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Prevalence of HIV-1 Drug Resistance after Failure of a First Highly Active Antiretroviral Regimen in KwaZulu Natal, South Africa

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Abstract

Background—Emergence of human immunodeficiency virus type 1 (HIV-1) drug resistance may limit the benefits of antiretroviral therapy in resource-limited settings. The prevalence of resistance was assessed among patients from KwaZulu Natal (KZN), South Africa following failure of their first highly active antiretroviral therapy (HAART) regimen.

Methods—Genotypic resistance testing was performed on plasma virus from patients experiencing virologic failure (VF) of a first HAART regimen at two clinics in KZN. Clinical and demographic data were obtained from medical records. Regression analysis was performed to determine factors associated with ≥ 1 significant resistance mutation.

Results—Between January 2005 and August 2006, 124 ART-treated adults with virologic failure VF were enrolled. The predominant subtype was HIV-1C. Samples from 83.5% carried ≥ 1 significant resistance mutation. Dual-class resistance was present in 64.3% of subjects; 2.6% had triple-class resistance. The most common mutation was M184V/I (64.3%); K103N was present in 51.3% and V106M in 19.1%. Thymidine analog resistance mutations were found in 32.2% of subjects, and protease resistance mutations in 4.4%.

Conclusions—Antiretroviral drug resistance was detected in more than 80% of South African patients with failure of a first HAART regimen. Patterns of resistance reflected drugs used in first-line regimens and viral subtype. Continued surveillance of resistance patterns is warranted to guide selection of second-line regimens.

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Keywords

HIV; drug resistance; antiretrovirals; resource-limited setting

The global threat of HIV/AIDS has reached pandemic proportions. UNAIDS estimates that by 2005, 33 to 46 million people were infected worldwide.[1] South Africa has been one of the hardest hit countries with 5.5 million infected persons. In 2005 an estimated 320,000 people died from AIDS-related complications in South Africa alone. Efforts to provide access to antiretroviral (ARV) therapy for infected persons in resource-limited settings have accelerated over the last several years. By the end of 2005, an estimated 1.3 million infected persons were receiving ARV therapy worldwide. Despite concerns regarding implementation [2,3], the rollout of ARV therapy has had a profound impact on AIDS-related morbidity and mortality among infected persons receiving treatment in resource-poor countries [4,5]. In South Africa, ARV treatment became available in many hospitals and clinics throughout the country after release of the Operational Plan for Comprehensive HIV and AIDS Care and Treatment for South Africa (www.gov.za). Approximately 190,000 South Africans were receiving ARV therapy by the end of 2005, accounting for a large share of the treatment scale-up in sub-Saharan Africa overall.

The emergence of antiretroviral drug resistance has been a major threat to sustained impact of these medications in resource-rich settings. One U.S. clinic reported a prevalence of triple-class resistance among treatment-experienced patients of 8% [6]. Among U.S. patients with newly diagnosed HIV-1 infection, the prevalence of resistance is approximately 10% [7].

Multiple factors that may contribute to drug resistance in resource-limited settings have been described, but the extent of drug resistance in the setting of recent rapid scale up in treatment has not been documented. On the one hand, an emphasis on adherence training and the lack of widespread use of single and dual therapy regimens prior to HAART might be expected to limit resistance. On the other hand, limited options for drug substitution in patients intolerant of certain regimens and interruptions in drug supply may lead increase the risk of drug resistance. Women who received single-dose nevirapine (NVP) to prevent mother-to-child transmission are also at risk for development of drug resistance [8–10]. We therefore assessed the prevalence of drug resistance after virologic failure in patients starting a first HAART regimen at two clinics in KwaZulu Natal Province, South Africa, where HIV seroprevalence rates are among the highest in Africa.

MATERIALS AND METHODS

Study Sites

This study was conducted at two clinics located in or near Durban, South Africa in the province of Kwa-Zulu Natal: the Sinikithemba Outpatient HIV/AIDS Clinic at McCord Hospital (MCH), Durban, and the iThemba Outpatient HIV/AIDS Clinic at the St. Mary's Hospital (SMH) in Mariannhill. Both sites are regional referral centers for antiretroviral therapy and receive partial support from the President's Emergency Plan for AIDS Relief (PEPFAR). Government funding for ARVs began in March 2003 at SMH and in February of 2004 at MCH, although some patients had received various privately-supported antiretroviral regimens as early as the year 2000. During the study period (January, 2005 to August, 2006) MCH followed 2,598 patients on antiretroviral therapy and SMH followed 781 patients. Both clinics attend only to patients receiving antiretroviral therapy. The study was approved by the respective ethics committees at both hospitals, and by the institutional review boards at Partners HealthCare Systems and Harvard Medical School in Boston, Massachusetts.

Study Participants

Between January 1, 2005 and August 15, 2006, all HIV–1 infected patients at MCH and SMH clinics aged 18 years or older who experienced virologic failure (defined below) after 24 weeks on their first HAART regimen were offered participation in this study. This regimen could have been a first or second-line regimen of the Operational Plan or some combination of available agents. The first-line regimen included weight-based dosing of stavudine (d4T) plus lamivudine (3TC) and either efavirenz EFV (regimen 1a) or NVP (regimen 1b). The second-line regimen included zidovudine (ZDV) plus didanosine (ddI) and lopinavir/ritonavir (LPV/RTV; fixed-dose combination). Second-line regimens were employed for patients who had prior treatment with a suboptimal (non-HAART) regimen or intolerance to first-line drugs. Patients had received adherence training prior to starting HAART and adherence counseling periodically thereafter. For the purposes of this study, virologic failure was defined as an HIV-1 RNA level of > 1,000 copies/mL (Roche Amplicor HIV-1 Monitor assay). Plasma HIV-1 RNA levels were not routinely obtained prior to the first treatment regimen but were determined every 24 weeks after initiation.

Subjects were categorized as either having “prior ARV therapy” or “first HAART”. Prior ARV therapy included subjects with a history of suboptimal therapy, defined as a non-HAART regimen that included single- or dual-drug therapy or use of a triple-nucleoside RT inhibitor combination. Those subjects with an uninterrupted (less than 2 weeks) inter-class switch for toxicity or to minimize side effects (e.g., efavirenz to lopinavir/ritonavir) were also considered to have prior ARV therapy. All other subjects were considered as first HAART, including those with an uninterrupted intra-class switch for toxicity or to minimize side effects (e.g., stavudine for zidovudine or nevirapine for efavirenz). Patients who had interrupted HAART (at least 2 weeks off therapy) were included as long as the same regimen was resumed for at least 4 weeks prior to enrollment; otherwise they were excluded.

All subjects gave signed, written informed consent; Zulu translation and interpretation were provided if needed.

Data collection

Resistance testing of plasma virus was performed at the Inkosi Albert Luthuli Hospital Department of Virology Laboratory, Nelson R. Mandela School of Medicine in Durban. using the TRUGENE® HIV-1 Genotyping Test on an OpenGene® DNA Sequencing System (Bayer HealthCare Diagnostics, Berkeley, CA) as directed by the manufacturer. Substitutions at the following positions were considered drug resistance mutations: for reverse transcriptase (RT), M41L, K65R, D67N, insertion 69, K70R, L74V, L100I, K103N, V106A/M, V108I, Q151M, Y181C, M184V, Y188C/L, G190A, L210W, T215Y/F, K219Q/E/N/R, P225H, and M230L; for protease (PR), D30N, V32I, L33F/I, M46I/L, I47V/A, G48V, I50V, V82A/T/F/S, I84V, and L90M. The PR and RT sequences have been deposited in the GenBank data bank under accession numbers EU307996–EU308110. In addition to genotypic resistance tests, laboratory data included CD4 count, plasma HIV-1 RNA level, complete blood count, hemoglobin, liver function tests, and serum creatinine at the time of enrollment.

Variables evaluated include age, gender, race, economic background, number and type of opportunistic infections diagnosed within six months prior to study enrollment, prior and current antiretrovirals, use of antimicrobials for tuberculosis, *Pneumocystis jiroveci* pneumonia (PCP) prophylaxis, use traditional medicines, and adherence. At St. Mary’s Hospital, adherence was estimated by pill counts; at McCord Hospital, adherence assessment was based on self-report.

Statistical Analysis

The prevalence of resistance was reported with 95% confidence limits, calculated based on normal approximation of binomial distribution. The median number of RT and PR resistance mutations was also reported. The association between presence of resistance and baseline explanatory variables in the pooled populations was tested using Fisher's exact test. Variables with prior knowledge of association with outcomes as well those independent variables that exhibited association with outcomes in bivariate analysis at p-values 0.1 or less or odds ratios of at least 1.5 (or at most 0.6) were advanced into multivariate analyses. Multivariate logistic regression analysis was performed to determine the independent effect of each factor under consideration. Variables tested included: CD4 cell count and virus load at the time of enrollment, history of prior ARV treatment, opportunistic infections within six months prior to study enrollment, WHO clinical stage at enrollment, age, and gender. Analyses were performed using SAS software version 9.1.3 service pack 4 (SAS, Cary, North Carolina, United States). All tests of significance were two-sided; associations with a p-values <0.05 were considered to be statistically significant.

RESULTS

Patient demographics and characteristics

Of 147 patients with virologic failure of antiretroviral therapy at the MCH and SMH clinics, 124 were still receiving the failing regimen and consented to enroll into the study. Data were incomplete for 2 subjects, and no genotype was obtained for 7, leaving 115 subjects for analysis. Table 1 shows subject characteristics and laboratory data at enrollment. The mean age was 37.3 years, 47.8% were male and 93.9% were black; and 97.4% reported heterosexual intercourse as the route of HIV infection. All but three were infected with HIV-1 subtype C (97.4%); other subtypes included A (1), B (1) and a C/J recombinant (1). The median CD4 count at enrollment was 161.5 cells/mm^3 (interquartile range [IQR], 104.0–243.5 cells/mm^3); 19.2% of subjects were classified as having WHO stage IV disease. The median HIV-1 RNA level at the time of study enrollment was 4.29 $\log_{10}$ copies/mL (IQR,3.73–4.90 $\log_{10}$ copies/mL).

The median duration of antiretroviral therapy prior to study enrollment was 10.8 months (IQR, 6.7–18.6 months). Fifty-six of 115 (48.7%) were receiving regimen 1a, 30 (26.1%) were receiving ZDV/3TC/EFV, and 6 (5.2%) were receiving regimen 1b. Eighteen subjects (15.7%) enrolled in the study had prior mono- or dual-therapy, 4.4% had prior single-dose NVP for prevention of mother-to-child transmission. Self-reported adherence was >95% in 82.7% of subjects. Symptoms recorded included headache, diarrhea, nausea, vomiting, dysphagia, weight loss, fever, night sweats, cough, dyspnea, rash, oral lesions, genital lesions, paresthesias and other.

Genotypic resistance test results

At least one resistance mutation was detected in samples from 83.5% of subjects (Table 2). Mutations conferring resistance to at least one drug in each of two classes were detected in 64.3%, and mutations associated with resistance to at least one drug in each of three classes were detected in 2.6%. Resistance patterns were not significantly different among subjects who were taking their first HAART versus those with prior ARV experience.

The most commonly detected mutations were M184V/I for 3TC and emtricitabine resistance (64.3%) and K103N for NNRTI resistance (51.3%) (Table 3); samples from 39.1% of subjects had both. Other NNRTI mutations detected included V106M (19.1%) and G190A/S (18 patients). Thymidine analog resistance mutations (TAMs) were found in samples from 32.2% of subjects. A total of 7.0% had mutations indicative of the TAM-1 pathway (M41L, L210W,

T215Y), 19.1% had TAM-2 mutations (67N, 70R, 215F, 219Q, R or E), and 6.1% had mutations common to both pathways (TAM-1 and TAM-2); 13.0% had ≥3 TAMs. Samples from three subjects had a K65R mutation (one with a TAM + NNRTI resistance, one with M184V, and one with Q151M) and two had an L74V mutation (one with 3 TAMs, M184V and NNRTI resistance and one with M184V only). Of note, one subject had a deletion at RT codon 69. Five subjects (4.4%) had PI resistance mutations: 2 had a history of PI therapy; PI resistance mutations in the other 3 could represent polymorphisms or (less likely) transmitted resistance.

Risk factors associated with genotypic drug resistance

An exploratory logistic regression analysis (Table 4) was performed to assess the risk factors associated with the presence of at least one significant drug resistance mutation in our subjects with virologic failure. In the univariate analysis, age less than 35 years old was associated with resistance (OR 3.60; 95% confidence interval [CI], 1.11–11.63; p=0.03), but age was not a significant independent risk factor in the multivariate models that adjusted for recent OI, CD4 count, and viral load (OR 3.27; 95% CI, 0.92–11.63; p=0.068). Subjects with viral loads at study enrollment of 5,000 to 99,999 copies/mL were more likely to have drug–resistant virus, but this association was of marginal statistical significance in the univariate analysis and was not significant in the multivariate analysis that adjusted for age, recent OI and CD4 cell count (Table 4).

DISCUSSION

Great strides have been made over the last few years in decreasing the morbidity and mortality resulting from HIV-1 infection in resource-limited settings by programs providing ARV treatment to those in need. This progress could be threatened by the widespread development of drug resistance. We documented the prevalence and pattern of drug resistance mutations in HIV-1 subtype C-infected patients with failure of a first HAART regimen in two large clinics in Durban, South Africa. Results of this study demonstrated the presence of at least one major resistance mutation in plasma virus from more than 83% of patients. Mutations conferring resistance to drugs in two classes were present in virus from more than half of the subjects we tested, but triple-class resistance was relatively uncommon (2.6%). A similarly high prevalence of antiretroviral drug resistance was reported in samples from patients with treatment failure in Zimbabwe and Uganda.[11–13]

The drug resistance mutations identified in this study were similar to those reported by other studies in patients infected with HIV-1C.[14–17] The relatively high frequency of V106M as compared to V106A in RT confirms previous reports that V106M is the favored NNRTI resistance mutation in HIV-1C.[18–20] The M184V mutation was the single most common mutation detected. In addition, samples from most subjects had at least one significant NNRTI resistance mutation, with K103N being the most common. As expected, there were few significant PI mutations, given the infrequent use of PI-containing regimens.

These results are consistent with the use of NNRTIs in the first-line regimens provided by the South Africa National Plan. By contrast, the prevalence of TAMs was relatively low (32%). This finding contrasts with data from the Development of Anti-Retroviral Therapy in Africa (DART) study, which noted presence of TAMs in more than half of viremic subjects receiving a regimen of tenofovir, lamivudine and zidovudine for 24 weeks, and in more than 80% after 48 weeks.[21] Although the precise duration of virologic failure in our patients is not known, it is likely that routine monitoring of plasma HIV-1 RNA led to shorter exposure to failing regimens, thereby reducing the opportunity for TAMs to accumulate. It is also possible that the South Africa National Plan regimens are less likely to select TAMs due to the combination of two NRTIs plus an NNRTI, compared to the triple-NRTI regimen used in DART.

Additionally, the finding of fewer TAMs in non-subtype B virus agrees with a previous report. [22]

Among those samples in which TAMs were detected, we found TAM-1, TAM-2 and mixed patterns of mutations. The TAM-1 mutations confer resistance to ZDV and d4T, as well as cross-resistance to multiple NRTI, whereas the resistance conferred by TAM-2 mutations usually is limited to ZDV and d4T.[23,24] Data from patients in Botswana suggest that in HIV-1C, T215Y occurs in combination with D67N and K70R, rather than with M41L and L210W, as in HIV-1B.[25] By contrast, we noted the presence of M41L together with T215Y (with or without L210W) in samples from 7 subjects. Similarly, K65R is thought to emerge commonly in HIV-1 subtype C [26], but we detected this mutation in samples from only 3 subjects. These findings suggest that resistance testing of a larger number of subtype C-infected patients with ART failure needs to be performed in order to define subtype C-specific patterns of resistance mutations.

Univariate analyses suggested that plasma HIV-1 RNA levels > 100,000 copies/mL and < 5,000 copies/mL were associated with a lower likelihood of drug resistance mutations, although this finding was of marginal statistical significance. This seemingly paradoxical finding could be explained if those with the highest virus loads were not taking their prescribed antiretroviral medications[27,28]. Additionally, those with much lower viral loads may have either less successful laboratory amplification for genotyping or may represent early virologic failure from nonadherence (prior to reaching the pretreatment setpoint). These findings could be potentially useful as a means of stratifying individuals likely to yield a relevant result on genotyping when seen in the clinic for locations with resource limitations. A surprising finding was the lack of association between prior suboptimal ART and resistance to the current failing regimen, since failure of a single- or dual-NRTI regimen would be expected to generate resistance to those drugs and predispose to failure of subsequent regimens. Also surprising was the lack of a statistically significant association between adherence and drug resistance. The metrics used to measure adherence–pill count and patient self-report–may overestimate adherence.[29] In fact, very few subjects in our study reported <95% adherence. Use of other tools such as a visual analog scale might improve the accuracy of adherence assessment without the need for more complex instruments such as a medication electronic monitoring system. [30]

This study has several limitations. Because we could not capture information on MHC and SMC patients who were not enrolled in this cohort, we were unable to compare the characteristics of patients with virologic failure to those who successfully maintained virologic suppression. Thus, we were not able to identify factors associated with virologic failure per se. In addition, because data on a number of risk factors such as plasma HIV-1 RNA levels were unavailable prior to the start of antiretroviral therapy and/or prior to virological failure, our analyses were unable to identify predictors of drug resistance at the time of or prior to virologic failure. Future results of ongoing prospective studies may help provide a more detailed picture of these predictors.

In conclusion, samples from a large percentage of subjects with virologic failure harbored HIV-1 drug resistance mutations. The most common mutations (K103N and M184V) were associated with NNRTI and NRTI resistance, respectively. The relatively limited number of TAMs and other NRTI resistance mutations, along with the low frequency of protease inhibitor resistance mutations suggests that these patients should respond well to second-line regimens containing a ritonavir-boosted protease inhibitor and appropriate NRTIs. Ensuring access to such regimens for patients in resource-limited settings is an urgent priority in order to provide treatment options for patients in whom first-line regimens have failed.

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Table 1

Subject characteristics at enrollment.

Characteristic	N=115
Mean age (years)	37.3 +/−8.4
Gender: male	55 (47.8)
Ethnicity: black	108 (93.9)
Employed	65 (56.5)
Route of Infection: heterosexual	112 (97.4)
Symptoms at the time of failure	80 (69.6)
OI within the six months prior to failure	75 (65.2)
PCP	3 (2.6)
Pulmonary TB	11 (9.6)
Disseminated TB	8 (7.0)
Cryptococcal meningitis	1 (0.9)
Herpes Zoster	3 (2.6)
Oropharyngeal Candidiasis	15 (13.0)
Recurrent respiratory infections	10 (8.7)
Other	5 (4.4)
WHO Stage	
I	22 (19.8)
II	25 (22.5)
III	42 (37.8)
IV	22 (19.8)
Median CD4 count (cells/mm^3)	162 (104–244)
Median plasma HIV-1 RNA (log_{10} copies/mL)	4.29 (3.73–4.90)
Required hospitalization within six months prior to failure	18 (15.7)
Regimen at the time of enrollment	
Regimen 1a (D4T/3TC/EFV)	56 (48.7)
Regimen 1b (D4T/3TC/NVP)	6 (5.2)
ZDV/3TC/EFV	30 (26.1)
ZDV/3TC/NVP	13 (11.3)
2 NRTI + LPV/r	5 (4.4)
Other	5 (4.4)
Median duration of ART prior to enrollment (months)	10.8 (6.7–18.6)
Subjects reporting >95% adherence	91 (82.7)
Prior dual- or mono-therapy	18 (15.7)
History of single-dose NVP for PMTCT	5 (4.4)
Concurrent medications	
Anti-tuberculosis therapy	17 (14.8)
PCP prophylaxis	93 (80.9)
Fluconazole	3 (2.6)
Traditional medicine(s)	16 (13.9)
Rash on exam	22 (19.3)
Lymphadenopathy on exam	14 (12.2)

Characteristic	N=115
Mean hemoglobin (g/dL)	12.2 +/−1.9

Data are n (%), mean ± SD or median (interquartile range). Percentages were calculated for complete data. IQR indicates interquartile range. PCP, *Pneumocystis jiroveci* pneumonia; TB, tuberculosis; WHO, World Health Organization; D4T, stavudine; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine; ZDV, zidovudine; LPV/r, lopinavir/ritonavir; ART, antiretroviral therapy; PMTCT, prevention of mother-to-child transmission.

† Symptoms included headache, diarrhea, nausea, vomiting, dysphagia, weight loss, fever, night sweats, cough, dyspnea, rash, oral lesions, genital lesions, paresthesias and other.

Table 2

Resistance mutations by regimen.

Current Regimen	No. of Subjects	≥ 1 significant mutation	Dual Class	Triple Class
Subjects on first ART[†]	**92**	**77 (83.7)** [‡]	**59 (64.1)**	**2 (2.2)**
- D4T/3TC + NNRTI	57	46 (80.7)	35 (61.4)	2 (3.5)
- ZDV/3TC + NNRTI	31	28 (90.3)	23 (74.2)	0
- 2 NRTI + LPV/r	1	1 (100.0)	0	0
- Other HAART	3	2 (66.7)	1 (33.3)	0
Subjects with prior ART[*]	**23**	**19 (82.6)**	**15 (65.2)**	**1 (4.3)**
- D4T/3TC + NNRTI	5	4 (80.0)	4 (80.0)	0
- ZDV/3TC + NNRTI	12	10 (83.3)	10 (83.3)	0
- 2 NRTI + LPV/r	4	3 (75.0)	1 (25.0)	1 (25.0)
- Other HAART	2	2 (100.0)	0	0
Total Cohort	**115**	**96 (83.5)**	**74 (64.3)**	**3 (2.6)**

[†] Includes intra-class uninterrupted switches (i.e. ZDV for D4T or NVP for EFV), n=25.

[‡] Number of subjects (%). No significant difference was found between first HAART group and Prior ART group (Fisher's exact test) or within groups comparing subjects with and without significant mutations using a chi-square analysis.

[*] Refers to either HAART (n=5) or dual NRTI (n=18) therapy.

ARV, antiretroviral; D4T, stavudine; 3TC, lamivudine; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; LPV/r, lopinavir/ritonavir; HAART, highly-active antiretroviral therapy.

Table 3

Frequency of selected resistance mutations in the reverse transcriptase and protease genes.

NRTI mutation	No. of mutations (%)	NNRTI mutation	No. of mutations (%)	PI mutation	No. of mutations (%)
M41L	12 (10.4)	L100I	4 (3.5)	D30N	0
A62V	2 (1.7)	K103N	59 (51.3)	V32I	0
K65R	3 (2.6)	V106A	1 (0.9)	L33F/I	2 (1.7)
D67N	23 (20.0)	V106M	22 (19.1)	M46I/L	2 (1.7)
insertion 69	0	V108I	14 (12.2)	I47V/A	0
K70R	17 (14.8)	Y181C/I	11 (9.6)	G48V	0
L74V	2 (1.7)	Y188C/L/H	12 (10.4)	I50V	0
V75I	3 (2.6)	G190A/S	18 (15.7)	I54V	1 (0.9)
F77L	0	P225H	8 (7.0)	V82A/T/F/S	1 (0.9)
Y115F	0	M230L	3 (2.6)	I84V	0
F116Y	0			L90M	1 (0.9)
Q151M	1 (0.9)				
M184V/I	74 (64.3)				
L210W	2 (1.7)				
T215Y	10 (8.7)				
T215F	6 (5.2)				
K219Q/E/N/R	13 (11.3)				
TAM 1 pathway	8 (7.0)				
TAM 2 pathway	22 (19.1)				
TAM 1&2	7 (6.1)				
Total TAMs	37 (32.2)				
≥ 1 NRTI mutation	81 (70.4)	≥ 1 NNRTI mutation	90 (78.3)	≥ 1 PI mutation	5 (4.4)
Total NRTI mutations	168	Total NNRTI mutations	152	Total PI mutations	7

TAM, thymidine analog mutation; TAM 1&2, percentage of subjects with at least one mutation in each of these two pathways (TAM 1: 41L, 210W, 215Y & TAM 2: 67N, 70R, 215F, 219Q/E/N/R); each of the three categories are mutually exclusive. Total TAMs, total percentage of subjects with TAM 1, TAM 2 or TAM 1&2 mutations; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitor.

Table 4

Univariate and multivariate analysis of variables associated with virologic failure and at least one significant resistance mutation.

		Univariate			Multivariate		
Variable	**Mutation Rate (%)**	**Odds Ratio**	**95%Confidence Interval**	**p**	**Odds Ratio**	**95%Confidence Interval**	**p**
Age							
<35	92	3.60	1.11–11.63	0.03	3.27	0.92–11.63	0.068
>=35	77	1.00	---		1.00		
Gender							
Male	82	0.79	0.30–2.13	0.65			
Female	85	1.00	---				
Employed							
Yes	78	0.40	0.14–1.21	0.10			
No	90	1.00	---				
Recent OI (within 6 months of study enrollment)							
Yes	88	2.44	0.90–6.64	0.08	2.20	0.70–6.88	0.175
No	75	1.00	---		1.00	---	
Symptoms (within 1 week of study enrollment)							
Yes	81	0.56	0.17–1.83	0.33			
No	89	1.00	---				
CD4 count at study enrollment(cells/μL)							
<200	84	0.87	0.30–2.57	0.81	0.87	0.23–3.33	0.838
>=200	86	1.00	---		1.00	---	
Plasma HIV-1 RNA level at study enrollment (copies/mL)							
< 5,000	77	1.37	0.39–4.88	0.08	1.05	0.23–4.81	0.103
5,000–29,999	90	4.39	1.01–19.20		3.91	0.84–18.15	
30,000–99,999	92	9.06	1.02–80.84		7.97	0.82–77.21	
>=100,000	71	1.00	---		1.00	---	
Hemoglobin (g/dL)							
<11	92	2.82	0.60–13.20	0.17			
>=11	81	1.00	---				
WHO clinical stage at study enrollment							
IV	86	1.39	0.37–5.26	0.63			

		Univariate			Multivariate		
Variable	**Mutation Rate (%)**	**Odds Ratio**	**95%Confidence Interval**	**p**	**Odds Ratio**	**95%Confidence Interval**	**p**
I–III	82	1.00	---				
Adherence (≥95%)							
Yes	85	1.96	0.61–6.32	0.25			
No	74	1.00	---				
Taking traditional medications							
Yes	75	0.54	0.15–1.88	0.33			
No	85	1.00	---				
ZDV/3TC vs D4T/3TC + NNRTI							
D4T/3TC	81	2.23	0.57–8.70	0.24			
ZDV/3TC	90	1.00	---				
Prior ART vs First HAART							
Prior ART	83	0.93	0.28–3.11	0.90			
First HAART	84	1.00	---				

OI, opportunistic infection; WHO, World Health Organization; ZDV, zidovudine; 3TC, lamivudine; D4T, stavudine; NNRTI, non-nucleoside reverse transcriptase inhibitor; ARV, antiretroviral; HAART, highly-active antiretroviral therapy.

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