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

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(See the editorial commentary by Smith and Schooley on pages 1598–1600)

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, South Africa, following failure of their first highly active antiretroviral therapy (HAART) regimen.

Methods. Genotypic resistance testing was performed on plasma virus samples from patients who experienced virologic failure of their first HAART regimen at 2 clinics in KwaZulu Natal. Clinical and demographic data were obtained from medical records. Regression analysis was performed to determine factors associated with $\geq$1 significant drug resistance mutation.

Results. From January 2005 through August 2006, a total of 124 antiretroviral-treated adults who experienced virologic failure were enrolled. The predominant subtype was HIV-1C. Virus samples from 83.5% of participants carried $\geq$1 significant drug resistance mutation. Dual-class drug-resistant virus was present in 64.3% of participants, and 2.6% had virus with triple-class drug resistance. The most common mutation was M184V/I (64.3% of patients); K103N was present in virus from 51.3%, and V106M was present in virus from 19.1%. Thymidine analog resistance mutations were found in virus from 32.2% of patients, and protease resistance mutations were found in virus from 4.4%.

Conclusions. Antiretroviral drug-resistant virus was detected in >80% of South African patients who experienced failure of a first HAART regimen. Patterns of drug 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.

The global threat of HIV infection and AIDS has reached pandemic proportions. The United Nations Joint Programme on HIV/AIDS estimates that, by 2005, 33–46 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 of 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 past 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 the release of the

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Operational Plan for Comprehensive HIV and AIDS Care and Treatment for South Africa [6]. 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 ARV drug resistance has been a major threat to the sustained impact of these medications in resource-rich settings. One US clinic reported a prevalence of triple-class ARV drug-resistant virus of 8% among treatment-experienced patients [7]. Among US patients with newly diagnosed HIV-1 infection, the prevalence of drug-resistant infection is ~10% [8].

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 a recent rapid scale-up in treatment has not been documented. On the one hand, an emphasis on treatment adherence training and the lack of widespread use of single-drug and dual-drug regimens prior to receipt of HAART might be expected to limit resistance. On the other hand, limited options for drug substitution for patients who are intolerant of certain regimens and interruptions in drug supply may lead to an increase in the risk of developing drug resistance. Women who have received single-dose nevirapine treatment to prevent mother-to-child transmission of HIV-1 infection are also at risk for development of drug-resistant infection [9–11]. Therefore, we assessed the prevalence of drug-resistant infection after virologic failure in patients starting a first HAART regimen at 2 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 the 2 following clinics located in or near Durban, South Africa, in the province of KwaZulu Natal: the Sinikithemba Outpatient HIV/AIDS Clinic at McCord Hospital (MCH) in Durban and the iThemba Outpatient HIV/AIDS Clinic at the St. Mary's Hospital (SMH) in Mariannhill. Both sites are regional referral centers for ARV therapy and receive partial support from the President's Emergency Plan for AIDS Relief. Government funding for ARV therapy began in March 2003 at SMH and in February 2004 at MCH, although some patients had received various privately supported ARV treatment regimens as early as 2000. During the study period (January 2005–August 2006), 2598 patients who received ARV therapy were observed at MCH, and 781 such patients were observed at SMH. Both clinics attend only to patients who receive ARV 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. From 1 January 2005 through 15 August 2006, all HIV-1–infected patients at MCH and SMH clinics who were aged $\geq$18 years and experienced virologic failure (defined below) after 24 weeks of receiving 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 dosages of stavudine plus lamivudine and either efavirenz (regimen 1A) or nevirapine (regimen 1B). The second-line regimen included zidovudine plus didanosine and lopinavir-ritonavir (fixed-dose combination). Second-line regimens were given to patients who had received prior treatment with a suboptimal (non-HAART) regimen or were intolerant to first-line drugs. Patients had received treatment 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 >1000 copies/mL (Roche Amplicor HIV-1 Monitor assay). Plasma HIV-1 RNA levels were not routinely determined prior to initiation of the first treatment regimen but were determined every 24 weeks after treatment initiation.

Participants were categorized as having either "prior ARV therapy" or "first HAART." Patients who had received prior ARV therapy included patients with a history of suboptimal therapy, defined as receipt of a non-HAART regimen that included single- or dual-drug therapy or receipt of a triple-nucleoside reverse-transcriptase inhibitor (NRTI) combination. Those patients who had an uninterrupted (<2 weeks between treatment regimens) interclass switch of treatment because of toxicity or to minimize adverse effects (e.g., switch from efavirenz to lopinavir-ritonavir) were also considered to have received prior ARV therapy. All other patients were considered to have received first HAART, including those who had an uninterrupted intraclass switch of treatment because of toxicity or to minimize adverse effects (e.g., switch from stavudine to zidovudine or from nevirapine to efavirenz). Patients who had received interrupted HAART (at least 2 weeks of not receiving therapy) were included if the same regimen was resumed for at least 4 weeks prior to enrollment (otherwise, the patient was excluded). All participants gave signed, written informed consent; Zulu translation and interpretation were provided when needed.

Data collection. Resistance testing of plasma virus samples was performed at the Inkosi Albert Luthuli Hospital Department of Virology Laboratory, Nelson R. Mandela School of Medicine (Durban, South Africa), using the TRUGENE HIV-1 Genotyping Test on an OpenGene DNA Sequencing System (Bayer HealthCare Diagnostics) as directed by the manufacturer. Substitutions at the following positions were considered to be drug resistance mutations: for reverse transcriptase, M41L, K65R, D67N, insertion 69, K70R/E, L74V, L100I, K103N, V106A/M, V108I, Q151M, Y181C, M184V, Y188C/L, G190A,

L210W, T215Y/F, K219Q/E/N/R, P225H, and M230L; and for protease, D30N, V32I, L33F/I, M46I/L, I47V/A, G48V, I50V, V82A/T/F/S, I84V, and L90M. The protease and reverse transcriptase sequences have been deposited in the GenBank database (accession numbers, EU307996–EU308110). In addition to genotypic resistance test results, laboratory data included CD4 cell count, plasma HIV-1 RNA level, complete blood count, hemoglobin level, liver function test results, and serum creatinine level at the time of enrollment in the study.

Variables evaluated include age, sex, race, economic background, number and type of opportunistic infections diagnosed within 6 months prior to study enrollment, prior and current ARV therapy, use of antimicrobials for tuberculosis, *Pneumocystis jiroveci* pneumonia prophylaxis, use of traditional medicines, and treatment adherence. At SMH, treatment adherence was estimated by pill counts; at MCH, treatment adherence assessment was based on self-report.

Statistical analysis. The prevalence of drug-resistant virus in the samples tested was reported with 95% CIs, calculated based on normal approximation of binomial distribution. The number of reverse-transcriptase inhibitor and protease inhibitor resistance mutations was also reported. The association between the presence of drug-resistant virus and baseline explanatory variables in the pooled populations was tested using Fisher's exact test. Variables that had a known association with outcomes, as well as those independent variables that exhibited an association with outcomes in bivariate analysis at $P \leqslant .1$ or ORs of $\geqslant 1.5$ (or $\leqslant 0.6$), 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 viral load at the time of study enrollment, history of ARV treatment, opportunistic infection within 6 months prior to study enrollment, World Health Organization clinical stage at enrollment, age, and sex. Analyses were performed using SAS software, version 9.1.3 (SAS Institute). All tests of statistical significance were 2-sided; associations with $P < .05$ were considered to be statistically significant.

RESULTS

Demographic characteristics. Of the 147 patients who experienced virologic failure of ARV therapy at the MCH and SMH clinics, 124 were still receiving the failing treatment regimen and consented to enroll in the study. Data were incomplete for 2 patients, and no genotype was obtained for 7, leaving 115 patients for analysis. Table 1 shows patient characteristics and laboratory data at study enrollment. The mean age was 37.3 years, 55 patients (47.8%) were male, and 108 (93.9%) were black; 112 patients (97.4%) reported heterosexual intercourse as the route of HIV infection. All but 3 patients were infected with HIV-1 subtype C (97.4% of patients); other subtypes included A (1 patient), B (1), and a C/J recombinant (1). The median CD4 cell count at enrollment was 161.5 cells/mm^3 (interquartile range, 104.0–243.5 cells/mm^3); 22 patients (19.2%) were classified as having World Health Organization stage IV disease. The median HIV-1 RNA level at the time of study enrollment was 4.29 $\log_{10}$ copies/mL (interquartile range, 3.73–4.90 $\log_{10}$copies/mL).

The median duration of ARV therapy prior to study enrollment was 10.8 months (interquartile range, 6.7–18.6 months). Fifty-six (48.7%) of 115 patients were receiving regimen 1A; 30 (26.1%) were receiving zidovudine, lamivudine, and efavirenz; and 6 (5.2%) were receiving regimen 1B. Eighteen patients (15.7%) who were enrolled in the study had received prior single-drug or dual-drug therapy, and 5 patients (4.4%) had received prior single-dose nevirapine therapy for prevention of mother-to-child transmission of HIV-1 infection. Self-reported treatment adherence was >95% for 82.7% of patients. Symptoms recorded included headache, diarrhea, nausea, vomiting, dysphagia, weight loss, fever, night sweats, cough, dyspnea, rash, oral lesions, genital lesions, paresthesias, and other symptoms.

Genotypic drug resistance test results. At least 1 drug resistance mutation was detected in virus from 83.5% of patients (table 2). Mutations conferring resistance to at least 1 drug in each of 2 classes were detected in samples from 64.3% of patients, and mutations associated with resistance to at least 1 drug in each of 3 classes were detected in samples from 2.6%. Resistance patterns were not statistically significantly different between patients who were receiving their first HAART regimen and those with prior ARV therapy experience.

The most commonly detected mutations were M184V/I for lamivudine and emtricitabine resistance (in virus from 64.3% of patients) and K103N for nonnucleoside reverse-transcriptase inhibitor (NNRTI) resistance (51.3%) (table 3); virus from 39.1% of patients had both. Other NNRTI mutations detected included V106M (in virus from 19.1% of patients) and G190A/S (15.7%). Thymidine analog resistance mutations (TAMs) were found in virus from 32.2% of patients. A total of 7.0% patients had virus with mutations indicative of the TAM-1 pathway (M41L, L210W, and T215Y), 19.1% had virus with mutations indicative of the TAM-2 pathway (67N, 70R, 215F, and 219Q, R, or E), and 6.1% had virus with mutations common to both pathways (TAM-1 and TAM-2); 13.0% of patients had virus $\geqslant$3 TAMs. Virus from 3 patients had a K65R mutation (1 with 1 TAM and an NNRTI resistance mutation, 1 with M184V, and 1 with Q151M), and virus from 2 patients had an L74V mutation (1 with 3 TAMs, M184V, and an NNRTI resistance mutation and 1 with M184V only). Of note, 1 patient had a deletion at reverse transcriptase codon 69. Five patients (4.4%) had virus with protease inhibitor resistance mutations; 2 of these patients had a history of protease inhibitor therapy,

Table 1. Patient characteristics at study enrollment.

Characteristic	Patients (n = 115)
Age, mean years ± SD	37.3 ± 8.4
Male sex	55 (47.8)
Black race	108 (93.9)
Employed	65 (56.5)
Heterosexual intercourse as route of infection	112 (97.4)
Symptoms at the time of treatment failure[a]	80 (69.6)
Opportunistic infection within the 6 months prior to treatment 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)
CD4 cell count, median cells/mm^3 (IQR)	162 (104–244)
Plasma HIV-1 RNA level, median $\log_{10}$copies/mL (IQR)	4.29 (3.73–4.90)
Required hospitalization within 6 months prior to treatment failure	18 (15.7)
Treatment regimen at the time of enrollment	
Regimen 1A (D4T, 3TC, and EFV)	56 (48.7)
Regimen 1B (D4T, 3TC, and NVP)	6 (5.2)
ZDV, 3TC, and EFV	30 (26.1)
ZDV, 3TC, and NVP	13 (11.3)
2 NRTI plus LPV/r	5 (4.4)
Other	5 (4.4)
Duration of ART prior to enrollment, median months (IQR)	10.8 (6.7–18.6)
Reported >95% treatment adherence	91 (82.7)
Prior dual- or single-drug therapy	18 (15.7)
History of single-dose NVP for PMTCT	5 (4.4)
Concurrent medications	
Anti-TB therapy	17 (14.8)
PCP prophylaxis	93 (80.9)
Fluconazole	3 (2.6)
Traditional medicine(s)	16 (13.9)
Examination findings	
Rash	22 (19.3)
Lymphadenopathy	14 (12.2)
Hemoglobin level, mean g/dL ± SD	12.2 ± 1.9

NOTE. Data are no. (%) of patients, unless otherwise indicated. Percentages were calculated for complete data. ART, antiretroviral therapy; D4T, stavudine; EFV, efavirenz; IQR, interquartile range; LPV/r, lopinavir plus ritonavir; NVP, nevirapine; PCP, *Pneumocystis jiroveci* pneumonia; PMTCT, prevention of mother-to-child transmission; TB, tuberculosis; 3TC, lamivudine; WHO, World Health Organization; ZDV, zidovudine.

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

Table 2. Resistance mutations by regimen.

ART experience, current treatment regimen	No. of patients	No. (%) of patients		
		⩾1 Significant mutation	Dual class	Triple class
First HAART[a]				
All	92	77 (83.7)	59 (64.1)	2 (2.2)
D4T, 3TC, and NNRTI	57	46 (80.7)	35 (61.4)	2 (3.5)
ZDV, 3TC, and NNRTI	31	28 (90.3)	23 (74.2)	0 (0)
2 NRTI and LPV/r	1	1 (100.0)	0 (0)	0 (0)
Other HAART	3	2 (66.7)	1 (33.3)	0 (0)
Prior ART[b]				
All	23	19 (82.6)	15 (65.2)	1 (4.3)
D4T, 3TC, and NNRTI	5	4 (80.0)	4 (80.0)	0 (0)
ZDV, 3TC, and NNRTI	12	10 (83.3)	10 (83.3)	0 (0)
2 NRTI and LPV/r	4	3 (75.0)	1 (25.0)	1 (25.0)
Other HAART	2	2 (100.0)	0 (0)	0 (0)
Total	115	96 (83.5)	74 (64.3)	3 (2.6)

NOTE. No significant difference was found between the first HAART group and the prior HAART group (using Fisher's exact test) or within groups between patients with and without significant mutations (using $\chi 2$ analysis). ART, antiretroviral therapy; D4T, stavudine; EFV, efavirenz; LPV/r, lopinavir plus ritonavir; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; 3TC, lamivudine; ZDV, zidovudine.

[a] Includes intraclass uninterrupted switches of therapy (i.e., from ZDV to D4T or from NVP to EFV; $n = 25$).

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

and protease inhibitor resistance mutations in virus from the other 3 might have represented 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 1 significant drug resistance mutation in virus from the patients who experienced virologic failure. In the univariate analysis, age <35 years was associated with drug-resistant infection (OR, 3.60; 95% CI, 1.11–11.63; $P = .03$), but age was not a statistically significant independent risk factor in the multivariate models that adjusted for recent opportunistic infection, CD4 cell count, and viral load (OR, 3.27; 95% CI, 0.92–11.63; $P = .068$). Patients with viral loads of 5000–99,999 copies/mL at study enrollment were more likely to have drug-resistant virus, but this association was of marginal statistical significance in the univariate analysis and was not statistically significant in the multivariate analysis that adjusted for age, recent opportunistic infection, and CD4 cell count (table 4).

DISCUSSION

Great strides have been made over the past 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 a cohort of HIV-1 subtype C–infected patients who experienced failure of a first HAART regimen in 2 large clinics in Durban, South Africa. Results of this study demonstrated the presence of at least 1 major drug resistance mutation in virus from plasma samples obtained from >83% of patients. Mutations conferring resistance to drugs in 2 classes were present in viruses from more than one-half of the patients tested, but triple-class resistance was relatively uncommon (occurring in 2.6% of viruses). A similarly high prevalence of ARV drug resistance was reported among samples from patients who experienced treatment failure in Zimbabwe and Uganda [12–14].

The drug resistance mutations identified in this study were similar to those reported by other studies involving patients infected with HIV-1subtype C [15–18]. The relatively high frequency of V106M, compared with V106A, in reverse transcriptase confirms previous reports that V106M is the favored NNRTI resistance mutation in HIV-1subtype C [19–21]. The M184V mutation was the most common mutation detected. In addition, virus from most patients had at least 1 significant NNRTI resistance mutation, with K103N being the most common. As expected, there were few significant protease inhibitor mutations because of the infrequent use of protease inhibitor–containing regimens.

These results are consistent with the use of NNRTIs in the first-line regimens provided by the South Africa National Plan. The prevalence of TAMs was relatively low (32%). This finding contrasts with data from the Development of Anti-Retroviral Therapy in Africa study, which noted the presence of TAMs in samples from more than one-half of viremic patients receiving

Table 3. Frequency of selected resistance mutations in the reverse transcriptase and protease genes.

Mutation	No. (%) of patients (n = 115)
NRTI resistance	
M41L	12 (10.4)
A62V	2 (1.7)
K65R	3 (2.6)
D67N	23 (20.0)
Insertion 69	0 (0)
K70R/E	19 (16.5)
L74V	2 (1.7)
V75I	3 (2.6)
F77L	0 (0)
Y115F	0 (0)
F116Y	0 (0)
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 and 2 pathways[a]	7 (6.1)
Total with any TAMs[b]	37 (32.2)
⩾1 NRTI resistance mutation	81 (70.4)
Total NRTI resistance mutations	170
NNRTI resistance	
L100I	4 (3.5)
K103N	59 (51.3)
V106A	1 (0.9)
V106M	22 (19.1)
V108I	14 (12.2)
Y181C/I	11 (9.6)
Y188C/L/H	12 (10.4)
G190A/S	18 (15.7)
P225H	8 (7.0)
M230L	3 (2.6)
⩾1 NNRTI resistance mutation	90 (78.3)
Total NNRTI resistance mutations	152
PI resistance	
D30N	0 (0)
V32I	0 (0)
L33F/I	2 (1.7)
M46I/L	2 (1.7)
I47V/A	0 (0)
G48V	0 (0)
I50V	0 (0)
I54V	1 (0.9)
V82A/T/F/S	1 (0.9)
I84V	0 (0)
L90M	1 (0.9)
⩾1 PI resistance mutation	5 (4.4)
Total PI resistance mutations	7

NOTE. NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor; TAM, thymidine analog mutation.

[a] Percentage of patients with at least 1 mutation in each of these 2 pathways: TAM 1 (41L, 210W, and 215Y) and TAM 2 (67N, 70R, 215F, and 219Q/E/N/R).

[b] Total percentage of patients with TAM 1 and/or TAM 2.

Table 4. Univariate and multivariate analysis of variables associated with virologic failure and at least 1 significant drug resistance mutation.

Variable	Mutation rate, %	Univariate analysis		Multivariate analysis	
		OR (95% CI)	*P*	OR (95% CI)	*P*
Age, years					
<35	92	3.60 (1.11– 11.63)	.03	3.27 (0.92– 11.63)	.068
⩾35	77	1.00		1.00	
Sex					
Male	82	0.79 (0.30– 2.13)	.65	…	
Female	85	1.00		…	
Employed					
Yes	78	0.40 (0.14– 1.21)	.10	…	
No	90	1.00		…	
Recent OI (within 6 months before study enrollment)					
Yes	88	2.44 (0.90– 6.64)	.08	2.20 (0.70– 6.88)	.175
No	75	1.00		1.00	
Symptoms (within 1 week before study enrollment)					
Yes	81	0.56 (0.17– 1.83)	.33	…	
No	89	1.00		…	
CD4 cell count at study enrollment, cells/mm^3					
<200	84	0.87 (0.30– 2.57)	.81	0.87 (0.23– 3.33)	.838
⩾200	86	1.00		1.00	
Plasma HIV RNA level at study enrollment, copies/mL					
<5000	77	1.37 (0.39– 4.88)	.08	1.05 (0.23– 4.81)	.103
5000–29,999	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 level, g/dL					
<11	92	2.82 (0.60– 13.20)	.17	…	
⩾11	81	1.00		…	
WHO clinical stage at study enrollment					
IV	86	1.39 (0.37– 5.26)	.63	…	
I, II, or III	82	1.00			
Treatment adherence ⩾95%					
Yes	85	1.96 (0.61– 6.32)	.25	…	
No	74	1.00		…	
Taking traditional medications					
Yes	75	0.54 (0.15– 1.88)	.33	…	
No	85	1.00		…	
ZDV and 3TC vs. D4T, 3TC, and NNRTI					
D4T and 3TC	81	2.23 (0.57– 8.70)	.24	…	
ZDV and 3TC	90	1.00		…	
Prior ART vs. first HAART					
Prior ART	83	0.93 (0.28– 3.11)	.90	…	
First HAART	84	1.00		…	

NOTE. ART, antiretroviral therapy; D4T, stavudine; NNRTI, nonnucleoside reverse-transcriptase inhibitor; OI, opportunistic infection; 3TC, lamivudine; WHO, World Health Organization; ZDV, zidovudine.

a regimen of tenofovir, lamivudine, and zidovudine for 24 weeks and in samples from >80% of patients after 48 weeks of such therapy [22]. Although the precise duration of virologic failure experienced by our patients is not known, it is likely that routine monitoring of plasma HIV-1 RNA levels 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 treatment regimens are less likely to select TAMs because of the combination of 2 NRTIs plus an NNRTI, compared with the triple-NRTI regimen used in

the Development of Anti-Retroviral Therapy in Africa study. In addition, the finding of fewer TAMs in non–subtype B virus agrees with a previous report [23].

Among those samples in which TAMs were detected, we found TAM-1, TAM-2, and mixed patterns of mutations. TAM-1 confers resistance to zidovudine and stavudine and cross-resistance to multiple NRTIs, whereas the resistance conferred by TAM-2 is usually limited to zidovudine and stavudine [24, 25]. Data from patients in Botswana suggest that, in HIV-1 subtype C, T215Y occurs in combination with D67N and K70R, rather than with M41L and L210W, as in HIV-1 subtype B [26]. By contrast, we noted the presence of M41L together with T215Y (with or without L210W) in samples from 7 patients. Similarly, K65R is thought to commonly emerge in HIV-1 subtype C [27], but we detected this mutation in samples from only 3 patients. These findings suggest that drug resistance testing of a larger number of HIV-1 subtype C–infected patients who have experienced ARV therapy failure needs to be performed to define subtype C–specific patterns of resistance mutations.

Univariate analyses suggested that plasma HIV-1 RNA levels >100,000 copies/mL and <5000 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 viral loads were not taking their prescribed ARV medications [28, 29]. In addition, patients with much lower viral loads may either have less successful laboratory amplification for genotyping or experience early virologic failure because of nonadherence (prior to reaching the pretreatment setpoint). These findings could be potentially useful as a means of stratifying individuals who are likely to yield a relevant result by genotyping when seen in clinics for locations with resource limitations. A surprising finding was the lack of association between prior suboptimal ARV therapy and resistance to the current failing regimen, because 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 treatment adherence and drug resistance. The metrics used to measure adherence—pill count and patient self-report—may overestimate adherence [30]. In fact, very few patients in our study reported <95% treatment 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 an electronic medication monitoring system [31].

This study has several limitations. Because we could not capture information about MHC and SMC patients who were not enrolled in this cohort, we were unable to compare the characteristics of patients who experienced virologic failure with the characteristics of 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 level, were unavailable prior to the start of ARV therapy and/or prior to virologic 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 to provide a more detailed picture of these predictors.

In conclusion, virus from a large percentage of patient who experienced 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, in addition to 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 to provide treatment options for patients who have experienced failure of a first-line treatment regimen.

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References

1. The United Nations Joint Programme on HIV/AIDS (UNAIDS). 2006 report on the global AIDS epidemic. A UNAIDS 10th anniversary special edition. Geneva: UNAIDS. Available at: http://www.unaids.org/en/knowledgecentre/hivdata/globalreport/default.asp. Accessed 15 August 2007.

2. Kuritzkes DR. Extending antiretroviral therapy to resource-poor settings: implications for drug resistance. AIDS **2004**; 18(Suppl 3):S45–8.
3. Lange JM, Perriens J, Kuritzkes D, Zewdie D. What policymakers should know about drug resistance and adherence in the context of scaling-up treatment of HIV infection. AIDS **2004**; 18(Suppl 3):S69–74.
4. Severe P, Leger P, Charles M, et al. Antiretroviral therapy in a thousand patients with AIDS in Haiti. N Engl J Med **2005**; 353:2325–34.
5. Egger M. Outcomes of ART in resource-limited and industrialized countries [abstract 62]. In: Program and abstracts of the 14th Conference on Retroviruses and Opportunistic Infections (Los Angeles). Alexandria, VA: Foundation for Retrovirology and Human Health, **2007**:62.
6. South Africa Government online: the Government and Information System, Pretoria, South Africa. Available at: http://www.gov.za. Accessed 15 August 2007.
7. Napravnik S, Keys JR, Quinlivan EB, Wohl DA, Mikeal OV, Eron JJ Jr. Triple-class antiretroviral drug resistance: risk and predictors among HIV-1–infected patients. AIDS **2007**; 21:825–34.
8. Wheeler W MK, Bodnar U, Kline R, Hall I, McKenna M; US Variant, Atypical and Resistant HIV Surveillance (VARHS) Group. Antiretroviral drug-resistance mutations and subtypes in drug-naïve persons newly diagnosed with HIV-1 infection, US, March 2003 to October 2006 [abstract 648]. In: Program and abstracts of the 14th Conference on Retroviruses and Opportunistic Infections (Los Angeles). Alexandria, VA: Retrovirology and Human Health, **2007**:305.
9. Jackson JB, Becker-Pergola G, Guay LA, et al. Identification of the K103N resistance mutation in Ugandan women receiving nevirapine to prevent HIV-1 vertical transmission. AIDS **2000**; 14:F111–5.
10. Eshleman SH, Mracna M, Guay LA, et al. Selection and fading of resistance mutations in women and infants receiving nevirapine to prevent HIV-1 vertical transmission (HIVNET 012). AIDS **2001**; 15: 1951–7.
11. Cunningham CK, Chaix ML, Rekacewicz C, et al. Development of resistance mutations in women receiving standard antiretroviral therapy who received intrapartum nevirapine to prevent perinatal human immunodeficiency virus type 1 transmission: a substudy of pediatric AIDS clinical trials group protocol 316. J Infect Dis **2002**; 186:181–8.
12. Weidle PJ, Malamba S, Mwebaze R, et al. Assessment of a pilot antiretroviral drug therapy programme in Uganda: patients' response, survival, and drug resistance. Lancet **2002**; 360:34–40.
13. Kantor R, Zijenah LS, Shafer RW, et al. HIV-1 subtype C reverse transcriptase and protease genotypes in Zimbabwean patients failing antiretroviral therapy. AIDS Res Hum Retroviruses **2002**; 18:1407–13.
14. Richard N, Juntilla M, Abraha A, et al. High prevalence of antiretroviral resistance in treated Ugandans infected with non-subtype B human immunodeficiency virus type 1. AIDS Res Hum Retroviruses **2004**; 20: 355–64.
15. Nukoolkarn S, Pongthapisith V, Panyim S, Leelamanit W. Sequence variability of the HIV type 1 protease gene in Thai patients experienced with antiretroviral therapy. AIDS Res Hum Retroviruses **2004**; 20: 1368–72.
16. Brindeiro PA, Brindeiro RM, Mortensen C, et al. Testing genotypic and phenotypic resistance in human immunodeficiency virus type 1 isolates of clade B and other clades from children failing antiretroviral therapy. J Clin Microbiol **2002**; 40:4512–9.
17. Petch LA, Hoffman IF, Jere CS, et al. Genotypic analysis of the protease and reverse transcriptase of HIV type 1 subtype C isolates from antiretroviral drug–naive adults in Malawi. AIDS Res Hum Retroviruses **2005**; 21:799–805.
18. Pillay D, Walker AS, Gibb DM, et al. Impact of human immunodeficiency virus type 1 subtypes on virologic response and emergence of drug resistance among children in the Paediatric European Network for Treatment of AIDS (PENTA) 5 trial. J Infect Dis **2002**; 186:617–25.
19. Kantor R, Katzenstein DA, Efron B, et al. Impact of HIV-1 subtype and antiretroviral therapy on protease and reverse transcriptase genotype: results of a global collaboration. PLoS Med **2005**; 2:e112.
20. Loemba H, Brenner B, Parniak MA, et al. Genetic divergence of human immunodeficiency virus type 1 Ethiopian clade C reverse transcriptase (RT) and rapid development of resistance against nonnucleoside inhibitors of RT. Antimicrob Agents Chemother **2002**; 46:2087–94.
21. Brenner B TD, Oliveira M, Moisi D, et al. A V106M mutation in HIV-1 clade C viruses exposed to efavirenz confers cross-resistance to nonnucleoside reverse transcriptase inhibitors. AIDS **2003**; 17:F1–5.
22. DART Virology Group and Trial Team. Virological response to a triple nucleoside/nucleotide analogue regimen over 48 weeks in HIV-1–infected adults in Africa. AIDS **2006**; 20:1391–9.
23. Montes B, Vergne L, Peeters M, Reynes J, Delaporte E, Segondy M. Comparison of drug resistance mutations and their interpretation in patients infected with non-B HIV-1 variants and matched patients infected with HIV-1 subtype B. J Acquir Immune Defic Syndr **2004**; 35:329–36.
24. Marcelin AG, Delaugerre C, Wirden M, et al. Thymidine analogue reverse transcriptase inhibitors resistance mutations profiles and association to other nucleoside reverse transcriptase inhibitors resistance mutations observed in the context of virological failure. J Med Virol **2004**; 72:162–5.
25. Miller MD, Margot N, Lu B, et al. Genotypic and phenotypic predictors of the magnitude of response to tenofovir disoproxil fumarate treatment in antiretroviral-experienced patients. J Infect Dis **2004**; 189: 837–46.
26. Novitsky V, Wester CW, DeGruttola V, et al. The reverse transcriptase 67N 70R 215Y genotype is the predominant TAM pathway associated with virologic failure among HIV type 1C-infected adults treated with ZDV/ddI-containing HAART in southern Africa. AIDS Res Hum Retroviruses **2007**; 23:868–78.
27. Doualla-Bell F, Avalos A, Brenner B, et al. High prevalence of the K65R mutation in human immunodeficiency virus type 1 subtype C isolates from infected patients in Botswana treated with didanosine-based regimens. Antimicrob Agents Chemother **2006**; 50:4182–5.
28. Bangsberg DR, Moss AR, Deeks SG. Paradoxes of adherence and drug resistance to HIV antiretroviral therapy. J Antimicrob Chemother **2004**; 53:696–9.
29. King MS, Brun SC, Kempf DJ. Relationship between adherence and the development of resistance in antiretroviral-naive, HIV-1–infected patients receiving lopinavir/ritonavir or nelfinavir. J Infect Dis **2005**; 191:2046–52.
30. Wagner GJ, Rabkin JG. Measuring medication adherence: are missed doses reported more accurately then perfect adherence? AIDS Care **2000**; 12:405–8.
31. Amico KR, Fisher WA, Cornman DH, et al. Visual analog scale of ART adherence: association with 3-day self-report and adherence barriers. J Acquir Immune Defic Syndr **2006**; 42:455–9.