that of NL4-3 was noted in the reports. Since all sequences of the study participants belonged to CRF01_AE subtype, data on frequencies of each mutation in the C-terminal half of RT in CRF01_AE and subtype B in treatment-naïve patients was obtained from the Stanford HIV Drug Resistance Database [http://hicdb.stanford.edu/index.html, accessed as late as July 20th, 2010] for reference. Nucleotide sequences of the C-terminal half of CRF01_AE RT from 38 patients have been deposited in the DDBJ database (accession numbers AB545813–AB545850).

2.4. Construction of recombinant HIV-1 harboring G335D and/or A371V with or without TAMs

To examine the influence of G335D and A371V on drug susceptibility to NRTIs, we constructed mutant HIV-1 recombinants that included G335D, A371V or both with or without TAMs. TAM-1 virus was constructed as combination of M41L, L210W and T215Y and TAM-2 as combination of D67N, K70R and T215F. Mutant recombinant plasmid clones of the virus were generated by oligonucleotide site-directed mutagenesis as described previously [10], using pBS-RT_{WT}, which contains the entire RT coding sequences (amino acid position 14–560) and three silent restriction sites (XmaI, NheI and XbaI from the 5′ to 3′ end of RT at codons 15, 267, and 560). After site-directed mutagenesis, the mutated RT was ligated into pNL4-3, which contains the entire genome of HIV-1 and the same silent restriction sites as pBS-RT_{WT}. The infectious virus was generated by transfection of each molecular clone into Cos-7 cells, harvested and stored at −80 °C until use. Infectivity was measured as blue cell-forming units (BFU) of MAGIC-5 cells. All mutations in recombinant viruses were confirmed by full-length sequencing of the entire RT coding region.

2.5. Drug susceptibility assay

Susceptibility to NRTIs was determined by using MAGIC-5 cells as described previously [35] in more than three experiments. MAGIC-5 cells were infected with diluted virus stock (100 BFU) in the presence of increasing concentrations of RTIs, cultured for 48 h, fixed and stained with X-Gal (5-bromo-4chloro-3-indolyl-βD-galactopyranoside). The stained cells were counted under a light microscope. Drug concentrations that reduced the cell count to 50% of that of the drug-free control (EC_{50}) were determined by referring to the dose–response curve.

2.6. Statistical analysis

Data are expressed as mean ± SD. The Student's t-test was used to compare two groups of continuous variables and a p-value less than 0.05 was considered statistically significant. Statistical analyses were performed using SPSSII software package for Windows, version 11.0J (SPSS Japan Inc, Tokyo, Japan).

3. Results

3.1. Characteristics of patients failing antiretroviral therapy

A total of 416 individuals on ART were consecutively enrolled in the present study and their pVLs were assayed between October 1, 2007 and June 30, 2008 at the NTHD in Hanoi. Among them, 49 individuals were confirmed as treatment failure by the definition described above and assigned for genotypic resistance analysis. The characteristics of the 49 individuals are listed in Table 1. All patients had received AZT or d4T plus 3TC combined with NVP, EFV or lopinavir/ritonavir (LPV/r) at the time of enrollment. The most frequently used combination was AZT, 3TC, and NVP, followed by d4T, 3TC and NVP. Protease inhibitors (PIs) were used by 17 (34.7%) patients, while the Vietnamese national ART guideline recommends d4T, 3TC plus 1 NNRTI for the first line regimen [2]. This was probably due to the inclusion of patients who had started ART when the guideline had not been issued yet. The median duration of ART exposure was 2.98 years (IQR 2.17–4.00).

3.2. Genotypic resistance patterns including C-terminal domain of RT

We successfully amplified the N-terminal half of RT and protease of all the 49 patients and C-terminal region of RT of 38 patients. The proportion of patients with at least one NRTI resistance mutation in the N-terminal half of RT was 89.8%.

Table 1
Characteristics of patients failing antiretroviral therapy.

		$n = 49$	(%)
Sex, n (%)			
males		33	(67.3)
females		16	(32.7)
Median Age, years (range)		31	(21–50)
Risk of HIV-1 infection (multiple choice), n (%)			
sexual contact		46	(93.9)
intravascular drug use		20	(40.8)
CD4 count, median cells/mm^3 (IQR)		145	(84–195)
Plasma viral load, median log copies/ml (IQR),		4.23	(3.59–4.94)
Duration of prior ART, median years (IQR)		2.98	(2.17–4.00)
Experienced ART, n (%)			
NRTI	AZT	39	(79.6)
	d4T	24	(49.0)
	3TC	49	(100)
	ddI	7	(14.3)
	ABC	2	(4.1)
	TDF	2	(4.1)
NNRTI	NVP	43	(87.8)
	EFV	15	(30.6)
PI	IDV	12	(24.5)
	SQV	6	(12.2)
	LPVr	3	(6.1)

IQR: interquartile range. ART: antiretroviral therapy. NRTI: nucleoside reverse transcriptase inhibitor. NNRTI: non-nucleoside reverse transcriptase inhibitor. PI: protease inhibitor.

Of those, M184V was the most common (81.6%) and TAMs were also observed frequently in 71.4%: M41L (22.4%), D67N (24.5%), K70R (18.4%), L210W (14.3%), T215F (16.3%), T215Y (28.6%), K219E (12.2%) and K219Q (6.1%), whereas K65R (6.1%), L74V (4.1%), Y115F (2.0%) and mutations driven by Q151M complex (4.1%) were relatively rare. Similar to previous reports on drug resistance in CRF01_AE [28–30], mutations classified into TAM type 2 (TAM-2): D67N, K70R, T215F and K219E/Q, were more frequently observed than those of TAM type 1 (TAM-1): M41L, L210W and T215Y/F (30.6% v.s. 26.5%), except for a patient having only T215F. With regard to codon 215, T215F were more frequently seen with other TAM-2 mutations (six out of eight sequences that contain T215F), concurring with the previous reports showing the introduction of T215F into TAM-2 backbone increase relative fitness in the presence of AZT but resulted in decreased viral fitness in TAM-1 backbone [37]. The resistance mutations of NNRTIs in the N-terminal half of RT were detected in 79.6%. The most frequent NNRTI-resistance mutations were Y181C/I/V (32.7%), K103N (26.5%) and G190A (26.5%). In 17 PI experienced patients, no major mutations were found, but 9 minor mutations were detected: L10I/V (11.8%), I13V (88.2%), G16E (11.8%), K20R (17.6%), M36I (100%), L63P (29.4%), H69K (100%), V82I (11.8%) and I93L (8.2%). However, the mutations in protease are considered as consensus amino acids in most non-B subtype HIV-1 (I13V, M36I and H69K) or common polymorphic mutations (L10V, G16E, K20R, L63P, V82I and I93L) and could not be determined as mutations that emerged after treatment.

The frequencies of mutations in the C-terminal half of the RT reported previously as NRTI or NNRTI resistance [7–20] are described in Table 2. As shown, G335D (100%), N348I (36.8%), A371V (100%), A376S (5.3%), E399D (28.9%) and A400T (97.4%) were detected in the patients failing ART. However, as we reported previously [20], G335D and A371V were also commonly observed in untreated patients infected with non-B subtype HIV-1 and the frequencies of G335D and A371V in CRF01_AE subtype shown in the Stanford HIV Drug Resistance Database are 95.2% and 97.1%, respectively, while those are rare in subtype B (G335D: 1.3%, A371V: 3.2%). A400T is also one of the known polymorphisms in CRF01_AE [16]. Therefore, it is unlikely that G335D, A371V and A400T in this population were selected by ART exposure or involved in the resistance mutations.

Table 2
Frequencies of mutations associated with RTI-resistance in the connection and RNase H domain of reverse transcriptase of HIV-1.

Mutations[b]	Study participants (Treatment failure)		Stanford database[a] (RTI-naïve)	
	CRF01_AE		CRF01_AE	Subtype B
	$n = 38$			
	%	(n)	%	%
G333	100	(38)		
D	0	(0)	0	0.7
E	0	(0)	0	7.5
G335	0	(0)		
C	0	(0)	0	0.5
D	100	(38)	92.0	1.3
N348	57.9	(22)		
I	36.8	(14)	0	0.5
T	5.3	(2)	0	0
A360	97.4	(37)		
I	0	(0)	0	0
V	0	(0)	0	0.7
S	2.6	(1)	1.1	0
V365	100	(38)		
I	0	(0)	0	3.2
T369	94.7	(36)		
I	0	(0)	0	0
A	2.6	(1)	19.3	3.3
V	2.6	(1)	2.8	1.2
A371	0	(0)		
V	100	(38)	97.1	3.2
A376	94.7	(36)		
S	5.3	(2)	1.7	5.8
E399	*68.4*	*(26)*		
D	*28.9*	*(11)*	*2.6*	*14*
K	*2.6*	*(1)*	*0*	*0.1*
A400	0	(0)		
T	97.4	(37)	89.2	25.3
L	2.6	(1)	0	1
Q475	100	(38)		
A	0	(0)	0	0
Q509	97.4	(37)		
L	0	(0)	0	0
R	2.6	(1)	0	0

[a] Available from http://hicdb.stanford.edu/index.html.
[b] Resistance mutations reported previously [8–21] are indicated in bold. Resistance was defined as greater than three fold increase of EC_{50} compared to that of NL4-3.

3.3. Drug susceptibility assay for mutant recombinant HIV-1

To address whether G335D or A371V have an impact on NRTI susceptibility depending on the pattern of TAMs, we constructed recombinant viruses containing G335D and/or A371V in the background of TAM-1 or TAM-2 by site-directed mutagenesis. As shown in Table 3, G335D, A371V or their double mutant did not increase the resistance levels to all NRTIs by themselves. In contrast, as shown in Table 4, variants with G335D, A371V or both exhibited higher resistance to AZT in the background of TAM-1 (8.2- to 23.2-fold) and the increased resistance level was the greatest in the double mutant G335D/A371V. Although G335D/A371V showed statistical increase in resistance to all the other NRTIs except 3TC, the fold increase from TAM-1 mutant was the greatest in AZT (Table 4). Similar to TAM-1 background, G335D, A371V or G335D/A371V with TAM-2 exhibited considerable increase in susceptibility to AZT (52.7-, 21.1-, 52.6-fold, respectively). In addition, there were marginal changes in d4T susceptibility (Table 5) in the three patterns of the mutants, G335D, A371V or G335D/A371V. In TAM-2 background, we also found G335D alone increased susceptibility to ABC (4.2-fold) and to TDF (2.4-fold), and that G335D/A371V increased susceptibility to ddI (7.2-fold), ABC (3.1-fold) and

Table 3
Drug susceptibilities of HIV-1 variants with G335D or A371V.

Mutation[a]	EC_{50} (μM)[b] (fold increase)											
	AZT		d4T		ddI		3TC		ABC		TDF	
Wild Type	0.050 ± 0.002		2.55 ± 0.07		1.90 ± 0.17		0.45 ± 0.035		2.48 ± 0.21		0.020 ± 0.0023	
335D	0.052 ± 0.004	(1)	3.19 ± 0.14	(1.3)	4.56 ± 0.20	(2.4)	0.45 ± 0.022	(1)	2.71 ± 0.17	(1.1)	0.018 ± 0.0019	(0.9)
371V	0.047 ± 0.003	(0.9)	3.26 ± 0.17	(1.3)	5.30 ± 0.02	(2.8)	0.55 ± 0.027	(1.2)	2.32 ± 0.09	(0.9)	0.027 ± 0.0014	(1.3)
335D/371V	0.052 ± 0.010	(1)	3.52 ± 0.06	(1.4)	3.38 ± 0.21	(1.8)	0.65 ± 0.023	(1.5)	2.39 ± 0.12	(1)	0.025 ± 0.0031	(1.2)

AZT, zidovudine; d4T, stavudine; ddI, didanosine; 3TC, lamivudine; ABC, abacavir; TDF, tenofovir.
[b] Data are mean ± SD from at least three independent experiments. Fold increase was the relative change in EC_{50} value compared with that of HIV-1 WT.
[a] See Materials and Methods for the construction of clones.

TDF (5.2-fold). Of note, the increased resistance levels to AZT, d4T, ddI and TDF were greater in G335D/A371V in TAM-2 background than that in TAM-1 background. Our data suggest double mutant G335D/A371V in TAM-2 background would have the most impact on NRTI susceptibility.

4. Discussion

In the present study, we described the drug resistance mutations in the entire RT of CRF01_AE HIV-1-infected Vietnamese patients who had high pVL levels despite 6-month ART. According to the criteria used for evaluation of drug resistance proposed by Shafer et al. [38,39], correlations between mutations and treatment should be confirmed by extensive resistance surveillance. However, limited sequences of CRF01_AE in the connection subdomain and RNase H domain of the RT have been available so far especially from treatment-experienced patients [40]. Santos et al. [19] previously compared amino acid variations between treatment-naïve and treatment-experienced patients in connection subdomain (280 naïve vs. 230 treated) and RNase H domain (334 naïve vs. 234 treated). Although their study included substantial number of patients, larger number of cases belonged to subtype B (80–82% of treatment-experienced patients) and the unique characteristics of CRF01_AE, accounting for only 10% of their study, could not be fully assessed. Since our present study focused on CRF01_AE sequence alone, the data provide direct information on the evaluation of drug resistance mutations in CRF01_AE, although sequences before ART initiation were not available. The largest study to date exploring treatment-related mutation in RT C-terminal site in CRF01_AE infection is the report from Thailand by Saeng-aroon et al. [40], in which significantly higher frequencies of N348I, E399D, P537S and I542M in treatment-exposed patients than treatment-naïve patients (76 naïve vs. 49 treated) was noted. Although the former two mutations have already known to be associated with exposure to NRTI or NNRTI and were detected in our treatment-experienced patients, the results of P537S and I542M were different from us: no patients in our study had P537S and I542M. Further studies are required to determine the prevalence of drug resistance mutations in the C-terminal half of RT in CRF01_AE.

Among the mutations previously reported as drug resistance in the connection subdomain and RNase H domain of RT, we found no mutations except G335D, N348I, A371V, A376S, E399D and A400T in treatment-experienced individuals with CRF01_AE infection. Of these mutations, N348I is one of the most extensively assessed mutations in the RT connection domain and has been established as multiclass resistance to both NRTIs and NNRTIs by being identified in clinical isolates in treatment-experienced individuals in subtype B and by *in vitro* drug susceptibility assay [9,10,12,13]. Since N348I is rare in treatment-naïve of both subtype B and CRF01_AE, N348I observed in 35.8% of CRF01_AE sequences in our study was considered to be treatment-related. The wide use of NVP in Viet Nam might be one of the causes of the higher prevalence of N348I in this population than in subtype B. In addition to N348I, E399D has been thought to be associated with resistance to AZT and to EFV when combined with K103R and 179D [41,42]. Although our results of E399D prevalence of in treatment-exposed patients (28.9%) was relatively higher than those in the Stanford database (9%), it was similar to the previous study by Saeng-aroon et al. of treatment-exposed patients with CRF01_AE infection (32.7%) and considered to be selected after treatment. In contrast, A376S detected in this study was not clearly identified as a treatment-related mutation because the frequency (5.3%) was similar to those of treatment-naïve

Table 4
Drug susceptibilities of HIV-1 variants with G335D or A371V in the TAM-1 background.

Mutation	EC_{50} (μM) (fold change)											
	AZT		d4T		ddI		3TC		ABC		TDF	
Wild Type	0.050 ± 0.002		2.55 ± 0.07		1.90 ± 0.17		0.45 ± 0.035		2.48 ± 0.21		0.020 ± 0.0023	
TAM-1	0.200 ± 0.016	**(4)**	4.78 ± 0.30	(1.9)	5.35 ± 0.79	(2.8)	2.37 ± 0.017	**(5.3)**	4.20 ± 0.25	(1.7)	0.043 ± 0.0030	(2.2)
TAM-1/335D	0.411 ± 0.028	**(8.2)**[a]	6.63 ± 0.05	(2.6)	5.71 ± 0.57	(3.0)	2.14 ± 0.099	**(4.8)**	3.17 ± 0.23	(1.3)	0.024 ± 0.0026	(1.2)
TAM-1/371V	0.473 ± 0.052	**(9.4)**[a]	6.07 ± 0.12	(2.4)	6.30 ± 0.48	**(3.3)**	2.45 ± 0.110	**(5.5)**	3.88 ± 0.32	(1.6)	0.046 ± 0.0018	(2.3)
TAM-1/335D/371V	1.160 ± 0.078	**(23.2)**[a]	9.01 ± 0.20	**(3.5)**[a]	7.87 ± 0.35	**(4.1)**[a]	2.40 ± 0.016	**(5.4)**	7.57 ± 0.57	**(3.1)**[a]	0.056 ± 0.0004	(2.8)

Boldface indicates an increase greater than threefold.
[a] Increases in fold change were significant compared to TAM-1 without G335D or A371V.

Table 5
Drug susceptibilities of HIV-1 variants with G335D or A371V in the TAM-2 background.

Mutation	EC_{50} (μM) (fold increase)											
	AZT		d4T		ddI		3TC		ABC		TDF	
Wild Type	0.050 ± 0.002		2.55 ± 0.07		1.90 ± 0.17		0.45 ± 0.035		2.48 ± 0.21		0.020 ± 0.0023	
TAM-2	0.3960 ± 0.076	**(7.9)**	6.18 ± 0.11	(2.4)	6.71 ± 0.57	**(3.5)**	2.57 ± 0.089	**(5.7)**	2.97 ± 0.29	(1.2)	0.033 ± 0.0026	(1.7)
TAM-2/335D	2.6390 ± 0.396	**(52.7)**[a]	7.97 ± 0.47	**(3.1)**[a]	5.74 ± 0.63	**(3)**	2.37 ± 0.082	**(5.3)**	10.43 ± 0.41	**(4.2)**[a]	0.049 ± 0.0014	(2.4)[a]
TAM-2/371V	1.0600 ± 0.131	**(21.1)**[a]	8.29 ± 0.23	**(3.3)**[a]	6.00 ± 0.64	**(3.2)**	2.58 ± 0.072	**(5.8)**	3.43 ± 0.21	(1.4)	0.036 ± 0.0012	(1.8)
TAM-2/335D/371V	2.6340 ± 0.132	**(52.6)**[a]	13.71 ± 0.76	**(5.4)**[a]	13.76 ± 0.51	**(7.2)**[a]	2.45 ± 0.062	**(5.5)**	7.57 ± 0.21	**(3.1)**[a]	0.105 ± 0.0030	**(5.2)**[a]

Boldface indicates an increase greater than threefold.
[a] Increases in fold change were significant compared to TAM-2 without G335D or A371V.

subtype B (5.8%) and CRF01_AE (1.7%) infected individuals in the Stanford database. On the other hand, G335D, A371V and A400T were found in almost all the patients in our study. Although these three mutations are thought to be related to NRTI resistance in subtype B [7,11,16], they are common polymorphisms of wild-type CRF01_AE HIV-1 with prevalence of more than 90% in our previous study [20] and in the Stanford database. Therefore, we conclude that G335D, A371V and A400T detected in the present study were not selected after treatment but had existed before the introduction of treatment. Consequently, N348I was the only drug resistance mutation in the C-terminal half of RT observed in our cohort of treatment-experienced Vietnamese infected with CRF01_AE HIV-1.

Our results demonstrated that common CRF01_AE polymorphisms G335D and A371V play considerable role in drug resistance to NRTIs. Recent studies suggested that each of G335D or A371V is associated with drug resistance; G335D emerged after AZT exposure exhibits greater AZT resistance (8 to 53-fold over WT) when combined with TAM [11] and A371V selected in the background of D67N and K70R by high concentrations of AZT *in vitro* shows strong resistance to AZT in the presence of TAMs [7]. In agreement with those reports, our results showed that mutant containing G335D or A371V did not increase the resistance levels to NRTIs by themselves but they conferred higher resistance when combined with TAMs, especially to AZT (8.2–52.7 fold increase). Furthermore, we found that the dual mutation G335D/A371V had the greater impact than each single mutation on resistance in the presence of TAM. As G335D and A371V always appear together in treatment-naïve CRF01_AE, this finding is more critical for CRF01_AE HIV-1 infection than for subtype B infection. In addition, the fold change increased by G335D and A371V was greater with TAM-2 than that with TAM-1. Since TAM-2 is more frequent in CRF01_AE than in subtype B [28–30], this data is important for CRF01_AE HIV-1. Although the impact of G335D and A371V was the greatest in AZT resistance and seemed to be minor in other NRTIs' resistance, the fold-increase in TDF of G335D/A371V plus TAM-2 variant were above the clinical cut-off values [43], which can cause treatment failure. As TDF is often used in second line ART [2], this data is crucial for decisions on the next therapeutic strategies for CRF01_AE HIV-1-infected patients failing first line ART. Since our recombinant viruses were created with pBS-RT_{WT}, which was derived from subtype B RT but not from CRF01_AE RT, our results cannot be applied directly to CRF01_AE infection. CRF01_AE/B recombinants have been emerged and highly prevalent in Southeast Asian countries [32,44,45] and the breakpoint analysis showed some CRF01_AE/B recombinants consisted of subtype B N-terminal site and CRF01_AE C-terminal sites [45]. Therefore, our data suggests the potential influence of those CRF01_AE/B recombinants as well as CRF01_AE strain on the selection of second line therapy in Southeast Asia.

In summary, we reported the frequencies of drug resistance mutations in the connection subdomain and RNase H domain of RT in CRF01_AE HIV-1-infected Vietnamese who experienced ART. Then we demonstrated that the combination of G335D and A371V, a common pattern of polymorphisms in wild-type CRF01_AE, confer significant resistance to various NRTIs in the presence of TAMs. Our findings emphasize the important role of polymorphisms in C-terminal half of RT in CRF01_AE HIV-1 on drug resistance, especially in consideration of the second line therapy. Further investigation is needed on drug resistance mutations in widely prevailing non-subtype B HIV-1.

Acknowledgments

We thank Nguyen Thi Bich Ha, Nguyen Thi Dung and Nguyen Hang Long for collecting the clinical data, Le Thi Hoa and Pham Hang Hai for sample preparation, Van Dinh Trang and Nguyen Nhu Ha for viral load measurement and Nguyen Thi Huyen for the dedicated assistance. No conflicts of interest declared by all authors. This work was financially supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan (the Program of Founding Research Centers for Emerging and Reemerging Infectious Diseases) and the Ministry of Health, Labor, and Welfare of Japan.

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INTERNAL MEDICINE

ORIGINAL ARTICLE

Clinical Symptoms and Courses of Primary HIV-1 Infection in Recent Years in Japan

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Abstract

Background The natural course of HIV-1 infection includes 10 years of an asymptomatic period before the development of AIDS. However, in Japan, the disease progression process seems faster in recent years.

Methods The study subjects were 108 new patients with primary HIV-1 infection during the period from 1997 through 2007. We evaluated their clinical symptoms and laboratory data, and then analyzed disease progression in 82 eligible patients. Disease progression was defined as a fall in CD4 count below 350/μL and/or initiation of antiretroviral therapy.

Results Ninety percent of the patients were infected via homosexual intercourse. All patients had at least one clinical symptom (mean; 4.75±1.99) related to primary HIV-1 infection, with a mean duration of 23.2 days (±14.8) and 53.3% of them had to be hospitalized due to severe symptoms. The mean CD4 count and viral load at first visit were 390/μL (±220.1) and 4.81 log10/mL (±0.78), respectively. None developed AIDS during the study period. Estimates of risk of disease progression were 61.0% at 48 weeks and 82.2% at 144 weeks. In patients who required antiretroviral therapy, the median CD4 count was 215/μL (range, 52-858) at initiation of such therapy. Among the patients with a CD4 count of <350/μL at first visit, 53% never showed recovery of CD4 count (>350/μL) without antiretroviral therapy.

Conclusion Despite possible bias in patient population, disease progression seemed faster in symptomatic Japanese patients with recently acquired primary HIV-1 infection than the previously defined natural course of the disease.

Key words: HIV-1, primary infection, disease progression

(Intern Med 50: 95-101, 2011)
(DOI: 10.2169/internalmedicine.50.4137)

Introduction

The natural course of HIV-1 infection has been well described in large cohorts from the United States and Europe before the introduction of highly active antiretroviral therapy (HAART); primary HIV-1 infection (PHI) is followed by a clinical latency, usually lasting around 10 years, which precedes the eventual collapse of the immune system (1, 2). However, there is a common feeling among clinicians at present that the natural disease progression of recently infected patients is faster than in previous years (3, 4). Disease progression depends on various factors such as HLA type (5), concomitant infections (6, 7), and available medical resources (8). In addition to these factors, events occurring during PHI could also determine the natural course of the disease. Initial studies suggested that patients with more symptoms related to primary PHI and longer duration of illness exhibit faster rates of progression to AIDS (9-13). Plasma viral load at a set point is also an independent predictor of disease progression (14, 15). However, to determine the viral set point is sometimes difficult. Therefore, for clinicians, the severity of clinical symptoms is the only predictor of subsequent disease progression. The latency be-

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Received for publication June 28, 2010; Accepted for publication September 29, 2010
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tween the development of PHI and commencement of HAART is also important in the present HAART era.

The main aim of this study was to evaluate the natural disease progression of recently infected Japanese patients. To determine whether or not the disease progression of recently infected patients is accelerated, their CD4 decline was compared with that of hemophiliacs infected before 1985 as the first HIV-1 infection in Japanese.

Furthermore, we also evaluated the correlation between initial CD4 count, viral load, and clinical events and subsequent changes in CD4 and/or time to start HAART in symptomatic Japanese patients with PHI.

Patients and Methods

Study site and patients with PHI

This study was conducted at the AIDS Clinical Center (ACC), National Center for Global Health and Medicine (NCGM: formerly International Medical Center of Japan). The NCGM (925 beds) is a tertiary general hospital located in central Tokyo and the ACC is the main referral clinic for treatment of HIV infected patients in Japan. As part of the follow-up service, HIV-1 infected patients usually visit the ACC on a monthly basis and CD4 count and viral load are measured at each visit. In the present retrospective study, we reviewed the medical records of 108 patients with PHI who were newly diagnosed with PHI between 1997 through 2007 at the ACC. We had conducted a clinical trial of structured treatment interruptions in patients with PHI from November 2000 through December 2002 and 26 patients were enrolled in that trial (16, 17). In terms of the data of these 26 patients, only the initial clinical and laboratory data were included in the present analysis, while all other data, such as time to events, were excluded from this study. To compare the natural CD4 decline of previously and recently infected patients, CD4 counts of 42 Japanese hemophiliacs recorded in the database in 1988 were analyzed as a previous control. Japanese hemophiliacs were infected with HIV-1 through contaminated blood products before 1985 (the estimated mean year of infection was 1983). Therefore, CD4 counts at the end of 1988 were the data at least 3 years after infection. In this comparison, the number of eligible recently infected patients was 59 patients; untreated and CD4 count at 3 years after infection was available.

Definition of PHI

PHI was diagnosed based on the presence of the following three criteria: 1) negative or incomplete western blot finding at the first visit with subsequent change to positive, 2) negative or weakly reactive enzyme-linked immunosorbent assay (ELISA) result for plasma HIV-1 RNA, and 3) confirmed HIV-1 infection on the first visit with documentation of negative ELISA result within 6 months. Symptomatic PHI was defined as PHI accompanied by at least one symptom related to acute retroviral syndrome, such as fever, lymphadenopathy, or skin rash.

Definition of disease progression

Disease progression was defined as fall in CD4 count below 350/μL and/or initiation of antiretroviral therapy. Specifically, patients with an AIDS-defined illness [listed under Centers for Disease Control and Prevention (CDC) category C], patients with AIDS requiring initiation of HAART, and those with severe symptomatic PHI on HAART were defined to have disease progression. The selection of a cutoff value of 350/μL for CD4 count was based on the fact that treatment is generally indicated during the chronic phase of infection when CD4 count falls below 350/μL (18). Patients were considered to be in immunologic progression at the first visit when the initial CD4 count was <350/μL and never subsequently reached 350/μL. For patients who showed a spontaneous increase in subsequent CD4 counts to ≥350/μL (such recovery occurred within 3 months from the first visit in all such patients), disease progression was set to have started at the time when such change in CD4 count occurred.

Statistical analysis

Continuous variables are presented as mean value ± SD. Categorical variables were presented as absolute numbers and proportions. Time to events was analyzed by the Kaplan-Meier survival curves, and compared using log-rank test. For patients who did not experience the events described above, data were censored at their last visit. To evaluate the differences between patients groups, the Student t test and χ^2 test were used when appropriate. The relationships between variables were analyzed by the Spearman rank-over correlation test. Statistical significance was defined as $p<0.05$. Data were analyzed using SPSS for Windows (version 15, SPSS, Inc., Chicago, IL).

Results

Table 1 lists the demographics of the enrolled patients with PHI. All patients had at least one documented symptom consistent with PHI (median 5; range 1-11). Fever, cervical lymphadenopathy, pharyngitis, and rash were found in more than 50% of patients (Table 2). The mean duration of symptoms was 23.2 days (SD ±14.8). Fifty-eight (53.7%) patients had to be hospitalized due to severe clinical symptoms. The initial viral loads in hospitalized patients were significantly higher than those of non-hospitalized patients. A longer duration of symptoms was associated with higher initial viral load (R=0.31, p=0.002) (Fig. 1A), and lower CD 4 count (R=-0.22, p=0.03) (Fig. 1B). Consequently, a higher viral load slightly was correlated with a lower CD4 count at the first visit (R=-0.22, p=0.033) (Fig. 1C).

Disease progression was analyzed in 82 patients. None of the patients had AIDS-defining events. Estimates of the risk of disease progression were 50.6% at 24 weeks, 61.0% at 48 weeks, 67.0% at 96 weeks, and 82.2% at 144 weeks

Table 1. Baseline Characteristics of 108 Patients with Primary HIV-1 Infection in this Study

Characteristics	Total number or mean (±SD) or %	Hospitalized patients (n = 58)	Non-hospitalized patients (n = 50)	p
Age (year)	31.8±8.48	32±9.07	31±7.82	NS
Sex				
Male	102	56	46	NS
Female	6	2	4	NS
Predisposing factor				
MSM	97	53	44	NS
Heterosexual	8	3	5	NS
IDU	1	0	1	NS
Unknown	2	2	0	NS
PMH of STD	75 (69.7)	44 (40.4)	31 (29.3)	NS
Syphilis	49 (45.5)	27 (25.3)	21 (20.2)	NS
Acute hepatitis A	11 (10.1)	6 (6.1)	5 (4.0)	NS
Acute hepatitis B	36 (33.3)	22 (20.2)	14 (13.1)	NS
Amebiasis	10 (9.1)	9 (8.0)	1 (1.1)	0.035
Others	7 (6.1)	2 (2.0)	5 (4.1)	NS
No. of symptoms	4.75±1.99	4.98±1.94	4.48±2.04	NS
Duration of symptoms (days)	23.2±14.8	27.8±13.1	18.0±15.1	0.001
Laboratory findings				
CD4 count/μL	390.0±220.1	356.1±204.1	443.7±236.0	0.06
HIV RNA $\log_{10}$/mL	4.81±0.78	5.03±0.68	4.48±0.81	0.001
STI trial*	26	12	14	NS

*Patients enrolled in a clinical trial of structured treatment interruptions in recently HIV-1-infected patients. Abbreviations; MSM: men who have sex with men, PMH of STD: past medical history of sexual transmitted diseases, STI: structured treatment interpretations, IDU: intravenous drug user, Others: genital herpes infection, chlamydial urethral infection condyloma acuminata, NS: not significant

Data are presented as mean ± SD or percentage (%) unless otherwise indicated

Table 2. Symptoms and Physical Findings Observed in the Patients with >10% Frequencies (n=108)

Symptoms and physical findings	frequency (%)
Fever	91
Lymphadenopathy	63
Pharyngitis	53
Rash	50
Diarrhea	37
Fatigue	32
Headache	26
Myalgia	20
Weight loss	19
Nausea	16
Appetite loss	14
Neurological sign	13
Hepatomegaly	13
Thrush	12

(Fig. 2). Eighteen of 34 (53.3%) patients with an initial CD 4 cell count below 350 cells/μL had immunologic progression at the first visit. Their CD4 counts never increased above 350/μL until initiation of HAART. Forty-eight (58.5%) required initiation of HAART in this study. The reasons for the initiation of HAART were severe clinical symptoms related to PHI in 16 patients and immunologic progression in 32 patients. The median CD4 count of those patients at initiation of HAART was 215/μL (range, 52-858).

We analyzed the clinical course in 66 patients (excluding 26 patients who enrolled in a clinical trial of structured treatment interruptions in PHI and 16 patients who received HAART for PHI) to determine the factors associated with disease progression. Half of these patients (33 patients) required hospitalization. As shown in Fig. 3A, the mean time to disease progression of the hospitalized patients [57.4 weeks, 95% confidence interval (95%CI); 34.9-79.8 weeks] was shorter than that of the non-hospitalized (33 patients, 94.4 weeks, 95%CI; 71-117 weeks, p=0.002). Among the 32 patients with CD4 count >350/μL at first visit, 24% had documented disease progression within 1 year, whereas among 34 patients with CD4 count <350/μL at first visit, 76.4% showed disease progression (Fig. 3B). The mean times to disease progression for the two groups were 111.9 weeks (95%CI; 92.8-131) and 39.5 weeks (95%CI; 18.6-60.5), respectively (p<0.001). Disease progression in 39 patients with high viral load (≥5.0 $\log_{10}$/mL) was not significantly different (p=0.41) from that in 27 patients with low viral load (<5.0 $\log_{10}$/mL) (Fig. 3C). The number of symptoms was not significantly different in each group (Fig. 3D). The mean time to disease progression was 69.8 weeks (95% CI; 47.2-92.5) in patients with a high viral load and 80.4 weeks (95%CI; 54.9-105.8) in those with a low viral load.

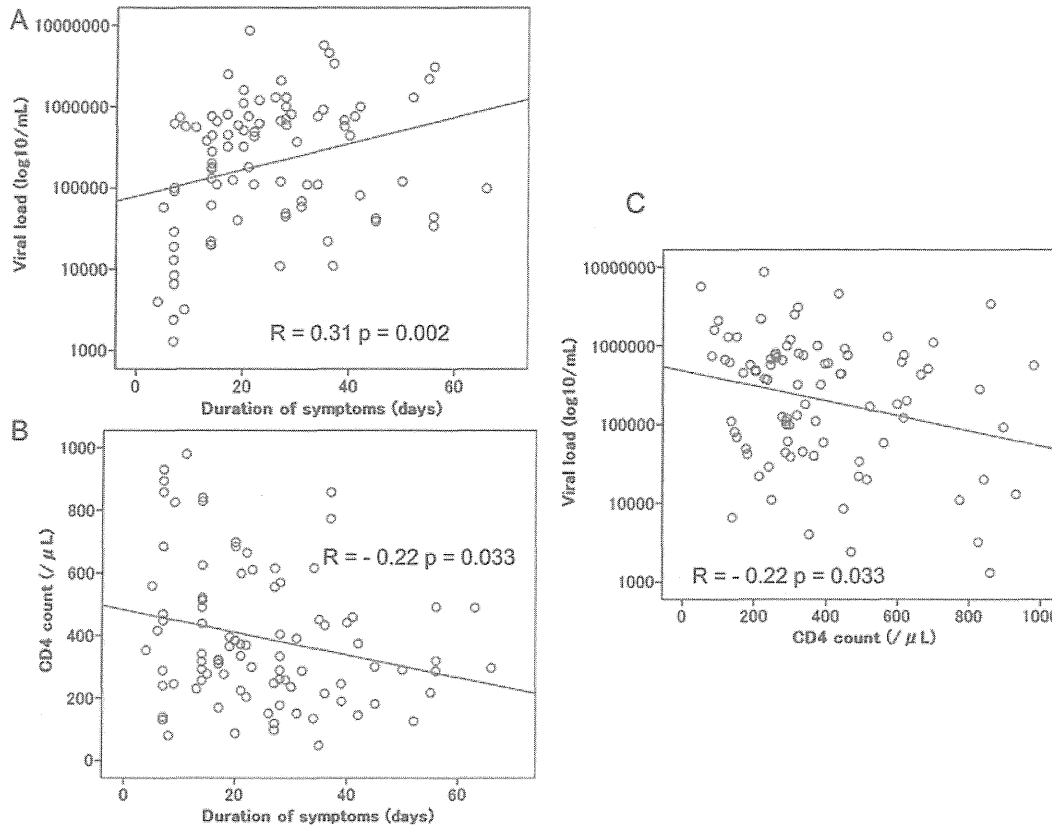

Figure 1. **Correlations among plasma viral load, CD4 count, and clinical symptoms. A; Plasma viral load correlated with duration of symptoms (R=0.31, p=0.002). B; CD4 count correlated inversely with duration of symptoms (R=-0.22, p=0.033). C; plasma viral load correlated inversely with CD4 count (R=-0.22, p=0.033).**

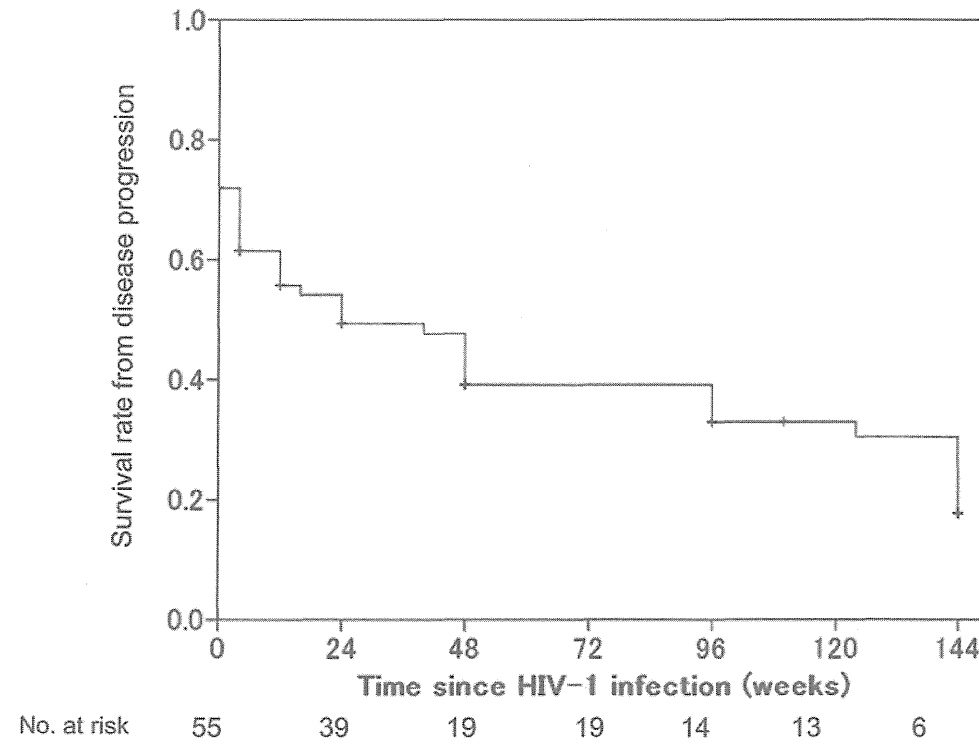

Figure 2. **Progression-free survival in 82 patients. Progression was defined as CD4 count <350/μL or initiation of HAART. No. at risk: the number of CD4 count >350/μL or HAART naïve patients**

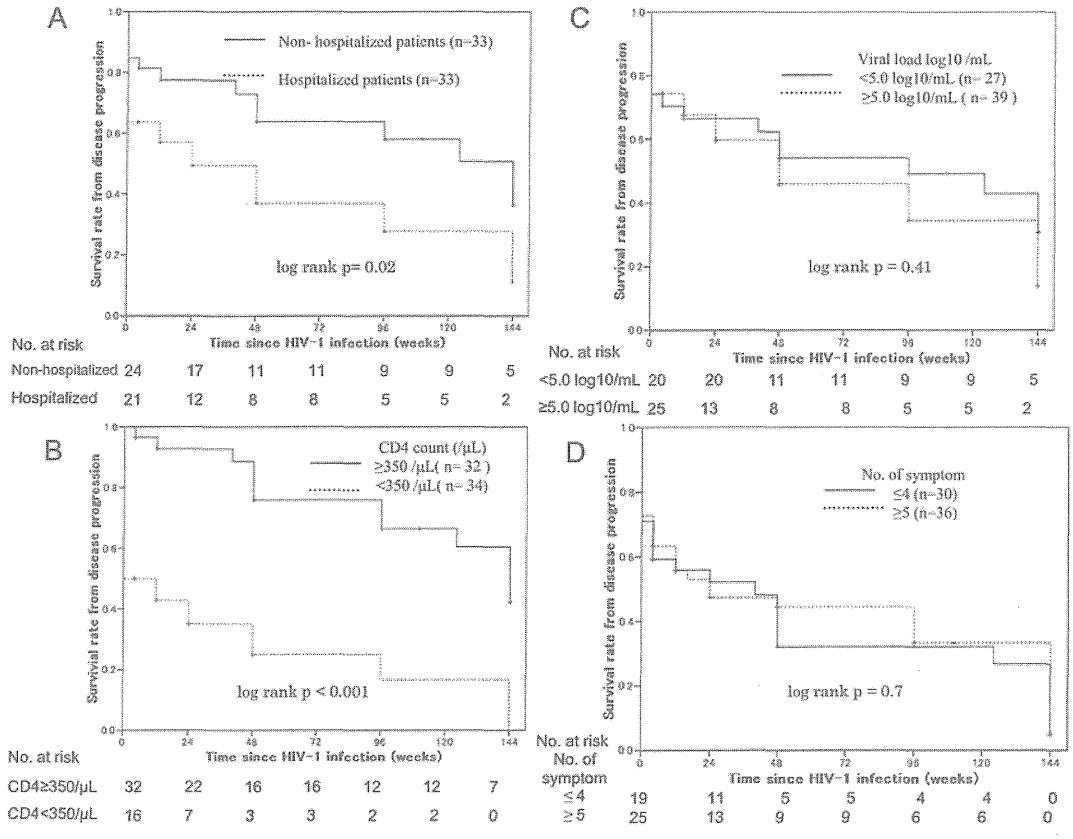

Figure 3. Progression-free survival among 66 patients according to rate of hospitalization, baseline CD4 count, and viral load. No. at risk: the number of CD4 count >350/μL or HAART naïve patients. A; Solid line: patients who required hospitalization due to PHI, dashed line: patients who did not require hospitalization (p=0.02, by log-rank test). B; Solid line: patients with CD4 count >350/μL at first visit, dashed line: patients with CD4 count <350/μL (p<0.001). C; Solid line: patients with viral load <5.0 log_{10}/mL, dashed line: patients with viral load ≥5.0 log_{10}/mL (p=0.41). Disease progression was defined as CD4 count <350/μL or initiation of HAART. D; Solid line: patients with the number of PHI symptoms ≤4, dashed line: patients with the number of PHI symptoms ≥5 (p=0.7, by log-rank test).

Comparison of percentage of recently infected patients with CD4 counts >350/μL at 3 years after infection and that of hemophiliacs as the first HIV-1 infected population in Japanese is shown in Fig. 4. The percentage (13.5%) of recently infected patients was significantly lower than that (47.6%) of Japanese hemophiliacs (p<0.001), clearly indicating the rapid decline of CD4 count in recently infected patients.

Discussion

In this study, we demonstrated rapid disease progression of symptomatic PHI Japanese patients in this decade. However, when we divided our study subjects into two groups according to the first half (1997-2002) and the latter half (2003-2007), disease progression of each group was not different (data not shown). In contrast, disease progression surrogated with natural CD4 decline of recently infected patients was significantly accelerated compared with Japanese hemophiliacs infected with HIV-1 before 1985. However, there are two quite different backgrounds; one is the route of infection and the other is the year of infection. Almost all hemophiliac patients are also co-infected with hepatitis C but do not have other sexually transmitted diseases (STDs). In contrast, most patients in the present study were infected via homosexual intercourse with many other STDs that may facilitate acceleration of the disease progression (7). In the present study, 69.7% patients had a past medical history of STDs, and the mean number of STDs was 1.08/patient (0: 31.3%, 1: 37.4%, 2: 23.2%, 3: 8.1%). In this regard, most published data on disease progression were obtained from men who have sex with men (MSM) cohorts (1, 2). Therefore, it is unlikely that the recent rapid disease progression is due to Japanese MSM. Whether or not the rapid disease progression in the recently HIV-1-infected Japanese can be generalized is to be elucidated in future studies.

Some HLA types are protective against disease progression such as HLA-B57 (19) and HLA-B51 (20) because HLA-restricted cytotoxic T lymphocytes (CTLs) play an important role on viral control. On the other hand, virus can easily escape from CTLs (17, 21). In some prevalent HLA types, escape virus can transmit and accumulate in the population (21). In this situation, some HLA types are no more

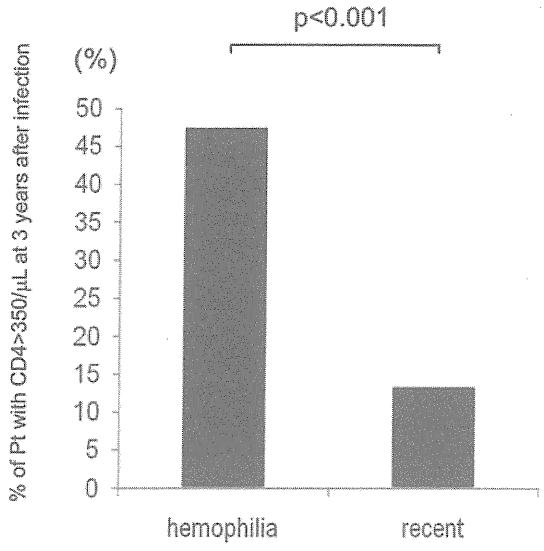

Figure 4. Comparison of percentage of previously and recently infected patients with CD4 counts >350/μL at 3 years after infection. In this analysis, Japanese hemophiliacs (designated "hemophilia" in the figure) were regarded as a previously infected patient, because they were infected with HIV-1 before 1985. The number of hemophiliacs was 42 patients. The eligible number of recently infected patients (designated "recent" in the figure) was 59 patients; infected with HIV-1 after 1997, untreated, and CD4 count at 3 years after infection.

protective. The HLA distribution is different in Americans compared to Japanese. Another possible hypothesis for the different disease progression is that Japanese hemophiliacs were exposed to HIV-1 through contaminated blood products imported from US as the first Japanese population infected with the virus around 1983. However, in recent years, most HIV-1 infection in Japanese is transmitted from Japanese patients. It can be postulated that current HIV-1 in Japan has adapted to the Japanese population, indicating acquisition and accumulation of escape virus from immune pressure of the otherwise protective HLA in Japanese population (21). From a negative point of view, the situation is similar to the epidemic of drug-resistance virus in treatment of naïve patients (22). The clinical relevance of the prevalence of immune escape virus in Japanese is a potentially serious matter in terms of the natural course of HIV-1 infection.

In the present study, all patients have had at least one symptom associated with PHI. During the follow-up period, no patient developed AIDS, whereas around 70% of the patients experienced immunologic progression as defined by a CD4 count <350/μL. It is noteworthy that the majority of these patients exhibited immunologic progression within 3 years and, surprisingly, >60% of them were documented within the first year. HAART was initiated in nearly 60% of patients during this period, including initiation for PHI-related severe symptoms in 20% of these patients. Previous studies on PHI have suggested that the number, duration, and/or severity of symptoms can predict faster disease progression to AIDS (23, 24). Our findings are compatible with these previous studies. Considered together, these results suggest that the duration of illness rather than the number of symptoms is more likely to be a major determinant of immunological progression. The estimated risks of disease progression were more than 50% by week 24 and 80% by week 144. Comparison with those observed elsewhere during the natural course of HIV-1 infection (24), these disease progression rates are surprisingly high. Among the patients with CD4 counts >350/μL at first visit, a quarter of them showed disease progression within 1 year. In contrast, in patients with CD4 count <350/μL, three quarters of them showed disease progression within the same period. Goujard et al (25) suggested possible recovery of CD4 count after the primary infection phase even in patients with very low count because it fluctuates during that period. In contrast, our results suggest that patients with a CD4 count of <350/μL during primary infection should be monitored carefully because spontaneous recovery of CD4 cell count during primary infection was rare. This cautionary remark could also apply to patients with a CD4 count of >350/μL because they exhibited nearly 60% risk of disease progression within 3 years. These observations may allow more targeted clinical monitoring and timely initiation of HAART. The impact of a short-term HAART during symptomatic primary infection on the subsequent disease progression needs to be elucidated in future study.

Although we included all recent seroconverters during the study period, it could be argued that this study carries some institution bias (i.e., a high proportion of cases with severe disease). However, the present finding of a surprisingly rapid disease progression in our patient population is new. Whether or not the natural course of disease progression has recently become accelerated in other countries or other cohorts is a matter of great interest.

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

Acknowledgement

The authors thank all clinical staffs of the AIDS Clinical Center. This study was supported in part by a Grant-in-Aid for AIDS Research from the Ministry of Health, Labour, and Welfare of Japan.

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INTERNAL MEDICINE

ORIGINAL ARTICLE

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

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Abstract

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

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

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

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

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

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

(Intern Med 50: 699-705, 2011)
(DOI: 10.2169/internalmedicine.50.4572)

Introduction

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

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

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

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

Objective

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

Subjects and Methods

Study design

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

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

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

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

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

Statistical analyses

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

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

Table 1. **Baseline Characteristics of Participants**

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

Results

Participants

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

Primary endpoint

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

Virologic success over time

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

Secondary endpoints

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

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

Discussion

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

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

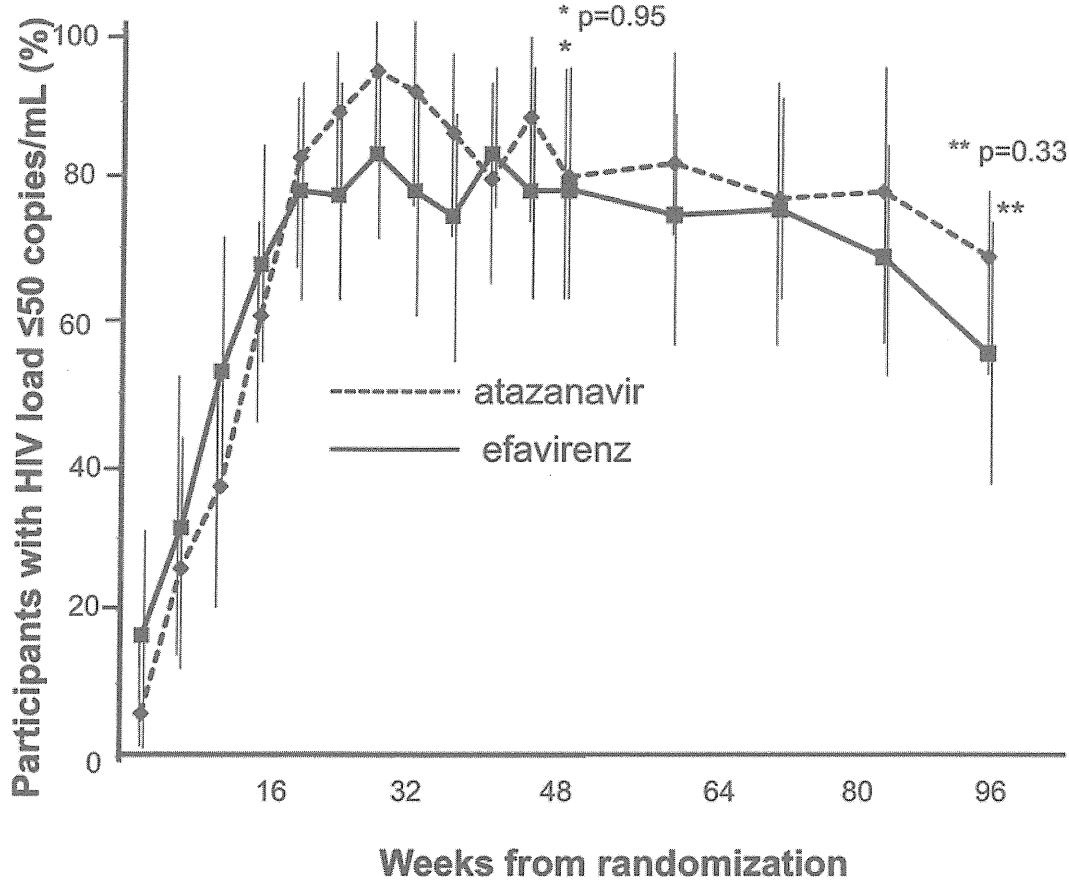

Figure 1. **Proportions of participants with HIV-RNA less than 50 copies/mL.**
The efficacies of the efavirenz arm and ritonavir-boosted atazanavir arm were compared with intent-to-treat analysis. There were no significant difference between arms at both week 48 (p=0.95) and week 96 (p=0.33).

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

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

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

Limitations of this study include a small sample size.

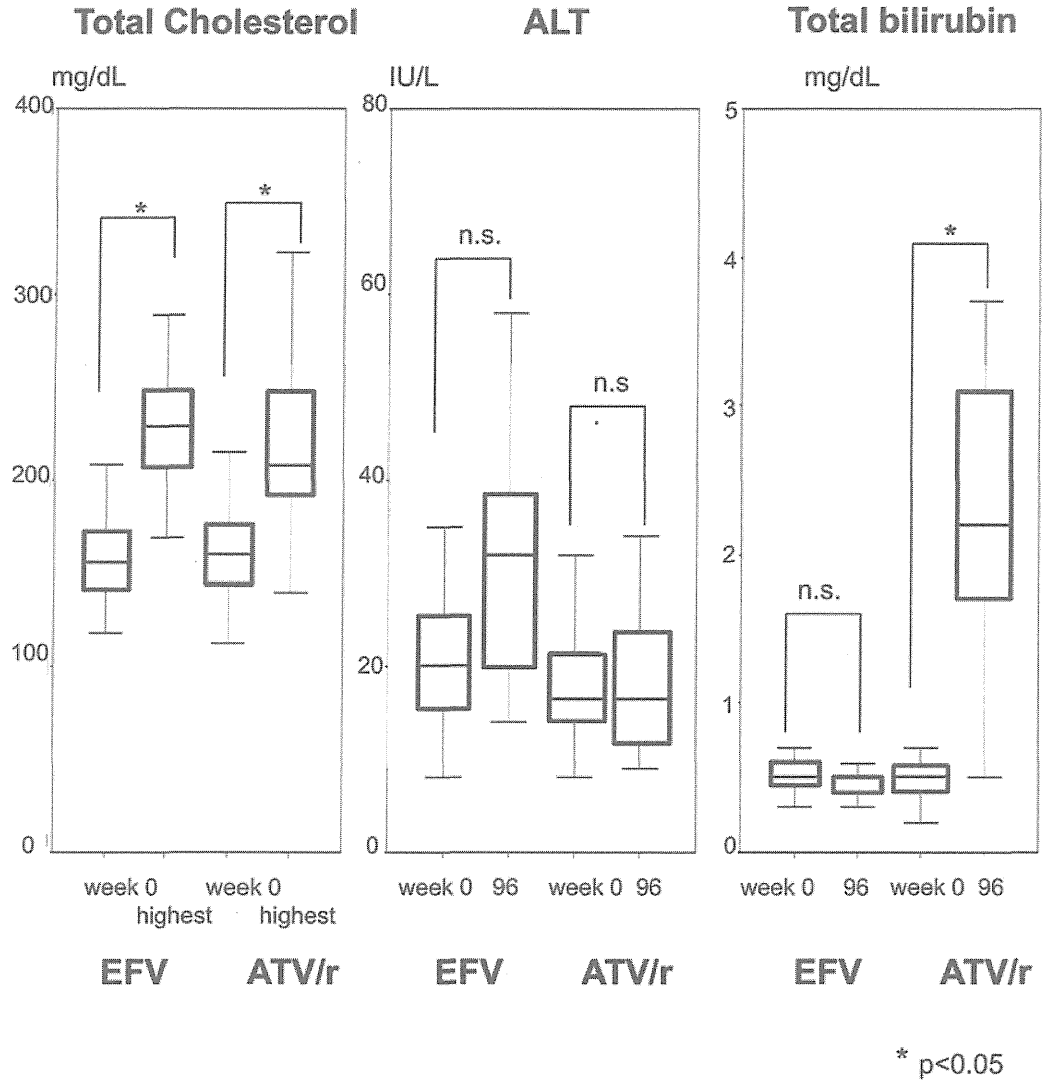

Figure 2. Changes from baseline in total cholesterol, ALT and total bilirubin.
ALT and total cholesterol at week 96 were compared with the baseline values. Since participants who developed hyperlipidemia were treated with lipid-lowering agents during the study period, the highest levels registered in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

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

Conclusion

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

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

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

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Acknowledgement

This study was supported in part by a grant from the Japanese Foundation for the Promotion of International Medical Research Cooperation and National Center for Global Health and Medicine.

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