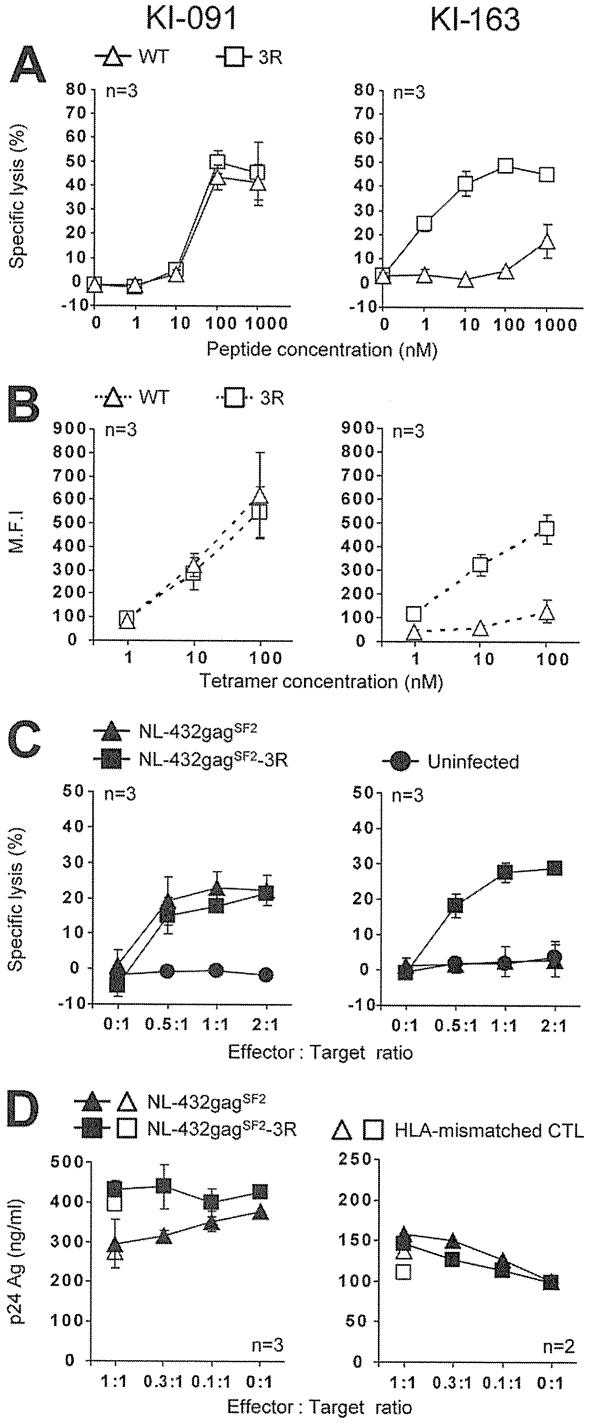

FIG 4 Antiviral activities of cross-reactive and 3R-specific CTL clones generated from patients KI-091 and KI-163 infected with 3R virus. Gag28-specific CTL clones were generated from chronic-phase PBMCs isolated from patients KI-091 and KI-163 after their stimulation with 3R peptide. The following activities of these CTL clones were analyzed. (A) Cytotoxic activity against 721.221-CD4-A2402 cells prepulsed with the WT or 3R peptide at concentrations of 1 to 1,000 nM. The cytotoxic activity was measured at an E:T ratio of 1:1. (B) Binding affinity toward WT and 3R tetramers at concentrations of 1 to 100 nM. The MFIs of the T cell clones are shown. (C) Cytotoxic activity against 721.221-CD4-A2402 cells infected with WT virus or 3R virus. WT-virus-infected and 3R virus-infected cells were used as target cells. The frequency of p24 Ag$^+$ cells among the HIV-1-infected cells was as follows: WT-virus-infected cells, 49.1% and 43.1% for CTL clones from KI-091 and KI-163, respectively, and 3R-virus-infected cells, 48.6% and 45.6% for CTL clones from KI-091 and KI-163, respectively. The cytotoxic activity was measured at E:T ratios of 0.5:1, 1:1, and 2:1. (D) Abilities of the clones to suppress the replication of WT or 3R virus. The abilities were tested at different E:T ratios. n, number of clones tested. The error bars indicate standard deviations.

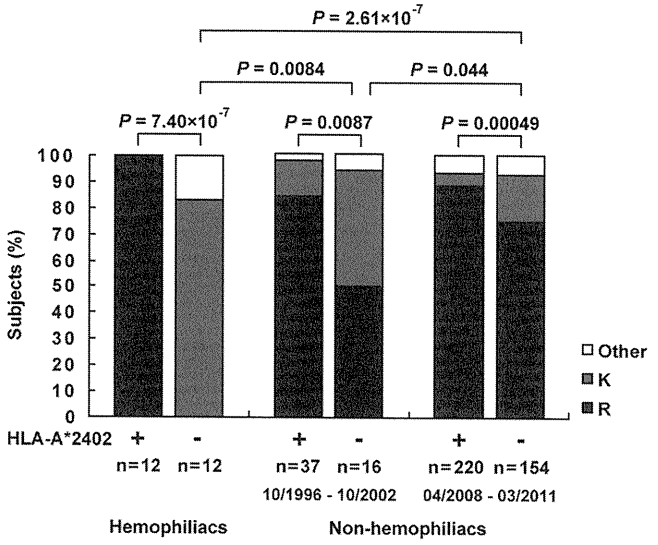

FIG 5 Frequencies of the 3R mutation in a Japanese hemophiliac cohort and nonhemophiliac cohorts recruited from 1996 to 2002 and from 2008 to 2011. The frequencies of mutations at position 3 of the Gag28 epitope in chronically HIV-1-infected HLA-A*24:02$^+$ or HLA-A*24:02$^-$ hemophiliac individuals and nonhemophiliac individuals recruited from 1996 to 2002 or from 2008 to 2011 are shown. The consensus sequence of this epitope in HIV-1 subtype B is KYKLKHIVW. The frequency of the 3R mutation between HLA-A*24:02$^+$ and HLA-A*24:02$^-$ subjects in each cohort or that in HLA-A*24:02$^+$ or HLA-A*24:02$^-$ subjects among the 3 cohorts was statistically analyzed by using Fisher's exact test.

CTL clones established from KI-091 did not have the ability to suppress the replication of the WT virus, although the CTL clones from individuals who had been infected with the WT virus had strong ability to suppress it. These findings suggest that this patient had been infected with the 3R virus rather than with the WT virus. However, it remains unknown why 3R-specific CTLs were elicited in the other 4 individuals but not in this patient. Thus, the abilities of CTLs to respond to WT peptide and to suppress the replication of WT virus together supported the idea that the individuals who had 3R virus in the early phase had been infected with 3R virus, although the possibility that they had been infected with WT virus cannot be completely excluded.

The 3R mutant epitope peptide would have been processed and presented to 3R-specific CTLs in 3R virus-infected cells, since 3R-specific and cross-reactive CTL clones effectively killed 3R virus-infected cells. However, these CTL clones failed to suppress the replication of the 3R virus. 721.221-CD4-A2402 cell lines were used as target cells for the killing assay, whereas CD4$^+$ T cells from healthy individuals were used for the replication suppression assay. The former cells express HLA-A*24:02 to a much higher degree than the latter cells. This difference between the 2 cell lines may account for the discrepancy of the results between the 2 assays. 3R-specific CTL clones failed to suppress the replication of the 3R virus, whereas cross-reactive CTLs from the individuals

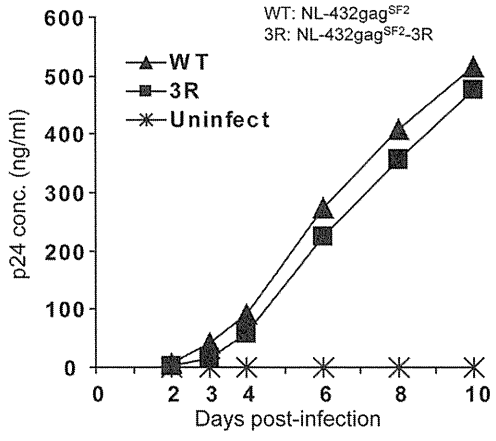

FIG 6 Replication kinetics of WT and 3R viruses in CD4$^+$ T cells. CD4$^+$ T cells (2×10^5) isolated from PBMCs from a healthy donor were infected with WT or 3R virus in triplicate at a blue-cell-forming unit of 500 (in MAGIC-5 cells) in a total volume of 0.2 ml and then incubated at 37°C for 2 h. The infected cells were washed twice with R10 and then cultured in 1 ml of complete medium plus rIL-2 at 37°C. A 0.1-ml volume of the culture supernatants was collected at days 2 to 10 postinfection. The concentration of p24 Ag was measured by using ELISA.

infected with WT virus effectively suppressed the replication of the WT virus but failed to suppress that of the 3R virus. These findings suggest that 3R virus-infected CD4$^+$ T cells could not effectively present the 3R mutant epitope. This finding also suggests that 3R virus-infected CD4$^+$ T cells were not the main source of antigen-presenting cells in 3R virus-infected individuals. A previous study showed that HIV-1-infected macrophages effectively present HIV-1 epitopes more than HIV-1-infected CD4$^+$ T cells (14), implying that 3R virus-infected macrophages are the main antigen-presenting cells and contribute to the elicitation of 3R-specific and cross-reactive CTLs in 3R virus-infected individuals. A further study should clarify the role of macrophages in the elicitation of 3R-specific and cross-reactive CTLs in 3R virus-infected individuals.

Cross-reactive CTLs were found in individuals infected with the WT virus or with the 3R virus. The CTL clones established from individuals infected with the WT virus had a strong ability to kill WT-virus-infected cells and to suppress the replication of the WT virus, whereas those established from an individual infected with the 3R virus showed moderate ability to kill WT-virus-infected cells and no ability to suppress the replication of WT virus. These findings indicate that cross-reactive CTLs from an individual infected with the 3R virus may have had less ability to recognize the WT epitope than those from an individual infected with the WT virus. Indeed, the former CTL clones exhibited lower sensitivity to reaction with WT peptide-pulsed cells than the latter CTLs, indicating that cross-reactive CTLs elicited in individuals infected with the WT virus had higher-affinity TCRs for WT peptide than those in an individual infected with the 3R virus. In addition, the latter CTL clones weakly killed 3R virus-infected cells, whereas the former clones showed the same killing activity against 3R virus-infected cells as against WT-virus-infected cells. Thus, cross-reactive CTLs in individuals infected with 3R virus have different characteristics than those in individuals infected with the WT virus. This finding suggests that cross-reactive CTLs elicited in individuals infected with the WT virus had TCRs with higher affinity for WT and 3R peptides than those in individuals infected with the 3R virus.

Japanese hemophiliacs were infected with HIV-1 via blood products from the United States around 1983, and HLA-A*24:02 is a rare allele in North America. Therefore, it may be speculated that HIV-1 in the blood product had not yet accumulated escape mutations. Indeed, the 3R mutation was not found in the 12 HLA-A*24:02$^-$ hemophiliacs tested, though other amino acid variants at position 3 were detected in 2 of these hemophiliacs. This mutation was found in 50.0% of HLA-A*24:02$^-$ individuals in the 1996 to 2002 cohort and in 74.7% of those in the 2008 to 2011 cohort, indicating that the mutation had accumulated in the Japanese population. The frequency of this mutation in HLA-A*24:02$^-$ individuals thus increased about 1.5-fold during the approximately 10-year period between these 2 nonhemophiliac cohorts. Thus, the mutation greatly accumulated over the last 10 years. Since HLA-A*24:02 is found in approximately 70% of Japanese, the high prevalence of the allele is the cause of the high accumulation of the 3R mutation in the Japanese population. In addition, this high accumulation resulted not only from a strong selection of the 3R mutation by WT-specific and cross-reactive CTLs elicited in the donors infected with WT virus, but also from a lack of reversion of the mutation in the HLA-A*24:02$^-$ individuals.

Our previous study concerning HLA-A*24:02-restricted Nef138-specific CTLs demonstrated that only WT epitope-dominant CTLs, which suppress the replication of WT virus but fail to suppress that of mutant virus, are elicited at an early phase in HLA-A*24:02$^+$ individuals infected with the WT virus and that mutant-epitope-dominant CTLs but not cross-reactive CTLs are elicited after the emergence of the mutant virus in them (15). In addition, only mutant-epitope-dominant CTLs are elicited in those individuals infected with the mutant virus. The mutant-epitope-dominant CTLs suppress the replication of WT virus but weakly suppress that of mutant virus (15). Thus, Nef138-specific CTLs elicited in individuals infected with WT or mutant viruses had different characteristics in terms of the recognition of WT and mutant epitopes than the Gag28-specific CTLs analyzed in the present study. The difference between Nef138-specific and Gag28-specific CTLs might be explained by a different CTL repertoire elicited at an early phase. These 2 studies suggest the elicitation of various HIV-1-specific CTLs in regard to recognition of escape mutations.

In the present study, we demonstrated that WT-specific and cross-reactive CTLs were elicited at an early phase in individuals infected with the WT virus and that cross-reactive CTLs were dominant in Gag28-specific CTLs after the emergence of the 3R virus. On the other hand, 3R-specific and cross-reactive CTLs were elicited in individuals infected with the 3R virus, though the former CTLs were predominantly elicited in these individuals. The CTLs elicited in the individuals infected with the WT virus, which had a strong ability to suppress the replication of WT virus, played a central role in the accumulation of the 3R mutation. In contrast, the CTLs elicited in those infected with 3R virus, which failed to suppress the replication of WT and 3R viruses, did not contribute to the control of the 3R virus infection. In addition, the high prevalence of HLA-A*24:02 and lack of effect of the 3R mutation on viral fitness may have strongly contributed to the high accumulation of the mutation in HIV-1-infeceted Japanese individuals.

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Trends in early and late diagnosis of HIV-1 infections in Tokyoites from 2002 to 2010

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SUMMARY

Objective: The objective of this study was to delineate the trends in early and late diagnosis of HIV-1 infection in newly diagnosed Tokyoites.
Methods: The BED assay was used to identify cases diagnosed at an early stage of infection. BED-positive non-AIDS cases with a CD4 cell count ≥200/μl were defined as cases with recent infection. The rates of AIDS and recent infection in 809 newly diagnosed Tokyoites during 2002–2010 were analyzed.
Results: The AIDS rate was 22.5%. AIDS patients were older (40.4 years) than non-AIDS patients (35.0 years), and a smaller proportion were men who have sex with men (MSM) in AIDS patients (81.7%) than in non-AIDS patients (89.9%). The AIDS rate was persistently lower (≤14.3%) in ≤29-year-old than in ≥30-year-old MSM. The rate of recent infection was 24.4%. Individuals with recent infection (33.0 years old) were younger than the others (37.2 years). The rate of recent infection was lower (≤18.5%) in MSM aged ≥40 years than in those aged ≤39 years during the study period, except for 2007 and 2008.
Conclusions: Younger MSM Tokyoites appear to be aware of the risk of their sexual behavior, sufficient to take voluntary HIV testing repeatedly, resulting in early diagnosis. Older MSM did not take HIV testing frequently enough and may be a good target for campaigns promoting testing.

1. Introduction

The overall growth of the global AIDS epidemic appears to have stabilized. The annual number of new cases of HIV infection has been in steady decline since the late 1990s.[1] In Japan, however, the annual number of newly diagnosed cases has almost doubled during the most recent decade (791 cases in 2000 and 1544 cases in 2010), although the prevalence of HIV in the adult population remains <0.1%.[2] The distribution of these cases is heavily concentrated in large cities, and approximately 35% of the newly diagnosed cases have been identified in Tokyo.[3]

Early diagnosis of HIV infection is critically important because some AIDS-defining diseases are fatal, even in the era of combination antiretroviral treatment (ART); also the introduction of ART after the development of AIDS is often complicated with immune reconstitution inflammatory syndrome (IRIS).[4,5] In this regard, the introduction of ART at the early stages seems to significantly reduce the sexual transmission of HIV-1.[6,7] Thus, it is important to identify newly infected individuals and provide early ART to reduce the incidence of AIDS and transmission of HIV. Knowledge about the proportion of patients diagnosed at the early stage of an HIV infection in the newly diagnosed cases is also useful for planning and evaluation of any prevention program and for resource allocation.[8,9] However, it is usually difficult to distinguish recent from long-standing HIV infections except for acute symptomatic infections.[10] Simple prediction of the infection time from CD4 cell counts appears inaccurate because the disease progression rate varies enormously among infected individuals.[11] The BED HIV-1 capture enzyme immunoassay (BED assay) uses the branched peptide to detect HIV-1 IgG antibodies from all subtypes (i.e., HIV-1 B, E, and D gp41 immunodominant sequences are included on a branched peptide used in the assay) and measures levels of anti-HIV-1 IgG relative to total IgG.[12] Since the ratio of anti-HIV-1 IgG to total IgG increases with time shortly after HIV-1 infection, the HIV-1-infected patient is considered to have recently acquired the infection when the normalized optical density (ODn) is less than 0.8 on the BED assay (ODn reaches 0.8 on average 197 days after seroconversion.).[13]

The present study was an attempt to delineate the trends in early diagnosis of HIV-1 infection in Tokyo from 2002 to 2010 by using the BED assay. The aim of this analysis was to enhance our understanding of the status of HIV-1 spread in Tokyo and to help in the design of strategies to control the HIV-1 epidemic in Japan.

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2. Materials and methods

2.1. Newly diagnosed patients

This study included all ART-naïve HIV-1-infected individuals who met the following criteria: (1) those who visited the AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, between 2002 and 2010 within 30 days of their diagnosis with an HIV-1 infection and (2) availability of plasma samples taken at the first visit under signed informed consent for use in viral, immunological, and epidemiological studies. Participant information including CD4 count, HIV-1 load, age at the first visit, gender, nationality, probable HIV-1 transmission route, and history of HIV testing, were collected from the medical records. According to the Japanese law for infection control, physicians are obliged to report newly diagnosed HIV/AIDS cases to the National AIDS Surveillance Committee (the Ministry of Health, Labor, and Welfare of the Japanese Government). A total of 11 673 HIV/AIDS cases nationally, including 4048 cases diagnosed in Tokyo (Tokyo cases), which were entered into the registry of this committee from 2002 to 2010, were used as the control populations to evaluate the representativeness of the patients enrolled in the present study (AIDS Clinical Center cases).[2,3] Plasma samples obtained from the participants were stored at −80 °C. The viral subtype in each case was determined from the HIV-1 protease–reverse transcriptase sequence (which was analyzed for drug resistance genotyping) by the neighbor-joining method using the Genetic-Win system (Software Development, Tokyo).[14]

This study was conducted according to the principles of the Declaration of Helsinki and was approved by the ethics committee of the National Center for Global Health and Medicine.

2.2. BED assay

The BED HIV-1 capture enzyme immunoassay (BED assay; Calypte Biomedical Corp., Portland, OR, USA) was used to estimate the time of HIV-1 infection.[12] In accordance with the manufacturer's instructions, 5 μl of plasma was diluted with 500 μl of the diluent in the kit, and the proportion of anti-HIV-1-specific IgG to the total IgG in the sample was measured by optical density (OD). The OD values of the test specimens were normalized (ODn) relative to the value of a calibrator (specimen OD/calibrator OD) to minimize inter-run variation. Samples with ODn ≤0.8 were considered to be from individuals who had seroconverted within 197 days and were defined as BED-positive.[13] BED-positive non-AIDS cases with CD4 cell counts ≥200/μl were defined as individuals with recent infection. The others were defined as chronic infection.

2.3. Statistical analysis

Differences in demographic data including age, gender, risk behavior, nationality, and AIDS development among the AIDS Clinical Center cases, national cases, and Tokyo cases, were examined for significance using one-way analysis of variance (ANOVA) and the Tukey test, or Pearson's Chi-square test. Differences in demographic data including age, CD4 count, logarithmic HIV-1 viral load, nationality, transmission category, HIV-1 subtype, cue for HIV diagnosis, and history of HIV testing, between AIDS and non-AIDS patients and between recent and chronic infection, were examined for significance using the *t*-test or Pearson's Chi-square test. To estimate the correlation with the development of AIDS, binominal logistic regression analysis including age, nationality (Japanese or not), and transmission category (men having sex with men (MSM) or not) was performed. A *p*-value of less than 5% denoted statistical significance. Statistical analyses were performed with SPSS Statistics 17.0 (IBM Japan Inc., Tokyo, Japan) and Stat Mate II (NANKODO, Tokyo).

3. Results

3.1. Newly diagnosed cases of HIV-1 infection

The study subjects were 809 ART-naïve HIV-1-infected patients. All of them had visited the AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, within 30 days of the diagnosis of HIV-1 infection (median 8 days) between 2002 and 2010. They included 741 Japanese, 35 Asians other than Japanese, and 33 from other countries. They represented 20.0% of the total number of newly diagnosed Tokyoite cases during the same period (Table 1). There were no significant differences in the proportion of AIDS (22.5% vs. 21.9%), percentage of males (96.2% vs. 94.3%), or proportion of Japanese (91.6% vs. 90.7%) between our study patients and those of the Tokyo registry, although our patients included a significantly smaller proportion of AIDS cases (22.5% vs. 30.4%) and significantly larger population of male patients (96.2% vs. 91.8%) and Japanese patients (91.6% vs. 88.5%) compared with the patients of the national registry. Furthermore, our patients were significantly younger than the patients of the Tokyo and national registries (36.2 vs. 37.7 and 38.0 years), and the proportion of MSM among male patients was significantly higher than in the Tokyo and national registries (88.0% vs. 72.8% and 59.8%).

Subtype analysis successfully determined the HIV-1 subtype in 807 patients (99.8%); the majority were infected with HIV-1 subtype B (742 patients, 91.9%), while 5.7% were infected with HIV-1 subtype AE, which is comparable to previously published subtype data in Japan.[14] The HIV-1 subtype could not be determined in two patients because the viral load was below the detection limit (<40 copies/ml), although they were not being treated with anti-HIV drugs.

3.2. Features of AIDS patients

Among the 809 cases, 182 (22.5%, 95% confidence interval (95% CI) 19.6–25.4) had already developed AIDS at the first visit, while the other 627 were non-AIDS cases (Table 2). AIDS cases were significantly older (40.4 years, 95% CI 38.8–41.9 vs. 35.0 years, 95% CI 34.2–35.9), and as expected, had lower CD4 counts (61.7/μl, 95% CI 50.6–72.8 vs. 318.0/μl, 95% CI 303.0–333.0) and higher viral loads (5.22 log VL/ml, 95% CI 5.13–5.31 vs. 4.63 log VL/ml, 95% CI 4.56–4.70) than non-AIDS patients. There were no significant differences in nationality (Japanese 91.8%, 95% CI 87.8–95.8 vs. 91.5%, 95% CI 89.4–93.7) or HIV-1 subtype (subtype B 89.0%, 95% CI 84.5–93.6 vs. 92.5%, 95% CI 90.4–94.6) between AIDS and non-AIDS

Table 1
New cases of HIV-1-infected patients diagnosed between 2002 and 2010

	Japan[a]	Tokyo[b]	This study
Number of cases	11 673	4048	809
Age, years (mean ± SD)	38.0 ± 11.8[c]	37.7 ± 11.9[d]	36.2 ± 11.0
Males	10 721 (91.8%)[c]	3819 (94.3%)	778 (96.2%)
Men having sex with men	6408 (59.8%)[c]	2780 (72.8%)[c]	685 (88.0%)
Japanese	10 335 (88.5%)[d]	3673 (90.7%)	741 (91.6%)
AIDS cases	3551 (30.4%)[c]	885 (21.9%)	182 (22.5%)

Statistical analyses were performed by one-way ANOVA and Tukey test, or Chi-square test.

[a] Provided by the National AIDS Surveillance Committee (the Ministry of Health, Labor, and Welfare of the Japanese Government).

[b] Provided by the Bureau of Social Welfare and Public Health, Tokyo.

[c] $p < 0.001$, compared with the study participants.

[d] $p < 0.01$ compared with the study participants.

Table 2
Demographics of participants with and without AIDS

	AIDS (n = 182)		Non-AIDS (n = 627)		p-Value[a]
	Mean	(95% CI)	Mean	(95% CI)	
Age (years)	40.4	(38.8–41.9)	35.0	(34.2–35.9)	<0.001
CD4 count /μl	61.7	(50.6–72.8)	318.0	(303.0–333.0)	<0.001
Log viral load/ml	5.22	(5.13–5.31)	4.63	(4.56–4.70)	<0.001
	n	% (95% CI)	n	% (95% CI)	
Nationality					0.424
Japan	167	91.8 (87.8–95.8)	574	91.5 (89.4–93.7)	
Asia other than Japan	11	6.0 (3.3–10.8)	24	3.8 (2.6–5.7)	
North and South America	2	1.1 (0.2–4.0)	17	2.7 (1.7–4.3)	
Africa	2	1.1 (0.2–4.0)	6	1.0 (0.4–2.1)	
East and West Europe	0	0 (0–2.0)	4	0.6 (0.2–1.6)	
Oceania	0	0 (0–2.0)	2	0.3 (0–1.1)	
Transmission category					
Male	175	96.2 (93.4–98.9)	603	96.2 (94.7–97.7)	0.024
MSM	143	81.7 (76.0–87.4)	542	89.9 (87.5–92.3)	
Heterosexual	21	12.0 (7.2–16.8)	43	7.1 (5.4–9.6)	
IDU	1	0.6 (0–3.2)	2	0.3 (0.1–1.2)	
Unknown	10	5.7 (3.0–10.5)	16	2.7 (1.6–4.3)	
Female	7	3.8 (1.7–7.9)	24	3.8 (2.6–5.7)	-
Heterosexual	7	100 (46.8–100)	24	100 (100–100)	
Subtype					0.351
B	162	89.0 (84.5–93.6)	580	92.5 (90.4–94.6)	
AE	16	8.8 (5.4–14.3)	30	4.8 (3.4–6.8)	
C	1	0.5 (0–3.0)	7	1.1 (0.5–2.3)	
G	2	1.1 (0.2–4.0)	3	0.5 (0.1–1.4)	
AG	1	0.5 (0–3.0)	3	0.5 (0.1–1.4)	
A	0	0 (0–2.0)	2	0.3 (0–1.1)	
Unknown	0	0 (0–2.0)	2	0.3 (0–1.1)	
Cue for HIV diagnosis					<0.001
Voluntary testing	12	6.6 (3.7–11.5)	283	45.1 (41.2–49.0)	
Provider-initiated testing	167	91.8 (87.8–95.8)	338	53.9 (50.0–57.8)	
Unknown	3	1.6 (0.4–4.8)	6	1.0 (0.4–2.1)	
Previous testing					<0.001
Yes	29	15.9 (10.6–21.3)	282	45.0 (41.1–48.9)	
No	65	35.7 (28.8–42.7)	254	40.5 (36.7–44.4)	
Unknown	88	48.4 (41.1–55.6)	91	14.5 (11.8–17.3)	
BED assay					<0.001
Recent (ODn ≤0.8)	47	25.8 (19.5–32.2)	255	40.7 (36.8–44.5)	
Chronic (ODn >0.8)	135	74.2 (67.8–80.5)	372	59.3 (55.5–63.2)	

CI, confidence interval; MSM, men who have sex with men; IDU, intravenous drug user; ODn, normalized optical density.
[a] By t-test or Pearson's Chi-square test.

cases (Pearson's Chi-square test). MSM activity was the most frequent transmission route in both groups, and still more frequent in non-AIDS cases (89.9%, 95% CI 87.5–92.3) than in AIDS cases (81.7%, 95% CI 76.0–87.4). A larger proportion of patients in the non-AIDS group than in the AIDS group had undertaken previous HIV testing (45.0%, 95% CI 41.1–48.9 vs. 15.9%, 95% CI 10.6–21.3) and had been diagnosed with HIV-1 infection by voluntary testing (45.1%, 95% CI 41.2–49.0 vs. 6.6%, 95% CI 3.7–11.5), suggesting that repeated voluntary testing may prevent disease progression to AIDS in the high-risk groups.

Binominal logistic regression analysis of age, nationality (Japanese or not), and transmission category (MSM or not) identified age as the most significant factor associated with the development of AIDS (per 1-year increment, (hazard ratio) HR 1.041, 95% CI 1.026–1.057; $p < 0.001$).

To delineate the trends in late diagnosis of HIV-1 infection, the annual rates of AIDS cases in newly-diagnosed HIV-1-infected patients were plotted through the study period. The rate of AIDS cases remained around 30% between 2002 and 2004. It decreased to 15.0% in 2005, but then showed a gradual increase annually, reaching 24.8% in 2010 (Figure 1). To identify the population that influenced the increase in the rate of AIDS cases in the most recent years, we selected and categorized the study participants based on their features. Specifically, we focused on MSM patients, because 85% of our patients were MSM. Based on the above results of the significance of age in the binominal logistic regression analysis in the development of AIDS, we examined the effect of age in more detail by dividing the MSM patients into three age groups: those aged ≤29 years (217 patients, 31.7%), 30–39 years (273 patients, 39.9%), and ≥40 years (195 patients, 28.5%). In the ≥40 years MSM group, the rate was higher than 50% between 2002 and 2004, but decreased to 21.4% in 2005 and further decreased to 14.3% in 2006, but gradually increased and reached ~30% in 2009 and 2010 (Figure 1). On the other hand, in the ≤29 years MSM group, the AIDS rate was steadily lower than 20%, indicating that most young HIV-1-infected MSM were diagnosed before the development of AIDS throughout the study period. The AIDS rate in the 30–39 years MSM group was between those of the other two groups during most of the study period. A significantly larger proportion of patients in the ≤29 years MSM group had undergone voluntary HIV testing (43.8%, $p = 0.002$, Pearson's Chi-square test) and diagnosis with HIV (48.8%, $p < 0.001$, Pearson's Chi-square test), compared with the 30–39 years MSM group (43.6% and 36.6%, respectively) and the ≥40 years MSM group (34.9% and 32.3%, respectively). These results suggest that repeated voluntary testing may have prevented disease progression to AIDS in the younger MSM groups. The high rate of AIDS in all the study participants observed in 2002–2004 seemed mainly due to the ≥40-year-old MSM. Furthermore, the gradual increase in the AIDS rate in the ≥40-year-old MSM since 2006 also seemed to have contributed to

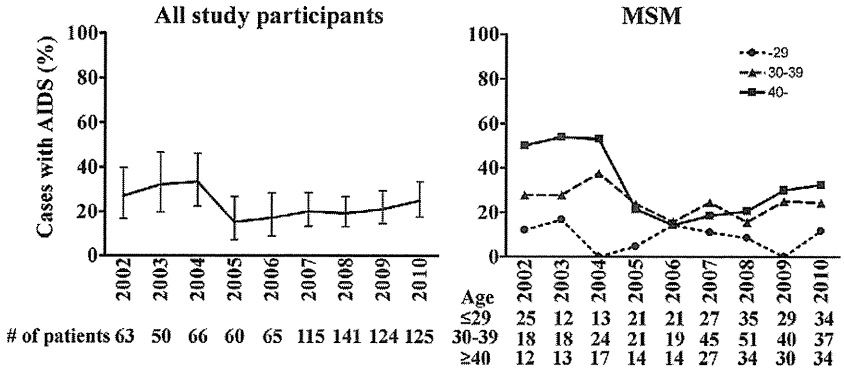

Figure 1. Annual rate of AIDS in newly diagnosed HIV-1-infected individuals. The annual AIDS rate for all study participants (809 patients; left panel), and men who have sex with men (MSM) categorized by age: ≤29 years (n = 217), 30–39 years (n = 273), and ≥40 years (n = 195) (right panel). The 95% confidence intervals are also shown in the left panel. Data including 95% confidence intervals for the MSM are provided in the **Supplementary Information** (Table S1).

the rising AIDS rate in all, suggesting that older MSM should be the main target for interventions aimed at promoting HIV testing for early diagnosis and prevention of the development of AIDS.

3.3. Trends in early HIV diagnosis

To identify individuals with recent HIV-1 infection, we performed a BED assay for the 809 study participants. Before analysis of the results, we dealt with the problem of potential misclassification. Previous studies reported small levels of anti-HIV-1-specific IgG relative to the total IgG in cases with both recent HIV-1 infection and long-standing chronic cases with severe immunodeficiency, which could result in false classification of chronic cases as recent infection.[12,15,16] To tackle this problem, previous studies classified AIDS cases and cases with CD4 cell counts <200/μl as chronic infection cases, in accordance with the Joint United Nations Programme on HIV/AIDS (UNAIDS)/World Health Organization (WHO) guidelines.[17–21] We applied

Table 3
Demographics of participants with recent and chronic infection

	Recent (n = 197)		Chronic (n = 612)		p-Value[a]
	Mean	(95% CI)	Mean	(95% CI)	
Age (years)	33.0	(31.7–34.3)	37.2	(36.3–38.1)	<0.001
CD4 count /μl	423.2	(399.2–447.3)	207.9	(193.3–222.4)	<0.001
Log viral load/ml	4.61	(4.46–4.76)	4.81	(4.74–4.87)	0.005
	n	% (95% CI)	n	% (95% CI)	
Nationality					0.101
Japan	189	95.9 (93.2–98.7)	552	90.2 (87.8–92.6)	
Asia other than Japan	2	1.0 (0.2–3.7)	33	5.4 (3.9–7.6)	
North and South America	3	1.5 (0.4–4.4)	16	2.6 (1.6–4.2)	
Africa	1	0.5 (0–2.8)	7	1.1 (0.5–2.4)	
East and West Europe	1	0.5 (0–2.8)	3	0.5 (0.1–1.4)	
Oceania	1	0.5 (0–2.8)	1	0.2 (0–0.9)	
Transmission category					
Male	192	97.5 (95.3–99.7)	586	95.8 (94.2–97.3)	0.314
MSM	177	92.2 (88.4–96.0)	508	86.7 (83.9–89.4)	
Heterosexual	11	5.7 (3.1–10.2)	53	9.0 (7.0–11.8)	
IDU	0	0 (0–1.9)	3	0.5 (0.1–1.5)	
Unknown	4	2.1 (0.7–5.3)	22	3.8 (2.5–5.7)	
Female	5	2.5 (1.0–5.9)	26	4.2 (2.9–6.2)	-
Heterosexual	5	100 (34.4–100)	26	100 (81.5–100)	
Subtype					0.029
B	188	95.4 (92.5–98.3)	554	90.5 (88.2–92.8)	
AE	4	2.0 (0.7–5.2)	42	6.9 (5.2–9.3)	
C	1	0.5 (0–2.8)	7	1.1 (0.5–2.4)	
G	1	0.5 (0–2.8)	4	0.7 (0.2–1.7)	
AG	1	0.5 (0–2.8)	3	0.5 (0.1–1.4)	
A	0	0 (0–1.9)	2	0.3 (0–1.2)	
Unknown	2	1.0 (0.2–3.7)	0	0 (0–0.6)	
Cue for HIV diagnosis					<0.001
Voluntary testing	102	51.8 (44.8–58.8)	193	31.5 (27.9–35.2)	
Provider-initiated testing	94	47.7 (40.7–54.7)	411	67.2 (63.4–70.9)	
Unknown	1	0.5 (0–2.8)	8	1.3 (0.6–2.6)	
Previous testing					<0.001
Yes	116	58.9 (52.0–65.8)	195	31.9 (28.2–35.6)	
No	57	28.9 (22.6–35.3)	262	42.8 (38.9–46.7)	
Unknown	24	12.2 (7.6–16.8)	155	25.3 (21.9–28.8)	

CI, confidence interval; MSM, men who have sex with men; IDU, intravenous drug user.
[a] By t-test or Pearson's Chi-square test.

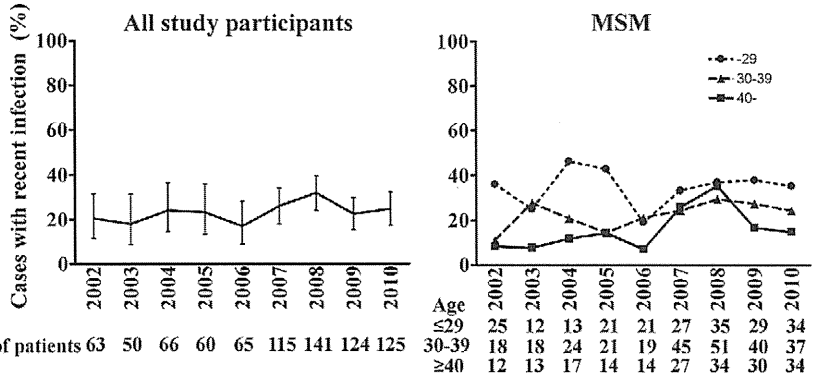

Figure 2. Annual rate of recent infection in newly diagnosed HIV-1-infected cases. The annual rate of recent infection in all study participants (809 patients; left panel), and in men who have sex with men (MSM) categorized by age: ≤29 years (n = 217), 30–39 years (n = 273), and ≥40 years (n = 195) (right panel). The 95% confidence intervals are also shown in the left panel. Data including 95% confidence intervals for the MSM are provided in the **Supplementary Information** (Table S2).

the same strategy in this study and thus defined only BED-positive non-AIDS cases with CD4 cell counts ≥200/μl as recent infection.

In the 456 non-AIDS cases with CD4 cell counts ≥200/μl, 197 cases were BED-positive and classified as recent infection (43.2%; 24.4% of the total cases) (Table 3). BED-negative cases, AIDS cases, and cases with CD4 cell counts <200/μl were classified as chronic infection. Patients with recent infection were younger (33.0 years, 95% CI 31.7–34.3 vs. 37.2 years, 95% CI 36.3–38.1) and had higher CD4 counts (423.2/μl, 95% CI 399.2–447.3 vs. 207.9/μl, 95% CI 193.3–222.4), as expected, and lower viral load (4.61 log VL/ml, 95% CI 4.46–4.76 vs. 4.81 log VL/ml, 95% CI 4.74–4.87), compared to patients with chronic infection. A larger proportion of recent infection (95.4%, 95% CI 92.5–98.3) was caused by HIV-1 subtype B than in those with chronic infection (90.5%, 95% CI 88.2–92.8). There were no significant differences in the nationality and transmission category between recent and chronic infection cases (Pearson's Chi-square test), although the proportion of Japanese patients was higher in recent infection (95.9%, 95% CI 93.2–98.7) than in chronic infection (90.2%, 95% CI 87.8–92.6) (p = 0.012, Chi-square test). A significantly larger proportion of patients underwent previous HIV testing (58.9%, 95% CI 52.0–65.8 vs. 31.9%, 95% CI 28.2–35.6) and were diagnosed with HIV-1 infection by voluntary testing (51.8%, 95% CI 44.8–58.8 vs. 31.5%, 95% CI 27.9–35.2) among recent infection cases than chronic infection cases (p < 0.001 in both, Pearson's Chi-square test).

To delineate the trends in early diagnosis of HIV-1 infection, the annual rate of recent infection in all 809 study participants was plotted over the study period (Figure 2). The rate was stable at ~20% between 2002 and 2010, except for 2007 (26.1%) and 2008 (31.9%), when a slight increase was evident. In order to identify the population that influenced the annual trends of early diagnosis, we focused on MSM patients and again divided them into three age groups: ≤29 years, 30–39 years, and ≥40 years. The rates of recent infection in the ≤29 and ≥40 years MSM groups were the highest and the lowest, respectively, in most years of the study period. The rate in the ≤29 years MSM group was high, ranging from 25.0% to 46.2% between 2002 and 2005, but it decreased to 19.0% in 2006, and increased again in 2007 and remained around 35% between 2007 and 2010. The rate of recent infection in the ≥40-year-old MSM group was steadily low at ~10% between 2002 and 2006, but increased in 2007 to 25.9% and 2008 to 35.3%, then decreased to around 15% in 2009 and 2010. The rate in the 30–39-year-old MSM ranged between those of the other two groups during most part of the study period. These results suggest that younger MSM tend to be diagnosed persistently earlier, whereas older MSM are usually diagnosed at a later stage of the HIV disease.

4. Discussion

The present study analyzed the trends in the proportion of AIDS patients and patients with recent infection among 809 new cases of HIV-1-infection diagnosed between 2002 and 2010. This group recruited from our AIDS Clinical Center represents 20.0% of the total number of newly diagnosed Tokyoites during the same period. We found that MSM, especially younger MSM, tend to be diagnosed at an earlier stage before the development of AIDS, probably because of frequent voluntary HIV testing. The proportion of AIDS cases remained at a steady low level and the rate of recent infection remained at a high level in younger MSM patients, indicating that younger MSM are aware of the risk of their sexual behavior sufficient to take HIV testing repeatedly. On the other hand, in the older MSM, the rate of AIDS was relatively high and the rate of recent infection comparatively low, but transiently increased in 2007 and 2008, suggesting that older MSM with a high-risk of HIV infection usually do not take HIV testing frequently and may respond to campaigns that promote such tests. Interestingly, the Japan Foundation for AIDS Prevention conducted several campaigns to promote voluntary HIV-1 testing in 2007. A popular male Japanese singer took part in one such campaign in July 2007, which was a great surprise among the Japanese in general, and this was followed by an increase in the number of voluntary HIV tests performed in 2007 and 2008.[2] The event may have prompted older MSM at high risk to take voluntary HIV testing, resulting in the transient increase in the rate of early diagnosis for 2007 and 2008. The sharp decline in the rate of early diagnosis observed in 2009 and 2010 in the older MSM group coincided with reductions in the number of voluntary tests,[2] and could be an omen of future increases in the number of AIDS patients in this population. Early diagnosis followed by early introduction of ART may reduce the spread of HIV-1 among MSM, which could help to prevent an HIV epidemic in this population.[6,7,22] A strategy based on the promotion of voluntary testing needs to be formulated, similar to the 2007 campaigns that resulted in significant increases in the rate of early diagnosis in older MSM.

Discordant shifts were observed between the rates of AIDS and recent infection. The reasons may be that AIDS usually develops several years after HIV infection and that disease progression varies enormously among infected individuals. Therefore, the variable length of time during which HIV infection was ignored resulted in the development of AIDS, the proportion of which does not always correlate with the rate of recent infection in the same year.[11] Furthermore, disease progression has been suggested to have become faster in a significant portion of Japanese patients, probably because the prevailing HIV-1 strains in Japan have

adapted to the Japanese population by acquiring escape mutations from immune pressure restricted by human leukocyte antigens (HLAs) popular among the Japanese.[23,24] Based on this point of view, early diagnosis is even more important due to the shorter asymptomatic period before the development of AIDS.

The majority of our study participants were infected with HIV-1 subtype B, and HIV-1 subtype B infection correlated significantly with MSM (crude odds ratio 37.9, $p < 0.001$; Chi-square test). The non-AIDS patients were more likely to be infected with subtype B than AIDS patients (crude odds ratio 1.59, $p = 0.098$). The same was true for recent infection than chronic infection (crude odds ratio 2.81, $p = 0.009$). A previous Japan-wide survey also showed a close relationship between subtype B and MSM in Japan; all cases diagnosed with primary HIV-1 infection ($n = 45$) were caused by subtype B, and such primary infections were significantly frequent among MSM.[14] Considered together, the results indicate that subtype B is the major currently prevalent strain in Japan, especially among MSM, and such strains are probably adapting to the Japanese population by repeated exposure to immune pressure of the Japanese.

This study used case reporting-based surveillance to estimate the number of new HIV-1 infections in Tokyoites between 2002 and 2010. The data were collected at a single center and thus may have included some institutional bias. The study participants were statistically younger and were more likely to be MSM than those of the Tokyo registry. The BED assay was used in this study to determine the rate of recent infection in the selection study group and not to determine the national incidence rate. However, the data from this study suggest the following target-specific differential strategies for controlling the HIV epidemic and for AIDS prevention in Tokyo: campaigns aimed at promoting testing should be directed at older MSM for early diagnosis to prevent/halt the progression of AIDS; commencement of ART for HIV-infected younger MSM at early stages of the disease may effectively reduce the number of new cases based on the control of current hot-spots of HIV transmission among this group.

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Conflict of interest: The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2011.11.003.

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Research Letter

AIDS 2012, **26**:000–000

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

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

Introduction

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

Methods

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

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

Results

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

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

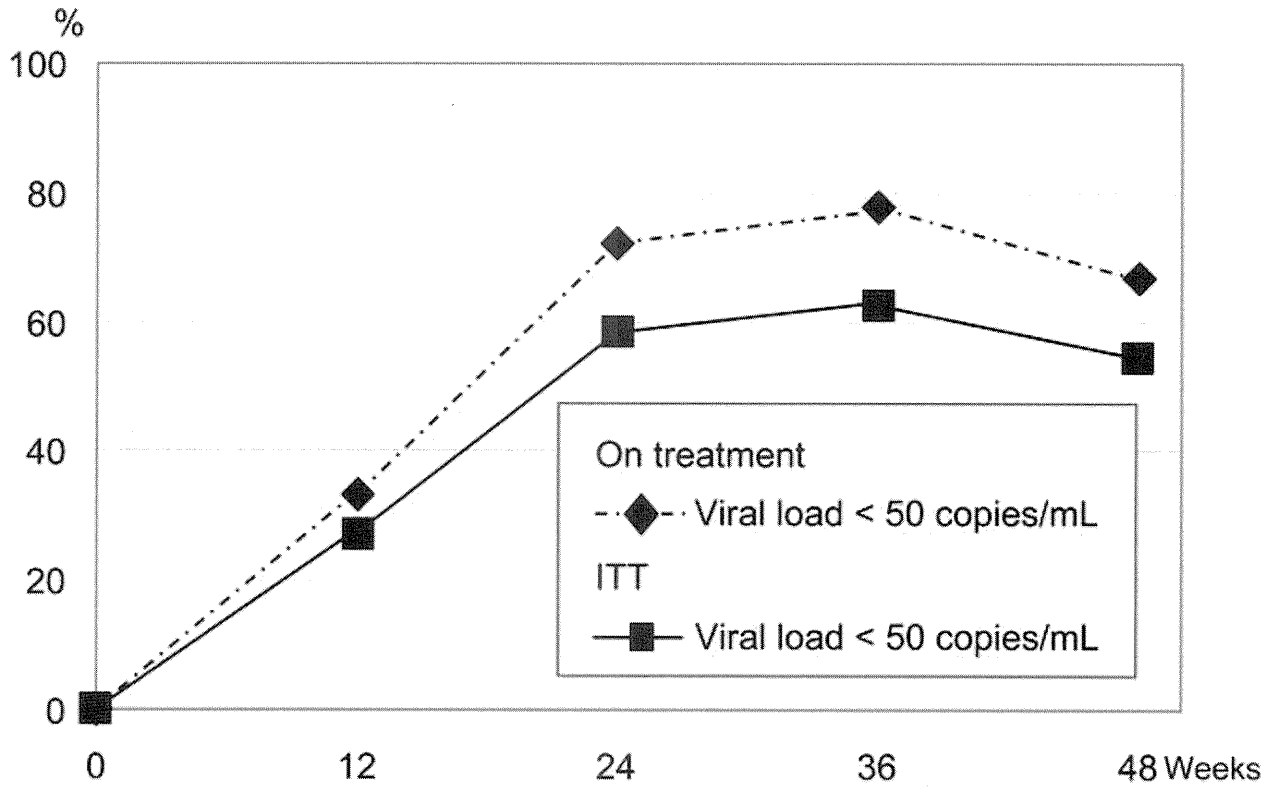

Fig. 1. Proportions of patients with viral load <50 copies/mL at 48 weeks with on-treatment and intention-to-treat (ITT) analysis.

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

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

Discussion

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

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

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

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Predictors and Outcomes of HIV-Infected Antiretroviral-Naive Patients With Discordant Responses to Combination Antiretroviral Treatment in Asian and Australian Populations: Results From APHOD

To the Editors:

Some patients experience a "discordant response" after the initiation of combination antiretroviral treatment (cART), whereby the HIV-1 RNA plasma level is below the limit of detection, but the CD4 T-cell count response is blunted.[1–6] Other patients exhibit a different pattern of discordant response, characterized by a sustained CD4 T-cell count response, despite persistent viremia.[1–6] Although there have been studies on discordant responses,[1–8] there is still limited knowledge about the clinical significance of such a response. Furthermore, there are no published data on discordant responses within the Asia-Pacific region. The objective of this analysis was to evaluate predictors of discordant response and their outcomes among HIV-infected antiretroviral-naive patients on cART from Asian and Australian populations.

We analysed the Asia-Pacific HIV Observational Database, a prospective observational cohort study of adults with HIV from 26 sites across Australia [the Australian HIV Observational Database (AHOD)][9] and 18 sites from the Asia-Pacific regions [the TREAT Asia HIV Observational Database (TAHOD)].[10] The structure of these databases and standardized mechanisms for data collection and follow-up have been previously described.[9,10] All antiretroviral-naive participants who initiated cART between 1996 and 2008, aged 16 years or older at treatment initiation, had a known date of therapy initiation, and had at least 1 measurement (CD4 T-cell count and HIV viral load) at 24 months of therapy (within a window of ± 3 months) were eligible for inclusion in analyses. Virologic response (VR) was defined as HIV viral load <500 copies per milliliter at 6 months, 12 months, and 24 months after initiation of cART. Immunologic response (IR) was defined as a rise in CD4 T-cell count of at least 50 cells per microliter or a CD4 T-cell count more than 350 cells per microliter at 6 months after initiation of cART. IR was also defined as a rise in CD4 T-cell count of at least 100 cells per microliter or a CD4 T-cell count more than 350 cells per microliter at 12 months and 24 months after initiation of cART. The exposures of interest were 6, 12, and 24 month responses to therapy, categorized according to VR and IR, considered jointly as complete response (VR+/IR+), virologic-only response (VR+/IR−), immunologic-only response (VR−/IR+), and complete failure (VR−/IR−). To evaluate predictors of poor immunologic recovery in the context of effective virologic suppression, we performed logistic regression analyses including subjects with virologic suppression at 6, 12, and 24 months after cART initiation. We investigated outcomes after concordant or discordant responses using survival analyses methods. The primary endpoint to evaluate outcomes was all cause mortality or new AIDS-defining illness documented after the 24-month time point. The survivor function for AIDS diagnosis or death of the discordant responses at 24 month was compared using Kaplan–Meier curve. Time was calculated from the 24-month time point after treatment initiation and ended at the appropriate endpoint (new AIDS or death) or the last follow-up visit. Discordant data at 6 or 12 months after cART initiation were included in the final multivariate model separately. The analyses were performed using SAS (version 9.1, SAS Institute Inc, Cary, NC) and STATA (version 10.1, StataCorp, College Station, TX).

A total of 2157 participants were eligible for this analysis: 1036 from AHOD and 1121 from TAHOD (see **Table, Supplemental Digital Content 1**, http://links.lww.com/QAI/A140). At 6 months, 68% patients had complete response; 15% had virologic-only response; 12% had immunologic-only response; and 5% showed complete failure (see **Table, Supplemental Digital Content 2**, http://links.lww.com/QAI/A141). At 12 months, 63% patients had complete response; 19% had virologic-only response; 12% had immunologic-only response; and 6% showed complete failure. At 24 months, 70% patients had complete response; 13% had virologic-only response; 11% had immunologic-only response; and 6% showed complete failure.

In patients with a VR, factors associated with a discordant IR at 6, 12, and 24 months on multivariate analyses were consistent at all 3 time points (see **Table, Supplemental Digital Content 3**, http://links.lww.com/QAI/A142), although the magnitude of effects and statistical significance did vary. Female patients were less likely than males to have a poor IR. Compared with patients reporting homosexual contact, patients reporting injecting drug use and heterosexual contact as their mode of HIV acquisition were more likely to have

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a poor IR. Prior AIDS diagnosis at cART initiation was associated with a poor IR at 12 months after cART initiation. There was a borderline significant association of hepatitis C coinfection with poor IR at 6 months, but this association was not observed at 12 and 24 months. Higher CD4 T-cell count and lower viral load (<50,000 copies/mL) at cART initiation were associated with lower risk of poor IR at all 3 time points. Finally, initial cART containing didanosine was associated with a higher risk of poor IR, most notable 12 months after cART initiation.

We evaluated the prognostic significance of discordant responses on disease progression (newly developed AIDS or death) (Table 1). There were 150 newly developed AIDS or death events reported during 10,876 person years of follow-up (an incidence of 1.38 per 100 person years, 95% confidence interval: 1.18 to 1.62). In multivariate model, hepatitis C coinfection was associated with a higher risk of disease progression. Patients having higher baseline CD4 T-cell counts, especially greater than 200 cells per microliter, were at significantly lower risk of disease progression. Compared with patients with complete response (VR+/IR+), patients with a virologic-only response (VR+/IR−) at 6, 12, and 24 months had similar risk of disease progression, however patients with an immunologic-only response (VR−/IR+) or complete failure (VR−/IR−) had significantly increased risk of disease progression. The Kaplan–Meier

TABLE 1. Cox Proportional Hazards Analysis of All-Cause Mortality or Newly Developed AIDS for 2157 HIV-Infected Individuals

Variable	Unadjusted HR	95% CI	*P*	Adjusted HR	95% CI	*P*
Age (year) at cART initiation						
≤25	1.00	—	—	—	—	—
26–35	0.76	(0.39 to 1.51)	0.438	—	—	—
36–45	0.79	(0.40 to 1.57)	0.497	—	—	—
46+	0.93	(0.46 to 1.89)	0.844	—	—	—
Gender						
Male	1.00	—	—	—	—	—
Female	0.93	(0.55 to 1.56)	0.770	—	—	—
Reported route of infection						
Homosexual contact	1.00	—	—	1.00	—	—
Injecting drug use	1.90	(1.05 to 3.42)	0.033	1.01	(0.52 to 1.97)	0.974
Heterosexual contact	0.99	(0.62 to 1.59)	0.978	0.90	(0.55 to 1.47)	0.668
Prior AIDS diagnosis at cART initiation						
No	1.00	—	—	—	—	—
Yes	1.13	(0.76 to 1.69)	0.539	—	—	—
Hepatitis B coinfection						
No	1.00	—	—	1.00	—	—
Yes	1.68	(0.98 to 2.90)	0.061	1.54	(0.89 to 2.67)	0.124
Hepatitis C coinfection						
No	1.00	—	—	1.00	—	—
Yes	1.93	(1.23 to 3.04)	0.004	1.93	(1.21 to 3.07)	0.005
CD4 T-cell count (cells/μL) at cART initiation						
≤50	1.00	—	—	1.00	—	—
51–100	0.77	(0.39 to 1.53)	0.458	0.68	(0.34 to 1.35)	0.269
101–200	0.72	(0.43 to 1.21)	0.212	0.61	(0.36 to 1.03)	0.065
201–300	0.39	(0.21 to 0.74)	0.004	0.36	(0.19 to 0.69)	0.002
301 or more	0.57	(0.35 to 0.93)	0.025	0.59	(0.36 to 0.98)	0.040
HIV viral load (copies/mL) at cART initiation						
50001+	1.00	—	—	1.00	—	—
<500	1.08	(0.68 to 1.71)	0.739	1.33	(0.83 to 2.14)	0.236
501–10000	0.99	(0.51 to 1.93)	0.975	1.15	(0.58 to 2.28)	0.699
10001–50000	0.16	(0.02 to 1.16)	0.070	0.17	(0.02 to 1.26)	0.084
Discordant data at 6 months after cART initiation						
VR+IR+	1.00	—	—	1.00	—	—
VR+IR−	1.07	(0.58 to 1.97)	0.838	0.90	(0.47 to 1.72)	0.743
VR−IR+	2.21	(1.39 to 3.50)	0.001	1.60	(0.98 to 2.59)	0.059
VR−IR−	2.08	(1.06 to 4.08)	0.033	0.92	(0.43 to 1.96)	0.835
Discordant data at 12 months after cART initiation						
VR+IR+	1.00	—	—	1.00	—	—
VR+IR−	0.98	(0.55 to 1.76)	0.955	0.88	(0.47 to 1.67)	0.705
VR−IR+	2.38	(1.49 to 3.79)	<0.001	1.73	(1.02 to 2.92)	0.041
VR−IR−	3.95	(2.41 to 6.47)	<0.001	2.04	(1.09 to 3.81)	0.026
Discordant data at 24 months after cART initiation						
VR+IR+	1.00	—	—	1.00	—	—
VR+IR−	1.16	(0.67 to 2.00)	0.602	1.36	(0.77 to 2.40)	0.287
VR−IR+	1.81	(1.15 to 2.84)	0.010	1.71	(1.08 to 2.69)	0.021
VR−IR−	4.47	(2.89 to 6.91)	<0.001	4.94	(3.17 to 7.72)	<0.001

Discordant information at 6 or 12 months after cART initiation was added separately into the multivariate model.
CI, confidence interval.

curve summarizing time to death or new AIDS diagnosis indicated that disease progression differed significantly according IR and IR at 24 months after initiating cART (log rank test, $P < 0.001$) (see **Figure, Supplemental Digital Content 4**, http://links.lww.com/QAI/A143). The prognosis was best for the VR+/IR+ and VR+/IR− group, worst for the VR−/IR− group, and intermediate for the VR−IR+ group. However, the difference between the VR+/IR+ group and the VR+/IR− group was not statistically significant ($P = 0.502$). When included in the final multivariate model, immunologic-only responses at 12 and 24 months had significantly higher risk for disease progression than complete response. However, those with virologic-only responses at 6, 12, and 24 months had a similarly good outcomes to those with complete responses.

The data suggest that in the first 2 years, VR is more important than IR in terms of survival and HIV disease progression. However, further research is warranted to establish the importance of IR beyond the initial 2-year treatment period because lower CD4 counts are associated with higher risk of AIDS and serious non-AIDS events.[11] A limitation of our study is the absence of some important variables such as adherence and concomitant medications, particularly cytotoxic drugs. In addition, the small number of patients with treatment response, either VR+/IR−, VR−/IR+, or VR−/IR−, may have limited statistical power. Collaboration with other cohorts is warranted to increase the number and the power. Further studies are needed to elucidate the prognostic significance of virologic-only and immunologic-only responses with clear definitions, and clinical trials are required to identify the most appropriate intervention in patients who are at elevated risk of disease progression.

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Impact of Adding Enfuvirtide to the Predictive Value of the Darunavir Genotypic Resistance Score

To the Editor:

INTRODUCTION

Development of HIV-1 resistance to the antiretroviral drug class of protease inhibitors (PIs) is caused by the selection of PI resistance–associated mutations in the protease coding region. High-level resistance to PI requires accumulation of resistance mutations in protease.[1] To predict viral susceptibility to a drug, genotypic resistance scores are generated, based on the assessment of the impact of genotypic patterns at baseline on the subsequent virological response. Different drug-resistance interpretation algorithms, all based on genotypic resistance scores, are mostly used in clinical practice to reliably select and adapt a new antiretroviral-based regimen after a virological failure, particularly in antiretroviral experienced patients.[2,3]

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