbo T, Aoki T, *et al.* **Renal function declines more in tenofovir-than abacavir-based antiretroviral therapy in low-body weight treatment-naive patients with HIV infection.** *PLoS One* 2012; **7**:e29977.
17. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M. **Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients.** *Clin Infect Dis* 2010; **51**:496–505.

DOI:10.1097/QAD.0b013e32835cadb7

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

Philipp Schommers[a,*], Christoph Wyen[a,*], Marcus Hentrich[b], Daniel Gillor[a], Alexander Zoufaly[c], Bjoern Jensen[d], Johannes R. Bogner[e], Jan Thoden[f], Jan C. Wasmuth[g], Gerd Fätkenheuer[a] and Christian Hoffmann[h]

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

Kunio Yanagisawa[1], Junko Tanuma[2], Shotaro Hagiwara[3], Hiroyuki Gatanaga[2], Yoshimi Kikuchi[2] and Shinichi Oka[2]

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

(Intern Med 52: 955-959, 2013)
(DOI: 10.2169/internalmedicine.52.9088)

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.

[1]Department of Medicine and Clinical Science, Graduate School of Medicine, Gunma University, Japan, [2]AIDS Clinical Center, National Center for Global Health and Medicine, Japan and [3]Department of Hematology, National Center for Global Health and Medicine, Japan
Received for publication October 9, 2012; Accepted for publication January 16, 2013
Correspondence to Dr. Junko Tanuma, jtanuma@acc.ncgm.go.jp

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.

Intern Med 52: 955-959, 2013 DOI: 10.2169/internalmedicine.52.9088

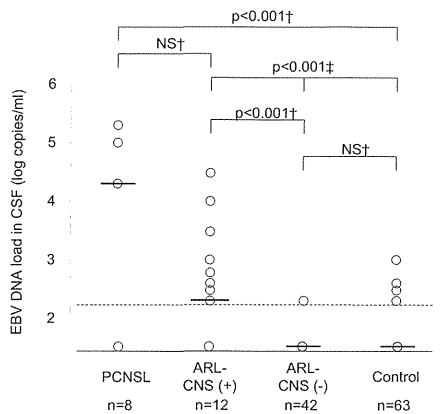

Figure 1. The EBV-DNA loads in the cerebrospinal fluid (CSF) of the patients with AIDS-related lymphoma and the control subjects. PCNSL: primary CNS lymphoma, ARL-CNS (+): systemic AIDS-related lymphoma with CNS involvement, ARL-CNS (-): systemic AIDS-related lymphoma without CNS involvement, NS: not 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.

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.

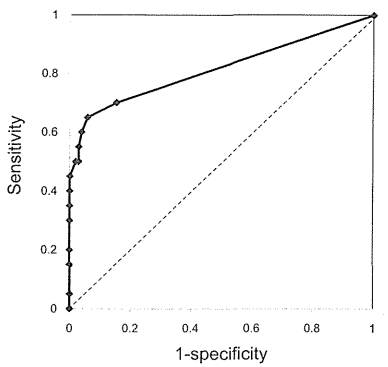

Figure 2. Receiver operating characteristic (ROC) curve for the cutoff values of the EBV-DNA load in the cerebrospinal fluid for the diagnosis of CNS involvement of systemic AIDS-related lymphoma. The dotted line is the reference line. The area under the ROC curve was 0.856 (95% confidence interval,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

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

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).

Acknowledgement

We thank the entire staff of the AIDS Clinical Center for caring for the patients.

This work was supported by a Grant for International Health Research (A21-104) and the Health Labour Sciences Research Grant (H22-AIDS-002) from the Ministry of Health, Labour and Welfare.

References

1. Vaccher E, Spina M, Talamini R, et al. Improvement of systemic human immunodeficiency virus-related non-Hodgkin's lymphoma outcome in the era of highly active antiretroviral therapy. Clin Infect Dis **37**: 1556-1564, 2003.
2. Desai J, Mitnick RJ, Henry DH, et al. Patterns of central nervous system recurrence in patients with systemic human immunodeficiency virus-associated non-Hodgkin lymphoma. Cancer **86**: 1840-1847, 1999.
3. Cingolani A, Gastaldi R, Fassone L, et al. Epstein-Barr virus infection is predictive of CNS involvement in systemic AIDS-related non-Hodgkin's lymphomas. J Clin Oncol **18**: 3325-3330, 2000.
4. Cinque P, Brytting M, Vago L, et al. Epstein-Barr virus DNA in cerebrospinal fluid from patients with AIDS-related primary lymphoma of the central nervous system. Lancet **342**: 398-401, 1993.
5. Arribas JR, Clifford DB, Fichtenbaum CJ, et al. Detection of Epstein-Barr virus DNA in cerebrospinal fluid for diagnosis of AIDS-related central nervous system lymphoma. J Clin Microbiol **33**: 1580-1583, 1995.
6. Roberts TC, Storch GA. Multiplex PCR for diagnosis of AIDS-related central nervous system lymphoma and toxoplasmosis. J Clin Microbiol **35**: 268-269, 1997.
7. Antinori A, De Rossi G, Ammassari A, et al. Value of combined approach with thallium-201 single-photon emission computed tomography and Epstein-Barr virus DNA polymerase. J Clin Oncol **17**: 554-560, 1999.
8. Ivers LC, Kim AY, Sax PE. Predictive value of polymerase chain reaction of cerebrospinal fluid for detection of Epstein-Barr virus to establish the diagnosis of HIV-related primary central nervous system lymphoma. Clin Infect Dis **38**: 1629-1632, 2004.
9. Tachikawa N, Goto M, Hoshino Y, et al. Detection of *Toxoplasma gondii*, Epstein-Barr virus, and JC virus DNAs in the cerebrospinal fluid in acquired immunodeficiency syndrome patients with focal central nervous system complications. Intern Med **38**: 556-562, 1999.
10. Bossolasco S, Cinque P, Ponzoni M, et al. Epstein-Barr virus DNA load in cerebrospinal fluid and plasma of patients with AIDS-related lymphoma. J Neurovirol **8**: 432-438, 2002.
11. Shide K, Henzan H, Nagafuji K, et al. Dynamics of Epstein-Barr virus load in pyothorax-associated lymphoma. J Med Virol **70**: 137-140, 2003.
12. Gulley ML, Glaser SL, Craig FE, et al. Guidelines for interpreting EBER in situ hybridization and LMP1 immunohistochemical tests for detecting Epstein-Barr virus in Hodgkin lymphoma. Am J Clin Pathol **117**: 259-267, 2002.
13. Khan G, Coates PJ, Kangro HO, et al. Epstein Barr virus (EBV) encoded small RNAs: targets for detection by in situ hybridisation with oligonucleotide probes. J Clin Pathol **45**: 616-620, 1992.
14. Boehme V, Zeynalova S, Kloess M, et al. Incidence and risk factors of central nervous system recurrence in aggressive lymphoma —a survey of 1693 patients treated in protocols of the German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNHL). Ann Oncol **18**: 149-157, 2007.
15. Bjorkholm M, Hagberg H, Holte H, et al. Central nervous system occurrence in elderly patients with aggressive lymphoma and a long-term follow-up. Ann Oncol **18**: 1085-1089, 2007.
16. Besien KV, Ha CS, Murphy S, et al. Risk factors, treatment, and outcome of central nervous system recurrence in adults with intermediate-grade and immunoblastic lymphoma. Blood **91**: 1178-1184, 1998.
17. Bossolasco S, Falk KI, Ponzoni M, et al. Ganciclovir is associated with low or undetectable Epstein-Barr virus DNA load in cerebrospinal fluid of patients with HIV-related primary central nervous system lymphoma. Clin Infect Dis **42**: e21-e25, 2006.
18. MacMahon EM, Glass JD, Hayward SD, et al. Epstein-Barr virus in AIDS-related primary central nervous system lymphoma. Lancet **338**: 969-973, 1991.
19. Wada N, Ikeda J, Hori Y, et al. Epstein-Barr virus in diffuse large B-Cell lymphoma in immunocompetent patients in Japan is as low as in Western Countries. J Med Virol **83**: 317-321, 2011.
20. Van Baarle D, Wolthers KC, Hovenkamp E, et al. Absolute level of Epstein-Barr virus DNA in human immunodeficiency virus type 1 infection is not predictive of AIDS-related non-Hodgkin lymphoma. J Infect Dis **186**: 405-409, 2002.
21. Gan YJ, Razzouk BI, Su T, Sixbey JW. A defective, rearranged Epstein-Barr virus genome in EBER-negative and EBER-positive Hodgkin's disease. Am J Pathol **160**: 781-786, 2002.

Intern Med 52: 955-959, 2013 DOI: 10.2169/internalmedicine.52.9088

INTERNAL MEDICINE

Intern Med 52: 735-744, 2013 DOI: 10.2169/internalmedicine.52.9155

□ ORIGINAL ARTICLE □

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

Takeshi Nishijima[1,2], Misao Takano[1], Michiyo Ishisaka[1], Hirokazu Komatsu[3], Hiroyuki Gatanaga[1,2], Yoshimi Kikuchi[1], Tomoyuki Endo[4], Masahide Horiba[5], Satoru Kaneda[6], Hideki Uchiumi[7], Tomohiko Koibuchi[8], Toshio Naito[9], Masaki Yoshida[10], Natsuo Tachikawa[11], Mikio Ueda[12], Yoshiyuki Yokomaku[13], Teruhisa Fujii[14], Satoshi Higasa[15], Kiyonori Takada[16], Masahiro Yamamoto[17], Shuzo Matsushita[2], Masao Tateyama[18], Yoshinari Tanabe[19], Hiroaki Mitsuya[20,21], Shinichi Oka[1,2], on behalf of the Epzicom-Truvada study team

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.

[1]AIDS Clinical Center, National Center for Global Health and Medicine, Japan, [2]Center for AIDS Research, Kumamoto University Graduate School of Medical Sciences, Japan, [3]Department of Community Care, Saku Central Hospital, Japan, [4]Department of Hematology, Hokkaido University Hospital, Japan, [5]Division of Respiratory Medicine, Higashisaitama National Hospital, Japan, [6]Department of Gastroenterology, National Hospital Organization Chiba Medical Center, Japan, [7]Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Japan, [8]Department of Infectious Diseases and Applied Immunology, Research Hospital of the Institute of Medical Science, The University of Tokyo, Japan, [9]Department of General Medicine, Juntendo University School of Medicine, Japan, [10]Department of Infectious Diseases and Infection Control, The Jikei University School of Medicine, Japan, [11]Department of Infectious Diseases, Yokohama Municipal Citizen's Hospital, Japan, [12]Immunology and Infectious Disease, Ishikawa Prefectural Central Hospital, Japan, [13]Clinical Research Center, National Hospital Organization Nagoya Medical Center, Japan, [14]Division of Blood Transfusion, Hiroshima University Hospital, Japan, [15]Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Japan, [16]Postgraduate Clinical Training Center, Ehime University Hospital, Japan, [17]Internal Medicine, Clinical Research Institute, National Hospital Organization Kyushu Medical Center, Japan, [18]Department of Infectious, Respiratory, and Digestive Medicine Control and Prevention of Infectious Diseases Faculty of Medicine, University of the Ryukyus, Japan, [19]Division of Infection Control and Prevention, Niigata University Medical and Dental Hospital, Japan, [20]Departments of Infectious Diseases and Hematology, Kumamoto University Graduate School of Medical Sciences, Japan and [21]Experimental Retrovirology Section, HIV and AIDS Malignancy Branch, National Cancer Institute, National Institutes of Health, USA

Received for publication October 18, 2012; Accepted for publication December 17, 2012

Correspondence to Dr. Shinichi Oka, oka@acc.ncgm.go.jp

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

(Intern Med 52: 735-744, 2013)
(DOI: 10.2169/internalmedicine.52.9155)

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

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 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).

Intern Med 52: 735-744, 2013 DOI: 10.2169/internalmedicine.52.9155

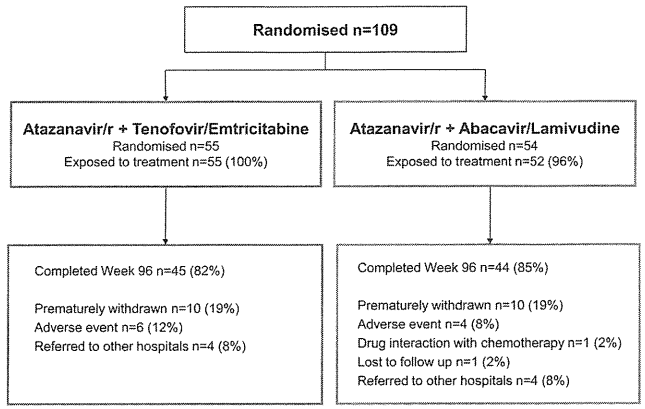

Figure 1. **Enrollment, randomization and disposition of patients.**

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

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

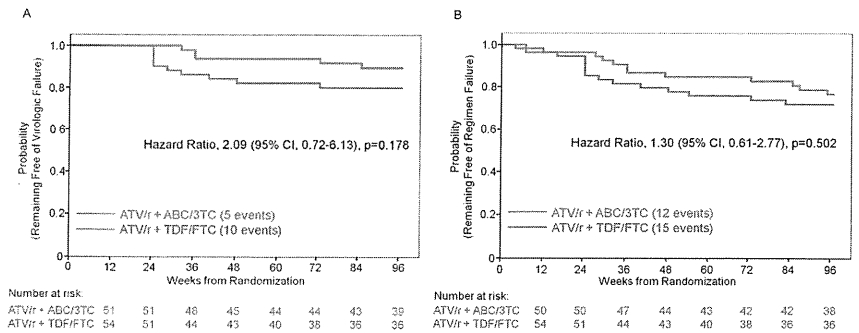

Figure 2. Efficacy results over 96 weeks. (A) Time to protocol-defined virologic failure. (B) Time to the first occurrence of either virologic failure or discontinuation of the initially randomized regimen. ATV/r: ritonavir-boosted atazanavir, ABC/3TC: abacavir/lamivudine, TDF/FTC: tenofovir/emtricitabine

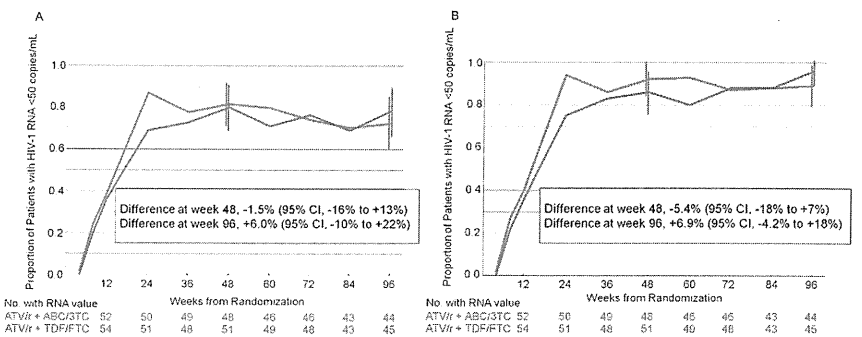

Figure 3. Efficacy results at 48 and 96 weeks. Proportion of patients with an HIV RNA level <50 copies/mL regardless of previous virologic failure with 95% binomial confidence intervals at 48 and 96 weeks. (A) Intention-to-treat analysis. (B) Per protocol analysis. ATV/r: ritonavir-boosted atazanavir,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

Intern Med 52: 735-744, 2013 DOI: 10.2169/internalmedicine.52.9155

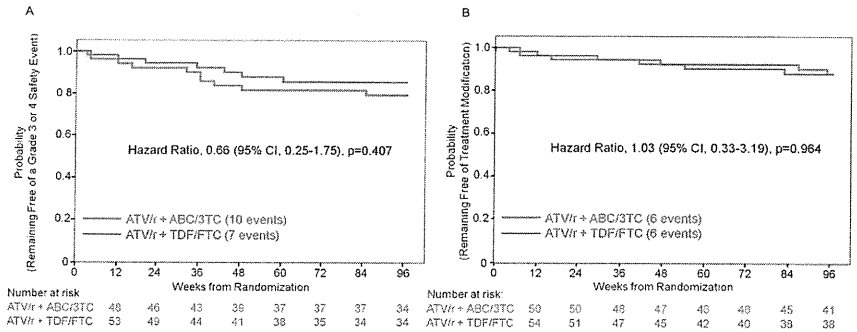

Figure 4. Safety and tolerability results over 96 weeks. (A) Time to first primary safety endpoint, defined as the first grade 3 or 4 event on the initial randomized regimen, which was at least one grade higher than baseline. (B) Time to tolerability endpoint, defined as the first change in regimen. 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

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

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

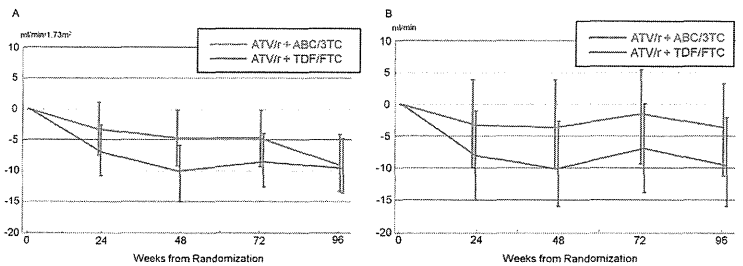

Figure 5. **Changes in the renal function between baseline and 96 weeks. (A) Changes in the estimated glomerular filtration rate calculated with the Modification of Diet in Renal Disease study equation adjusted for the Japanese population. (B) Changes in creatinine clearance calculated with the Cockcroft-Gault 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/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).
Uchiumi H: Research funding, ViiV Healthcare. Koibuchi T: Research funding, Nihon Ultmarc Inc. Naito T: Research funding,

MSD K.K. and Janssen Pharmaceutical K.K. Takada K: Research funding, ViiV Healthcare. Oka S: Research funding, MSD K.K., Abbott Japan, Co., Janssen Pharmaceutical K.K., Pfizer, Co., and Roche Diagnostics K.K.

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.

Acknowledgement

We thank the patients for participating in this study. The authors are indebted to Mikiko Ogata and Akiko Nakano for their support in this study as a data manager and research coordinator, respectively. The Epzicom-Truvada study team includes the following members: Takao Koike[1], Mitsufumi Nishio[1], Keisuke Yamaguchi[1], Katsuya Fujimoto[1], Satoshi Yamamoto[1], Ikumi Kasahara[1], Tetsuro Takeda[2], Takafumi Tezuka[2], Hiroshi Moro[2], Takeharu Kotani[3], Mieko Yamada[3], Yoshiyuki Ogawa[4], Kunio Yanagisawa[4], Aikichi Iwamoto[5], Takeshi Fujii[5], Takashi Odawara[5], Nahoko Miyazaki[5], Kazufumi Matsumoto[5], Kumiko Sumino[5], Mizue Saita[6], Mai Suzuki[6], Rino Sakamoto[6], Satoshi Kimura[7], Yukihiro Yoshimura[8], Motohiro Hamaguchi[9], Naoto Mamiya[9], Atsuyoshi Imamura[9], Ayumi Kogure[9], Mayumi Imahashi[9], Takuma Shirasaka[10], Munehiro Yoshino[10], Sawada Akihiro[11], Tazuko Tokugawa[11], Seiji Saito[12], Noboru Takata[12], Fumiko Kagiura[12], Rumi Minami[13], Soichiro Takahama[13], Toshikazu Miyagawa[14], Daisuke Tasato[15], Hideta Nakamura[15], Naoki Ishizuka[16], Katsuji Teruya[16], Miwako Honda[16], Kunihisa Tsukada[16], Hirohisa Yazaki[16], Junko Tanuma[16], Haruhito Honda[16], Ei Kinai[16], Koji Watanabe[16], Takahiro Aoki[16], Tamayo Watanabe[16], Mahoko Kamimura[16], Masako Ito[16], Jiro Mikami[16], Atsushi Kubota[16], Toshikatsu Kawasaki[16]

[1]Hokkaido University Hospital, Japan; [2]Niigata University Medical and Dental Hospital, Japan; [3]Ishikawa Prefectural Central Hospital, Japan; [4]Gunma University Graduate School of Medicine, Japan; [5]Research Hospital of the Institute of Medical Science, The University of Tokyo, Japan; [6]Juntendo University School of Medicine, Japan; [7]Tokyo Teishin Hospital, Japan; [8]Yokohama Municipal Citizen's Hospital, Japan; [9]National Hospital Organization Nagoya Medical Center, Japan; [10]National Hospital Organization Osaka Medical Center, Japan; [11]Hyogo College of Medicine, Japan; [12]Hiroshima University Hospital, Japan; [13]National Hospital Organization Kyushu Medical Center, Japan; [14]Kumamoto University Graduate School of Medical Sciences, Japan; [15]University of the Ryukyus, Okinawa, Japan and [16]National Center for Global Health and Medicine, Japan.

References

1. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services [http : / / www. aidsinfo. nih. gov / contentfiles / lvguidelines / adultandadolescentgl.pdf].
2. European AIDS Clinical Society Guidelines version 6 [http://www.europeanaidsclinicalsociety. org / images / stories / EACS-Pdf / EACSGuidelines-v6.0-English.pdf].
3. Sax PE, Tierney C, Collier AC, et al. Abacavir-lamivudine versus tenofovir-emtricitabine for initial HIV-1 therapy. N Engl J Med **361**: 2230-2240, 2009.
4. Smith KY, Patel P, Fine D, et al. Randomized, double-blind, placebo-matched, multicenter trial of abacavir/lamivudine or tenofovir/emtricitabine with lopinavir/ritonavir for initial HIV treatment. AIDS **23**: 1547-1556, 2009.
5. Hetherington S, McGuirk S, Powell G, et al. Hypersensitivity reactions during therapy with the nucleoside reverse transcriptase inhibitor abacavir. Clin Ther **23**: 1603-1614, 2001.
6. Mallal S, Phillips E, Carosi G, et al. HLA-B*5701 screening for hypersensitivity to abacavir. N Engl J Med **358**: 568-579, 2008.
7. Gatanaga H, Honda H, Oka S. Pharmacogenetic information derived from analysis of HLA alleles. Pharmacogenomics **9**: 207-214, 2008.
8. Sabin CA, Worm SW, Weber R, et al. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the D:A:D study: a multi-cohort collaboration. Lancet **371**: 1417-1426, 2008.
9. Ding X, Andraca-Carrera E, Cooper C, et al. No association of abacavir use with myocardial infarction: findings of an FDA meta-analysis. J Acquir Immune Defic Syndr **61**: 441-447, 2012.
10. Peyrière H, Reynes J, Rouanet I, et al. Renal tubular dysfunction associated with tenofovir therapy: report of 7 cases. J Acquir Immune Defic Syndr **35**: 269-273, 2004.
11. Verhelst D, Monge M, Meynard JL, et al. Fanconi syndrome and renal failure induced by tenofovir: a first case report. Am J Kidney Dis **40**: 1331-1333, 2002.
12. Gallant JE, Winston JA, DeJesus E, et al. The 3-year renal safety of a tenofovir disoproxil fumarate vs. a thymidine analogue-containing regimen in antiretroviral-naive patients. AIDS **22**: 2155-2163, 2008.
13. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M. Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. Clin Infect Dis **51**: 496-505, 2010.
14. McComsey GA, Kitch D, Daar ES, et al. Bone mineral density and fractures in antiretroviral-naive persons randomized to receive abacavir-lamivudine or tenofovir disoproxil fumarate-emtricitabine along with efavirenz or atazanavir-ritonavir: Aids Clinical Trials Group A5224s, a substudy of ACTG A5202. J Infect Dis **203**: 1791-1801, 2011.
15. Guidelines for antiretroviral therapy. Japanese Ministry of Health and Welfare, in Japanese. [http://www.haart-support.jp/pdf/guideline2012.pdf].
16. Sax PE, Tierney C, Collier AC, et al. Abacavir/lamivudine versus tenofovir DF/emtricitabine as part of combination regimens for initial treatment of HIV: final results. J Infect Dis **204**: 1191-1201, 2011.
17. Post FA, Moyle GJ, Stellbrink HJ, et al. Randomized comparison of renal effects, efficacy, and safety with once-daily abacavir/lamivudine versus tenofovir/emtricitabine, administered with efavirenz, in antiretroviral-naive, HIV-1-infected adults: 48-week results from the ASSERT study. J Acquir Immune Defic Syndr **55**: 49-57, 2010.
18. Nishijima T, Gatanaga H, Komatsu H, et al. Renal function declines more in tenofovir- than abacavir-based antiretroviral therapy in low-body weight treatment-naïve patients with HIV infection. PLoS One **7**: e29977, 2012.
19. Chaisiri K, Bowonwatanuwong C, Kasettratat N, Kiertiburanakul S. Incidence and risk factors for tenofovir-associated renal function decline among Thai HIV-infected patients with low-body weight. Curr HIV Res **8**: 504-509, 2010.
20. Nelson MR, Katlama C, Montaner JS, et al. The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. AIDS **21**: 1273-1281, 2007.
21. Nishijima T, Komatsu H, Gatanaga H, et al. Impact of small body weight on tenofovir-associated renal dysfunction in HIV-infected patients: a retrospective cohort study of Japanese patients. PLoS One **6**: e22661, 2011.
22. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. BMJ **340**: c869, 2010.
23. Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents October 10, 2006. [http://aidsinfo.nih.gov/contentfiles/AdultandAdolescentGL000629.pdf].
24. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron **16**: 31-41, 1976.
25. Hattori J, Shiino T, Gatanaga H, et al. Trends in transmitted drug-resistant HIV-1 and demographic characteristics of newly diagnosed patients: nationwide surveillance from 2003 to 2008 in Japan. Antiviral Res **88**: 72-79, 2010.
26. Rodriguez-Novoa S, Labarga P, Soriano V, et al. Predictors of kidney tubular dysfunction in HIV-infected patients treated with tenofovir: a pharmacogenetic study. Clin Infect Dis **48**: e108-e116, 2009.
27. DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS VERSION 1.0, DECEMBER, 2004; CLARIFICATION AUGUST 2009. [http://www.mtnstopshiv.org/sites/default/files/attachments/Table_for_Grading_Severity_of_Adult_Pediatric_Adverse_Events.pdf].
28. Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis **53**: 982-992, 2009.
29. Kudo K, Konta T, Mashima Y, et al. The association between renal tubular damage and rapid renal deterioration in the Japanese population: the Takahata study. Clin Exp Nephrol **15**: 235-241, 2011.
30. Ando M, Yanagisawa N, Ajisawa A, Tsuchiya K, Nitta K. Kidney tubular damage in the absence of glomerular defects in HIV-infected patients on highly active antiretroviral therapy. Nephrol Dial Transplant **26**: 3224-3229, 2011.
31. Gatanaga H, Tachikawa N, Kikuchi Y, et al. Urinary β_2-microglobulin as a possible sensitive marker for renal injury caused by tenofovir disoproxil fumarate. AIDS Res Hum Retroviruses **22**: 744-748, 2006.
32. Phillips EJ. Genetic screening to prevent abacavir hypersensitivity reaction: are we there yet? Clin Infect Dis **43**: 103-105, 2006.
33. Sun HY, Hung CC, Lin PH, et al. Incidence of abacavir hypersensitivity and its relationship with HLA-B*5701 in HIV-infected patients in Taiwan. J Antimicrob Chemother **60**: 599-604, 2007.
34. Martin MA, Klein TE, Dong BJ, Pirmohamed M, Haas DW, Kroetz DL. Clinical pharmacogenetics implementation consortium guidelines for HLA-B genotype and abacavir dosing. Clin Pharmacol Ther **91**: 734-738, 2012.

J Infect Chemother (2013) 19:1170–1172
DOI 10.1007/s10156-013-0588-7

CASE REPORT

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

Takuma Shindo · Takeshi Nishijima · Katsuji Teruya · Daisuke Mizushima · Hiroyuki Gatanaga · Shinichi Oka

Received: 22 January 2013 / Accepted: 10 March 2013 / Published online: 27 March 2013

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

T. Shindo · T. Nishijima · K. Teruya · D. Mizushima · H. Gatanaga (✉) · S. Oka
AIDS Clinical Center, National Center for Global Health and Medicine, 1-21-1, Toyama, Shinjuku-ku, Tokyo 162-0052, Japan
e-mail: higatana@acc.ncgm.go.jp

T. Nishijima · H. Gatanaga · S. Oka
Center for AIDS Research, Kumamoto University, Kumamoto, Japan

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 bleeding). To rule out drug-induced thrombocytopenia, ART, together with clopidogrel and aspirin, which had been administered for years, were discontinued on admission. Although platelet transfusion was initiated for a couple of days, no change in platelet count was noted. Bone marrow examination on day 5 showed hypocellularity with a low number of megakaryocytes. No histopathological findings specific to myelodysplastic syndrome or leukemia were noted. On day 10, the patient developed intermittent epistaxis with a platelet count of 4,000/μl. On that day, a four-day course of orally administered HD-DXM of 40 mg/day was initiated, and ART with DRV/r plus TDF/FTC was reinitiated. The platelet count increased to 66,000/μl on day 10 after the above treatment, but it decreased to 12,000/μl on day 14. A second course of HD-DXM of 40 mg/day was initiated. The platelet count improved to 115,000/μl on day 10 after the second course, and 142,000/μl on day 15. Based on such improvement, no third course was considered necessary. The patient was discharged on day 39 from admission. No adverse event of dexamethasone was observed. The platelet count remained stable after discharge despite the re-initiation of clopidogrel and aspirin (Fig. 1). Three months after re-initiation of ART and thereafter, the HIV-1 viral load was suppressed to <100 copies/ml with good medication adherence. The patient experienced no relapses of HIV-ITP for 9 months.

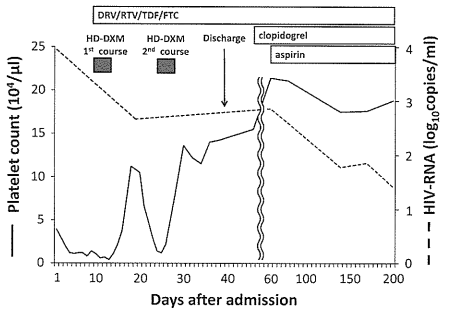

Fig. 1 Clinical course during hospitalization. *DRV* darunavir, *RTV* ritonavir, *TDF* tenofovir, *FTC* emtricitabine, *HD-DXM* high-dose dexamethasone

Discussion

We reported here a patient with HIV-ITP who was treated successfully with a combination with two courses of HD-DXM and ART. The ITP likely relapsed when the platelet count diminished to <90,000/μl on day 10 after the first course of HD-DXM [3], thus justifying the second course of HD-DXM. No additional courses were provided once the platelet count was above 90,000/μl on day 10 after the second course. That platelet count remained stable after re-initiating clopidogrel and aspirin negated any drug-induced thrombocytopenia. To our knowledge, this is the first case describing the use and effectiveness of the combination of HD-DXM and ART in the treatment of HIV-ITP.

In adult patients with non-HIV ITP, HD-DXM is preferred to conventional long-term prednisone [2], because of fewer adverse events, mainly due to the shorter term of steroid administration. The major side effect of steroid is immunosuppression, and it is important to avoid such complication, especially in immunocompromised hosts, such as HIV-1 infected patients. For the treatment of HIV-ITP, HD-DXM is probably as effective as in non-HIV ITP, because both diseases are considered to have a similar etiology [8]. Although about one-fifth of non-HIV ITP patients on HD-DXM treatment relapse by 8 months after treatment [5], the use of ART in patients with HIV-ITP can maintain long-term remission despite the lack of an immediate effect [9, 10]. In our patient, HD-DXM was applied when the clinical condition was severe with bleeding and thrombocytopenia, and it resulted in rapid improvement in platelet count. Thereafter, administration of ART resulted in suppression of viral load, which probably promoted long-term remission of HIV-ITP.

Notably, HD-DXM is cost-effective, compared to other treatments for ITP, such as immunoglobulin or rituximab. Although further studies are needed to confirm the efficacy and safety of the combination therapy, HD-DXM and ART is potentially suitable for treatment of HIV-ITP patients with severe thrombocytopenia or bleeding.

Acknowledgments The authors thank all the clinical staff at the AIDS Clinical Center, National Center for Global Health and Medicine. No financial support was received for this article.

Conflict of interest All authors declare no conflict of interest.

References

1. Ambler KL, Vickars LM, Leger CS, Foltz LM, Montaner JS, Harris M, et al. Clinical features, treatment, and outcome of HIV-associated immune thrombocytopenia in the HAART era. Adv Hematol. 2012;2012:910954.
2. Neunert C, Lim W, Crowther M, Cohen A, Solberg L Jr, Crowther MA. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. Blood. 2011;117:4190–207.
3. Cheng Y, Wong RS, Soo YO, Chui CH, Lau FY, Chan NP, et al. Initial treatment of immune thrombocytopenic purpura with high-dose dexamethasone. N Engl J Med. 2003;349:831–6.
4. Godeau B, Bierling P. High-dose dexamethasone as initial treatment for immune thrombocytopenic purpura. N Engl J Med. 2003;349:2267–8. (author reply 2267-2268).

5. Mazzucconi MG, Fazi P, Bernasconi S, De Rossi G, Leone G, Gugliotta L, et al. Therapy with high-dose dexamethasone (HD-DXM) in previously untreated patients affected by idiopathic thrombocytopenic purpura: a GIMEMA experience. Blood. 2007; 109:1401–7.
6. Marroni M, Gresele P. Detrimental effects of high-dose dexamethasone in severe, refractory, HIV-related thrombocytopenia. Ann Pharmacother. 2000;34:1139–41.
7. Ramratnam B, Parameswaran J, Elliot B, Newstein M, Schiffman FJ, Rich JD, et al. Short course dexamethasone for thrombocytopenia in AIDS. Am J Med. 1996;100:117–8.
8. Bettaieb A, Fromont P, Louache F, Oksenhendler E, Vainchenker W, Duedari N, et al. Presence of cross-reactive antibody between human immunodeficiency virus (HIV) and platelet glycoproteins in HIV-related immune thrombocytopenic purpura. Blood. 1992; 80:162–9.
9. Arranz Caso JA, Sanchez Mingo C, Garcia Tena J. Effect of highly active antiretroviral therapy on thrombocytopenia in patients with HIV infection. N Engl J Med. 1999;341:1239–40.
10. Carbonara S, Fiorentino G, Serio G, Maggi P, Ingravallo G, Monno L, et al. Response of severe HIV-associated thrombocytopenia to highly active antiretroviral therapy including protease inhibitors. J Infect. 2001;42:251–6.

Prophylactic Effect of Antiretroviral Therapy on Hepatitis B Virus Infection

Hiroyuki Gatanaga,[1,2] Tsunefusa Hayashida,[1,2] Junko Tanuma,[1] and Shinichi Oka[1,2]

[1]AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, and [2]Center for AIDS Research, Kumamoto University, Japan

Background. Hepatitis B virus (HBV) infection is common in individuals infected with human immunodeficiency virus, especially in men who have sex with men (MSM). Almost all currently used regimens of antiretroviral therapy (ART) contain lamivudine (LAM) or tenofovir disoproxil fumarate (TDF), both of which have significant anti-HBV activity. However, the prophylactic effect of ART on HBV infection has not been assessed previously.

Methods. Non-HBV-vaccinated HIV-infected MSM were serologically evaluated for HBV infection using stocked serum samples. Cases negative for HBV surface antigen (HBsAg), antibody to HBsAg (anti-HBs), and antibody to HBV core antigen (anti-HBc) in first serum samples were serologically followed until last available stocked samples. HBV genotype and LAM-resistant mutation (rtM204V/I) were analyzed in cases that became HBsAg-positive.

Results. The first stocked samples were negative for all analyzed HBV serological markers in 354 of 1434 evaluated patients. The analysis of their last samples indicated HBV incident infection in 43 of them during the follow-up period. The rate of incident infections was lower during LAM- or TDF-containing ART (0.669 incident infections in 100 person-years) than during no ART period (6.726 incident infections in 100 person-years) and other ART (5.263 incident infections in 100 person-years) ($P < .001$). Genotype A was most prevalent (76.5%), and LAM-resistant HBV was more frequent in incident infections during LAM-containing ART (50.0%) than in those during no ART and other ART (7.1%) ($P = .029$).

Conclusions. LAM- and TDF-containing ART regimens seem to provide prophylaxis against HBV infection, although drug-resistant strains seem to evade these effects.

Keywords. lamivudine; tenofovir disoproxil fumarate; resistant; chronic infection.

Patients with human immunodeficiency virus (HIV) infection are at high risk for both hepatitis B virus (HBV) infection and development of chronic infection [1–4]. Based on information from Western countries, the rate of coinfection varies according to risk categories; the highest rate is in men who have sex with men (MSM), with a slightly lower rate among intravenous drug users, and much lower in individuals infected through heterosexual contacts [5–8]. In Japan, HIV/HBV coinfection is also significantly associated with MSM [9, 10]. The progression of chronic HBV infection to cirrhosis, end-stage liver diseases, and/or hepatocellular carcinoma is more rapid in HIV-infected persons than in those with chronic HBV infection alone [11, 12]. Vaccination of non-HBV-immunized HIV-infected individuals is recommended to prevent HBV infection [13]. However, all current recommended antiretroviral therapy (ART) regimens contain lamivudine (LAM) or tenofovir disoproxil fumarate (TDF), both of which have significant anti-HBV activity [14]. Do these ART regimens provide any prophylaxis against HBV infection? This is an important question, as a positive answer could influence the strategy applied to prevent HBV infection in HIV-infected individuals. To delineate the hepatitis B prophylactic effect of ART, we used stocked samples for serological evaluation of HBV infection in HIV-infected MSM. The present

Received 6 November 2012; accepted 25 February 2013; electronically published 13 March 2013.

Correspondence: Hiroyuki Gatanaga, MD, AIDS Clinical Center, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan (higatana@acc.ncgm.go.jp).

Clinical Infectious Diseases 2013;56(12):1812–9

DOI: 10.1093/cid/cit145

study included those patients who had tested negative for hepatitis B surface antigen (HBsAg), antibody to HBsAg (anti-HBs), and antibody to hepatitis B core antigen (anti-HBc) using their first stocked blood samples, who were followed up serologically to identify new HBV incident infections among them. The other part of the study covered analysis of the relation between the frequency of incident infection and ART regimens.

METHODS

Patients

Since April 1997, we have stocked serum samples taken at routine clinical practice from HIV type 1 (HIV-1)–infected patients who visited the Outpatient Clinic of the AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan, under signed informed consent for use in virologic research. Every patient had been interviewed at the first visit by clinical nurse specialists at the HIV outpatient clinic using a structured questionnaire that includes items on sexuality and history of HBV vaccination. Most of the patients regularly visited our clinic every 1–3 months, and we had collected and stored their sera at almost all visits. The ethics committee of the National Center for Global Health and Medicine approved the collection and analysis of the samples. First, we selected HIV-1–infected MSM who met the following inclusion criteria: (1) the first visit to our clinic was between April 1997 and December 2009, (2) they had not received HBV vaccination before the first visit, and (3) at least 2 serum samples were available and collected at least 6 months apart. The first sample was defined as the baseline serum sample, and baseline clinical data were defined as those recorded on the date of sampling of the first stocked serum. Patients' baseline characteristics, including age, race, hepatitis C virus antibody, results of *Treponema pallidum* hemagglutination assay, and $CD4^+$ cell count were collected from the medical records.

HBV Analysis

In order to identify new HBV incident infection, we excluded patients with previously confirmed HBV infection. The baseline samples of the patients who met the inclusion criteria described above were serologically evaluated for HBsAg, anti-HBs, and anti-HBc using ARCHITECT HBsAg QT assay, anti-HBs assay, and anti-HBc assay, respectively (Abbott Laboratories, Chicago, Illinois) [15, 16]. Patients positive for any of HBsAg, anti-HBs, and anti-HBc at baseline were excluded from the serological follow-up. The remaining patients were considered to have never been infected with HBV before the baseline. Their last stocked sample taken before or in December 2010, or before HBV vaccination if performed during the follow-up period, was analyzed for HBsAg, anti-HBs, and anti-HBc. If the last sample was negative for all 3, the patient was considered to have never been infected with HBV up to the sampling date of the last stocked serum. If HBsAg, anti-HBs, or anti-HBc was positive in the last stocked serum, the patient was considered to have HBV incident infection during the follow-up period. In the latter case, the baseline samples were subjected to polymerase chain reaction (PCR) analysis for HBV DNA [17, 18], and all the stocked samples during the follow-up period were serologically analyzed to determine the date of HBV incident infection. The date of incident infection was defined as the sampling date of the first positive serum for any HBV serological marker. The time from the baseline to HBV incident infection was analyzed by the Kaplan-Meier method. The data were censored at the sampling date of the last stocked sample if it was negative for all analyzed HBV serological markers. Patients' age and $CD4^+$ cell count at the date of incident infection and alanine aminotransferase (ALT) values within 3 months of incident infection were collected. If an HBsAg-positive sample was available, HBV genotype and LAM-resistant mutation (rtM204V/I) were analyzed by PCR-invader assay [17–19]. The diagnosis of chronic HBV infection was considered when HBsAg was still positive in sera taken at 6 months or longer after the incident infection.

Antiretroviral Therapy

To determine the type of ART under which HBV incident infection occurred, the regimen information of ART was collected from medical records over the period spanning from the baseline to the incidence infection or to the end of follow-up. The treatment status was divided into 4 categories: (1) No ART, no treatment with any antiretroviral agent; (2) Other-ART, ART with regimens that did not contain LAM, TDF, or emtricitabine (FTC); (3) LAM-ART, ART with LAM-containing regimens that did not contain TDF or FTC; and (4) TDF-ART, ART with TDF-containing regimens with or without LAM or FTC. Data were censored on the sampling date of the last stocked sample if it was negative for all analyzed HBV serological markers. When the treatment category was modified, the data were censored on the date of category change for the previous treatment category and a new follow-up as a different case was initiated for the replacement treatment category.

Statistical Analysis

The time from the baseline to HBV incident infection was analyzed by the Kaplan-Meier method. The Cox proportional hazards regression analysis was used to assess the risk of HBV incident infections. The impact of patients' baseline characteristics, year of entry, the use of antiretroviral agents (any antiretroviral, and any of LAM, TDF, or FTC), and the frequency of changing ART regimen during the follow-up period was estimated with univariate analysis, and those with statistical significance were incorporated into multivariate analysis. The

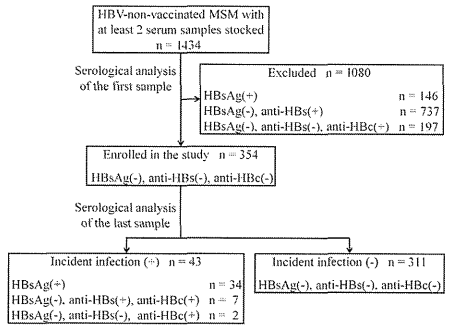

Figure 1. Patient selection process: 1434 patients met the inclusion criteria. Of these patients, 1080 were excluded because of positive hepatitis B virus serology in the first samples. The results of various serological tests are shown. The remaining 354 were enrolled for serological follow-up. Of these, 43 were positive in the last sample analysis. Their stocked samples were analyzed serologically and the results of HBV serology using the first positive samples are indicated. Abbreviations: anti-HBc, antibody to HBV core antigen; anti-HBs, antibody to HBsAg; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; MSM, men who have sex with men.

frequency and risk of HBV incident infection during each treatment category was also assessed by univariate Cox proportional hazards regression analysis. We used hazard ratios and 95% confidence intervals to estimate the impact of each variable on incident infection. Patients' age and $CD4^+$ cell count on the date of incident infection, and peak value of ALT within 3 months of incident infection were compared between transient infection and chronic infection with Wilcoxon rank-sum test. The differences in rates of HBV genotype A and rtM204V/I mutation were compared with χ^2 test (ie, the Fisher exact test). Statistical significance of difference was defined as a 2-sided P value of <.05. All statistical analyses were performed with the Statistical Package for Social Sciences version 17.0 (SPSS, Chicago, Illinois).

RESULTS

Figure 1 shows the patient selection procedure. A total of 1434 HIV-1–infected MSM met the inclusion criteria described in the Methods section. Of these, 146 patients (10.2%) were positive for HBsAg, 737 (51.4%) were positive for anti-HBs, and 197 (13.7%) were solely positive for anti-HBc using baseline samples. The remaining 354 patients (24.7%; negative for HBsAg, anti-HBs, and anti-HBc at baseline), who were considered to have never been infected with HBV, were enrolled for serological follow-up. Table 1 lists their baseline characteristics. Serological analysis of the last sample of each of these patients showed HBV incident infection during follow-up in 43 (12.1%). Their baseline samples were found to be PCR-negative for HBV DNA, confirming that the incident infection in these patients occurred during the follow-up period. All stocked samples of the 43 patients were analyzed serologically to determine the date of HBV incident infection. HBV incident infections occurred every year between 1997 and 2010 except in 1998. The median time period from the baseline to HBV incident infection was 1.6 years (interquartile range [IQR], 192–1151 days; range, 28–4068 days). The total observation period was 1607 person-years (median, 3.7 years [IQR], 1.9–6.5 years). Figure 2 shows the Kaplan-Meier curve for the HBV incident infection for the whole cohort of enrolled patients.

In order to assess the risk of HBV incident infections, patients' baseline characteristics, year of entry, the use of any antiretroviral agents, the use of any of LAM, TDF, or FTC, and the frequency of changing ART regimen during the follow-up

Table 1. Baseline Characteristics of the 354 Enrolled Patients

Characteristic	Total (n = 354)	Year of Entry			
		1997–2000 (n = 61)	2001–2003 (n = 79)	2004–2006 (n = 112)	2007–2009 (n = 102)
Age, y, median (IQR)	32.0 (27.0–38.0)	32.0 (27.8–37.3)	31.0 (27.0–37.8)	32.0 (27.0–38.0)	35.0 (27.0–42.0)
Race/ethnicity					
Japanese	340 (96.0)	59 (96.7)	78 (98.7)	109 (97.3)	94 (92.2)
Asian other than Japanese	4 (1.1)	0 (0.0)	0 (0.0)	1 (0.9)	3 (2.9)
Caucasian	10 (2.8)	2 (3.3)	1 (1.3)	2 (1.8)	5 (4.9)
HCV antibody, positive	8 (2.3)	1 (1.6)	2 (2.5)	1 (0.9)	4 (3.9)
TPHA positive	101 (28.5)	23 (37.7)	20 (25.3)	30 (26.8)	28 (27.5)
$CD4^+$ cell count, cells/mm^3, median (IQR)	277 (151–404)	277 (169–417)	313 (97–443)	316 (176–413)	252 (129–359)
HIV RNA, log_{10} copies/mL, median (IQR)	4.6 (3.8–5.2)	4.5 (3.6–5.2)	4.8 (3.9–5.4)	4.4 (3.8–4.9)	4.7 (3.9–5.2)

Data are No. (%) unless otherwise specified.

Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; TPHA, *Treponema pallidum* hemagglutination assay.