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Chapter 5

HIV-1 drug resistance mutations are present in six percent of persons initiating antiretroviral therapy in Lusaka, Zambia

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ABSTRACT

Objective

To assess the mutational patterns and factors associated with baseline drug-resistant HIV-1 present at initiation of first-line antiretroviral therapy (ART) at 3 sites in Lusaka, Zambia, in 2007–2008.

Methods

Population sequencing of the HIV-1 pol gene was performed in the PharmAccess African Studies to Evaluate Resistance Monitoring cohort. Drug resistance–associated mutations (DRMs) were identified using the WHO 2009 Surveillance DRM list. Multiple logistic regression was used to assess factors associated with baseline resistance.

Results

The overall prevalence of baseline resistance was 5.7% [31 of 548 participants; 95% confidence interval (CI): 3.9 to 7.9]; the prevalence of DRMs associated with nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors was 1.1%, 4.0%, and 1.1%, respectively. Resistance prevalence was 5.2% (27 of 523) in antiretroviral-naive and 16.0% (4 of 25) in antiretroviral-experienced (ie, previous use of ART or antiretroviral prophylaxis for prevention of mother-to-child transmission) participants (P = 0.022). Dual-class resistance to NRTIs and NNRTIs was observed in 0.6% of participants. HIV-1 subtype C was identified in 98.0% (537 of 548) of participants. Prior antiretroviral experience (odds ratio: 4.32, CI: 1.34 to 14.0, P =0.015) and hemoglobin level (highest tertile versus lowest tertile odds ratio: 2.74, CI: 1.09 to 6.89, P = 0.033) were independently associated with baseline resistance.

Conclusions

Baseline resistance may compromise the response to standard NNRTI-based first-line ART in 6% of patients in Lusaka, Zambia. Continuous resistance monitoring is warranted to maintain individual and population-level ART effectiveness.

INTRODUCTION

Access to combination antiretroviral therapy (ART) for HIV-1–infected persons and antiretroviral (ARV) prophylaxis for prevention of mother-to-child transmission of HIV-1 (PMTCT) in sub-Saharan Africa has greatly expanded during the past 5 years [1]. In resource-limited settings where access to routine HIV-1 viral load monitoring is lacking and where the unregulated use of ARV drugs may be common, the selection of drug-resistant HIV-1 variants [2] and their subsequent transmission to newly infected individuals [3–6] is of particular concern, especially since second-line treatment options are limited. Few studies have assessed the mutational patterns associated with HIV-1 drug resistance among pre-treatment populations in sub-Saharan Africa in which drug pressure is increasing after ARV rollout [7–12].

The government of Zambia, a country in southern Africa, which is among the countries worst affected by the HIV-1 pandemic, initiated a comprehensive HIV-1 care and treatment program with support from international agencies [13]. By the end of 2007, nation-wide ART coverage was 46% of those in need [1]. Standard first-line ART regimens combine a dual nucleoside/nucleotide reverse transcriptase inhibitor (NRTI) backbone with a non-nucleoside reverse transcriptase inhibitor (NNRTI) [14].

The objective of this study was to evaluate the mutational patterns and factors associated with baseline drug resistance in HIV-1–infected individuals present at time of initiating first-line ART in the geographical setting of Lusaka, Zambia, where ART first became available in the country.

METHODS

Study population and design

The PharmAccess African Studies to Evaluate Resistance Monitoring Study is a multicenter prospective observational cohort of HIV-1–infected patients who receive ART in routine circumstances at 13 clinical sites in 6 African countries [15]. We conducted a cross-sectional analysis including 3 clinical sites in Lusaka, Zambia: Lusaka Trust Hospital, a private general hospital (Woodlands area); KARA Clinic, a free nongovernment sector clinic (city center); and, Coptic Hospital, a free faith-based general hospital (Manda Hill area). The 3 sites have provided HIV-1 care and treatment since 1997, 2004, and 2006, respectively. The Academic Medical Center Institutional Review Board and the University of Zambia Research Ethics Committee approved all study procedures. Confirmed HIV-1 seropositive individuals aged ≥18 years who were eligible to initiate first-line ART

as defined by national guidelines (i.e., advanced immunodeficiency as defined by CD4 count, 200 cells/mL or advanced disease according to the World Health Organization (WHO) clinical stages) [14] were consecutively enrolled. All participants provided written informed consent for use of routinely collected demographic, clinical, and laboratory data and additional phlebotomy for assessment of HIV-1 RNA and genotypic resistance. Exclusion criteria were pregnancy at study screening and re-initiation on first-line ART less than 30 days after stopping previous first-line ART. Re-initiation on first-line ART more than 30 days after stopping previous first-line ART and/or any previous use of ARV prophylaxis or non-suppressive mono/dual therapy were not exclusion criteria (ARV-experienced group).

Laboratory methods

HIV-1 RNA determination was performed on EDTA-anticoagulated plasma using the NucliSens EasyQ real-time assay version 1.2 (bioMérieux, Lyon, France). Population-based sequencing was performed on all plasma specimens which had HIV-1 RNA >1000 copies per milliliter using an in-house method [16]. Briefly, HIV-1 RNA was extracted from 200 mL of plasma using the automated Roche MagNa Pure LC analyzer and the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche, Germany). Genotyping encompassed protease and codons 1–230 of reverse-transcriptase, using an in-house sequencing method with an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences were assembled and manually edited using Sequencher version 4.5 software (Genecodes, Ann Arbor, MI). GenBank accession numbers: HM119603–HM120150.

Genotypic resistance analysis and subtyping

Baseline resistance was defined as the presence of ≥1 Drug resistance–associated mutation (DRM) according to the WHO 2009 Surveillance Drug Resistance Mutation list [17] using the Stanford Calibrated Population Resistance analysis tool (version 4.1 beta, available at http://hivdb.stanford.edu/). HIV-1 subtypes were determined using the REGA HIV-1 subtyping algorithm (version 2.0, available at http://www.bioafrica.net/subtypetool/html/) [18] and additional phylogenetic analysis using neighbor-joining method if required.

Statistical methods

Univariate and multivariate analyses were used to determine factors associated with drug resistance using logistic regression and expressed as odds ratios (ORs) (95% confidence interval, CI) and P values ($P < 0.05$ statistically significant). Prevalence values were calculated with a CI based on the binomial distribution. Categorical data were compared using Chi^2 test. Continuous data were investigated using Student t test. All analyses were performed using Stata version 10 (StataCorp LP, TX).

RESULTS

Study population

Between March 2007 and September 2008, a total of 839 adult men and non-pregnant women were recorded to initiate first-line ART at the 3 sites. Screening efforts resulted in the enrolment in the study of 584 individuals who met eligibility criteria and provided consent (i.e., 70% recruitment rate). A valid baseline HIV-1 RNA result was available for 576 (98.6%) participants. HIV-1 RNA was >1000 copies per milliliter in 556 (96.5%); of those, sequence analysis was successful in 548 (98.6%). Table 1 summarizes the demographic characteristics of all 584 participants. Females comprised 54.8% (n = 320) and were younger than males [36.1 (SD 8.8) versus 40.0 (SD 8.8) years, P<0.0001]. All participants were native Zambians. 321 (55.0%) participants had advanced stage disease (ie, WHO stage III or IV). Median CD4 count was 132 cells per microliter (interquartile range, IQR: 69–203). Mean HIV-1 RNA was 5.0 (SD: 0.9) $\log_{10}$ copies/mL. Twenty-seven (4.6%) participants had previous ARV-experience, either as (highly active) ART (n = 14), single-dose nevirapine for PMTCT (n = 5), combination therapy for PMTCT (n = 1), or unspecified (n = 7). Patient characteristics neither differed between ARV-naive versus ARV-experienced participants (table 1) nor between sites (data not shown).

Table 1. Characteristics of participants (n=584) with and without previous antiretroviral experience *

Characteristic	Total	ARV naive	ARV experienced *	p-value
Participants – no. (%)	584	557 (95.4)	27 (4.6)	
Age (years) – mean (sd)	37.9 (9.0)	38.0 (9.1)	35.4 (7.6)	.1454
Sex – no. (%)				
Female	320 (54.8)	301 (54.0)	19 (70.4)	.096
Male	264 (45.2)	256 (46.0)	8 (29.6)	
WHO clinical stage – no. (%)				.318
Early (I/II)	263 (45.0)	252 (45.2)	11 (40.7)	
Advanced (III/IV)	321 (55.0)	305 (55.8)	16 (59.4)	
Hemoglobin (g/dL) - median (IQR) †	11.2 (9.9-12.7)	11.2 (2.7)	11 (2.9)	.6645
CD4 count (cells/µl) - median (IQR) ᶜ	132 (69-203)	130 (63-193)	152 (81-223)	.4063
HIV-1 RNA ($\log_{10}$ c/mL) - mean (SD) ‡	5.0 (.9)	4.9 (.9)	5.1 (.7)	.2993

Data are no. (%) of participants, unless otherwise indicated. *Previous antiretroviral experience was defined as re-initiation on first-line ART (more than 30 days after stopping previous first-line ART), and/or any previous use of ARV prophylaxis or non-suppressive mono/dual therapy; previous ARV experience among n = 27 participants comprised previous (highly active) ART (n = 14), single-dose nevirapine for PMTCT (n = 5), combination therapy for PMTCT (n = 1), and unspecified (n = 7). †Data available for n = 580. ‡Data available for n = 576. ART, antiretroviral therapy; ARV, antiretroviral; IQR, interquartile range; NVP, nevirapine; WHO, World Health Organization.

Frequencies of subtypes and drug resistance–associated mutations

Subtype C was identified in 98.0% of participants (537 of 548). Other subtypes and circulating recombinant forms (CRFs) were subtype A1 (0.5%, 3 of 548), CRF02_AG (0.5%, 3 of 548), G (0.4%, 2 of 548), CRF09_cpx (0.4%, 2 of 548), and D (0.2%, 1 of 548). The overall prevalence of resistance was 5.7% (31 of 548 participants; CI: 3.9% to 7.9%); the prevalence of DRMs associated with NRTIs, NNRTIs, and protease inhibitors (PIs) was 1.1% (6 of 548), 4.0% (22 of 548), and 1.1% (6 of 548), respectively (figure 1). Among ARV-naive participants, the prevalence of resistance was 5.2% (27 of 523; CI: 3.4% to 7.4%); the prevalence of DRMs associated with NRTIs, NNRTIs, and PIs was 1.0% (5 of 523), 3.6% (19 of 523), and 1.1% (6 of 523), respectively. Among ARV-experienced participants, the prevalence of resistance was 16.0% (4 of 25 participants; CI: 4.5% to 36.1%); the prevalence of DRMs associated with NRTIs, NNRTIs, and PIs was 4.0% (1 of 25), 12.0% (3 of 25), and 0.0% (0 of 25), respectively. Detected DRMs were Y181C (1.6%, 9 of 548), K103N (1.3%, 7 of 548), K103S (0.7%, 4 of 548), G190A (0.7%, 4 of 548), L100I (0.4%, 2 of 548), K101E (0.4%, 2 of 548), M184V (0.4%, 2 of 548), V106M (0.2%, 1 of 548), Y188C (0.2%, 1 of 548), G190S (0.2%, 1 of 548), K65R (0.2%, 1 of 548), T69D (0.2%, 1 of 548), K70R (0.2%, 1 of 548), K70E (0.2%, 1 of 548), L74I (0.2%, 1 of 548), V75S (0.2%, 1 of 548), V75T (0.2%, 1 of 548), and K219E (0.2%, 1 of 548) in reverse transcriptase and L90M (0.7%, 4 of 548), I85V (0.2%, 1 of 548), and I50L (0.2%, 1 of 548) in protease. DRM frequencies did not differ significantly across sites (data not shown). Table 2 provides an overview of demographic and virologic characteristics of the 31 participants who harbored ≥1 DRM. Dual-class re-

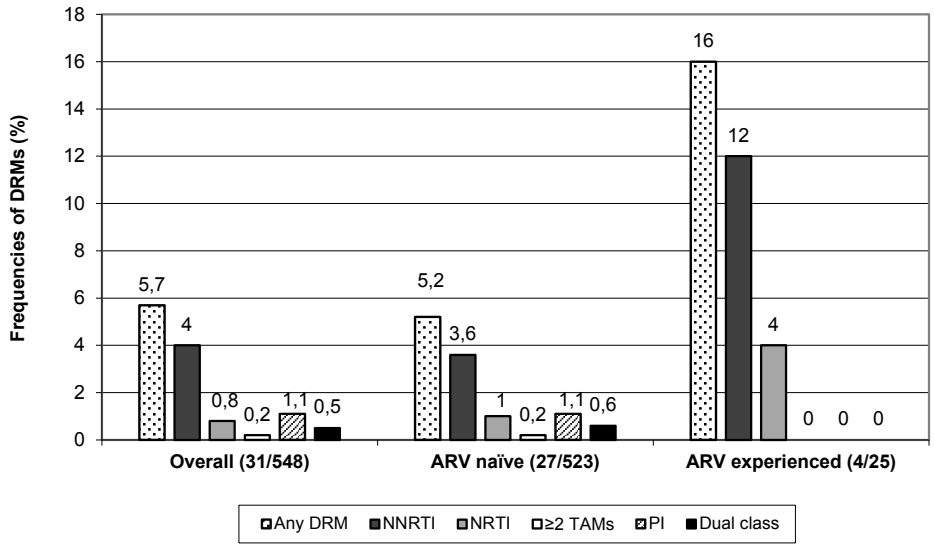

Figure 1. Frequencies of drug resistance–associated mutations in all participants and separately for antiretroviral-naive and antiretroviral-experienced participants.

Table 2. Demographic and virologic characteristics of participants (n=31) who harbored ≥1 drug resistance-associated mutation.

#	Specimen ID	Age (years)	Sex	ARV history	CD4 count (cells/µl)	HIV-1 RNA (log_{10} c/ml)	Genetic sub-type*	Drug resistance-associated mutations†		
								NRTI	NNRTI	PI
1	00011	27	female	naive	42	5.5	C		Y181C, G190A	
2	00035	25	female	naive	122	3.8	C		L100I	
3	00061	48	male	naive	98	4.8	C	M184V	K103S, V106M	
4	00067	38	female	naive	32	5.9	C		K103N, Y181C	
5	00068	40	male	naive	193	5.0	C		G190S	
6	00073	49	male	naive	156	5.3	C			L90M
7	00083	40	female	naive	46	5.9	C			L90M
8	00138	29	female	naive	83	5.7	C		K101E	
9	00143	32	female	single-dose NVP (2002)	169	5.2	C	L74I, V75S		
10	00145	37	female	3TC+d4T+NVP (Sep05-Jun06)	9	5.9	C		G190A	
11	00182	39	female	naive	99	5.7	C		K103N	
12	00197	40	male	naive	16	4.7	C			I50L
13	00279	38	female	naive	26	4.5	C	K65R, V75T	Y181C	
14	00409	18	male	naive	187	5.0	C			L90M
15	00417	36	male	naive	219	4.3	C			L90M
16	00447	36	female	naive	211	4.2	C		L100I	
17	00455	38	female	naive	161	5.2	C		G190A	
18	00483	22	female	naive	122	5.3	C		K103N	
19	00515	56	male	naive	24	3.6	C		K103N, K103S	
20	00521	55	female	naive	159	4.1	C		K103S	
21	00539	42	male	naive	77	4.1	C		Y181C	

Table 2 (continued)

#	Specimen ID	Age (years)	Sex	ARV history	CD4 count (cells/µl)	HIV-1 RNA (log_{10} c/ml)	Genetic subtype *	Drug resistance-associated mutations†		
22	00570	34	male	naive	118	4.9	C	T69D		
23	00580	35	male	AZT+3TC+NVP (Mar-May06)	159	5.0	C		Y181C	
24	00624	37	male	naive	249	4.0	C		K103N	
25	00652	27	female	naive	106	4.7	C	K70R, M184V, K219E	K101E, Y181C, G190A	
26	00666	30	male	AZT+3TC+NVP (Nov05-Apr06)	216	4.4	C		Y181C	
27	00670	45	male	naive	44	5.3	C			I85V
28	00671	32	female	naive	181	4.3	C		K103S	
29	02368	33	female	naive	93	6.2	C		K103N, Y181C	
30	02449	39	male	naive	80	5.1	C		K103N, Y181C, Y188C	
31	03977	46	male	naive	81	4.9	C	K70E		

* HIV-1 subtypes were determined from the pol sequences using the REGA HIV-1 subtyping algorithm (version 2.0, available at http://www.bioafrica.net/subtypetool/html/) and, for sequences with ambiguous subtype assignment, additional phylogenetic analysis (neighbor-joining method). † HIV-1 genotypic sequence analysis encompassing protease and partial reverse transcriptase; drug-resistance–associated mutations according to the WHO 2009 Surveillance Drug Resistance Mutation list. AZT, zidovudine; 3TC, lamivudine; d4T, stavudine; NVP, nevirapine

Table 3. Factors associated with HIV-1 genotypic baseline drug resistance.

				Univariate		Multivariate	
Characteristic *	**Total**	**DR**	**No DR**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
Participants – no. (%)	548	31 (5.7)	517 (94.3)				
Age (years) – mean (SD)	37.8 (8.9)	37.3 (8.8)	37.8 (8.9)	.99 (.95 to 1.03)	.732		
Sex – no. (%)							
Female	296 (54.0)	16 (51.6)	280 (54.2)	reference			
Male	252 (46.0)	15 (48.4)	237 (45.8)	1.11 (.54 to 2.3)	.782		
WHO clinical stage III/IV – no. (%)							
Early (I/II)	244 (44.5)	17 (54.8)	227 (43.9)	reference			
Advanced (III/IV)	304 (55.5)	14 (45.2)	290 (56.1)	.64 (.31 to 1.34)	.237		
History of ARV drug use – no. (%)							
ARV naive	523 (95.4)	27 (87.1)	496 (95.9)	reference		reference	
ARV experienced †	25 (4.6)	4 (12.9) [a]	21 (4.1)	3.50 (1.12 to 10.9)	.031	4.32 (1.34 to 14.0)	.015 ‡
Site							
LTH	109 (19.9)	6 (19.4)	103 (19.9)	reference			
KC	213 (38.9)	13 (41.9)	200 (38.7)	1.12 (.41-3.02)	.829		
CH	226 (41.2)	12 (38.7)	214 (41.4)	.96 (.35-2.64)	.941		
HIV-1 subtype					1.0 §		
C	537 (98.0)	31 (100.0)	506 (97.9)	reference			
Other «	11 (2.0)	0 (0.0)	11 (2.1)	n/a [f]	n/a §		
Hemoglobin (g/dL) - median (IQR) ¶	11.1 (9.8-12.7)	11.9 (10.5-13.4)	11.1 (9.6-12.6)				
Lowest tertile	189 (34.7)	8 (25.8)	181 (35.3)	reference		reference	
Middle tertile	178 (32.7)	8 (25.8)	170 (33.1)	1.06 (.39 to 2.90)	.902	1.20 (.43 to 3.32)	.723
Highest tertile	177 (32.5)	15 (48.4)	162 (31.6)	2.09 (.87 to 5.07)	.101	2.74 (1.09 to 6.89)	.033 ‡
CD4 count (cells/µl) - median (IQR)	129.5 (68 – 200)	106 (44.5-167.5)	132 (65-199)	.73 (.47 to 1.13) #	.162	0.65 (.41 to 1.02) #	.063
HIV-1 RNA (log_{10} c/mL) - mean (SD)	5.06 (0.7)	4.91 (0.7)	5.07 (0.8)	.76 (.48 to 1.22)	.261		

Data are no. (%) of participants, unless otherwise indicated. *Characteristics describe participants from whom a baseline HIV-1 genotypic sequence analysis was available (n = 548). † Previous antiretroviral experience was defined as re-initiation on first-line ART more than 30 days after stopping previous first-line ART and/or any previous use of ARV prophylaxis or non-suppressive mono/dual therapy; ARV-experience among n = 25 participants with versus without DR comprised previous (highly active) ART (n = 3 versus 10), single-dose nevirapine for PMTCT (n = 1 versus 4), combination therapy for PMTCT (n = 0 versus 1) and unspecified (n = 0 versus 6). ‡Statistically significant results (P<0.05).§ Logistic analysis not valid for this variable; P value by Fisher exact. « Other subtypes and circulating recombinant forms comprised A1 (n = 3), CRF02_AG (n = 3), G (n = 2), CRF09_cpx (n = 2), and D (n = 1). ¶ Data available for n = 544. # OR for a 100-cell increase of CD4 count. ART, antiretroviral therapy; ARV, antiretroviral; CH, Coptic Hospital; CI, confidence interval; CRFs, circulating recombinant forms; DR, genotypic drug resistance; IQR, interquartile range; KC, KARA Clinic; LTH, Lusaka Trust Hospital; n/a, not applicable; NVP, nevirapine; OR, odds ratio; WHO, World Health Organization.

sistance to NRTIs and NNRTIs was detected in 0.6% (3 of 523) of ARV-naive participants, and no triple-class resistance was observed. One ARV-naive participant harbored M184V plus 2 thymidine analogue mutations (TAMs) by the TAMII pathway (K70R and K219E).

Factors associated with baseline drug resistance

In univariate analysis, participants with versus without resistance did not differ for age, sex, WHO clinical stage, median serum hemoglobin level, median CD4 count, mean HIV-1 RNA, and subtype, whereas previous ARV experience was more frequent among participants with drug resistance compared with those without resistance (12.9% versus 4.1%, P = 0.031) (table 3). Multiple logistic regression analysis of patient characteristics demonstrated that previous ARV use (versus ARV-naive status; OR: 4.32, CI: 1.34 to 14.0, P = 0.015) and hemoglobin level (highest tertile versus lowest tertile; OR: 2.74, CI: 1.09 to 6.89, P = 0.033) were independently associated with the presence of baseline resistance (table 3).

DISCUSSION

In this study, we investigated patterns of drug-resistant HIV-1 present at time of initiating first-line ART among 584 predominantly HIV-1 subtype C–infected patients in Lusaka, Zambia, enrolled in the PharmAccess African Studies to Evaluate Resistance Monitoring study during 2007–2008. Six (CI 3.9 to 7.9) percent of participants were found to harbor ≥1 DRM, based on population genotyping. Baseline resistance may reflect the combined effect of drug-resistant strains transmitted during infection and acquired during previous ARV exposure. The majority (95.4%) of participants were reported to be ARV naive at baseline; compared with ARV-naive status, resistance was more frequent after prior use of ART or PMTCT (16.0% versus 5.2%, P = 0.022). NNRTI-associated DRMs were observed in the highest frequency (4.0%), whereas dual-class or triple-class resistance was rarely observed. An important strength of the study was its large multisite patient sample, representative of a variety of clinic populations within the geographic setting of Lusaka.

Primary HIV-1 drug resistance in Zambia had only been assessed in 1 small study before ARV drugs became widely available, reporting no major drug resistance–associated mutations (DRMs) among 28 ARV-naive persons [19]. Population surveys [7–12] and mathematical models [20] to date have reported low levels of drug-resistant infections in African populations with increasing selective ARV drug pressure, but recent preliminary reports have suggested transmission of resistant strains directly after national ARV rollout programs [21, 22]. The predominance of NNRTI-associated DRMs (mostly Y181C and K103N/S) in this baseline study probably results from the widespread use of NNRTIs

as part of standard first-line ART and PMTCT regimens. Most solitary NNRTI-associated DRMs cause a complete loss of activity of efavirenz and nevirapine [23] which may compromise the initial response to the standard first-line therapy [22]. Moreover, NNRTI-associated DRMs have been shown to have only a modest impact on viral replicative fitness [23, 24], allowing them to persist in the absence of the drug and to establish infection in a new host. Sex was not found to be associated with NNRTI resistance, suggesting either a limited effect on baseline resistance from PMTCT in females or secondary transmission of PMTCT-acquired NNRTI-resistant strains.

We found baseline NRTI-associated resistance to be limited. This contrasts not only with industrialized countries where transmission of NRTI-associated resistance is predominant [3–6] but also with several recent African studies in subtype C–infected patients experiencing treatment failure which reported considerable rates of NRTI-associated DRMs, including TAMs and K65R [25–28]. Several mechanisms could be expected to play a role. First, ARV rollout in sub-Saharan Africa is based on (highly active) ART, and widespread access has been established only recently, whereas in the industrialized world, ARV drugs have been widely used for many years including non-suppressive mono and dual therapies with thymidine analogues in the past. Second, the reduced replicative fitness of variants harboring multiple TAMs and/or K65R might reduce transmission efficiency [29]. Third, underestimation of NRTI-associated DRMs is possible due to reversion to wild type and/or outgrowth of minority wild-type species over time in absence of drug-selective pressure resulting in a reduction of the mutant strains with poor replicative fitness to minor variants below the limit of detection of genotypic analysis [30–33].

Because of the infrequent use of PIs, we observed very few significant DRMs in protease. A few participants harbored clades D, G, CRF02_AG, and CRF09_cpx, which to our knowledge have not been described in Zambia before. Twenty specimens (3.5%) had unexpectedly low (<1000 copies/mL) HIV-1 RNA levels, which is most likely due to inter-individual variations in viral replication rates and immunologic control [34]; other reasons could include partial viral suppression because of any undisclosed recent ARV exposure and varied assay performance between viral subtypes [35–37]. As an additional observation, the presence of drug-resistant virus at baseline was found to be associated with a non-reduced serum hemoglobin level. A non-causal relation seems plausible, that is, advancing HIV infection leading to anemia as a result of bone marrow suppression [38], in parallel to the diminution of poorly replicating drug-resistant minor variants, as described above [30–33].

The study has several limitations. First, it cannot be completely ruled out that reportedly ARV-naive participants had unknown previous exposure to therapy and/or prophylaxis.

Second, the potential for selection bias exists, although the lack of heterogeneity in resistance pattern between the established private versus the 2 more recently introduced free ART programs argues against this. Third, data on route, country and duration of HIV-1 infection, and the source's ARV history, as possible associated factors of resistance, were not available.

In conclusion, this study on baseline HIV-1 resistance in routine ART programs in Lusaka, Zambia, adds important information regarding the predicted population-level response to standard first-line ART. Patients with previous ARV-experience are particularly at risk for a compromised initial response to standard NNRTI-based regimens. If baseline NNRTI resistance levels further increase, reassessment of first-line guidelines may be warranted to maintain individual and population-level benefits of ART. It is mandatory to monitor worldwide for the presence and spread of drug-resistant HIV-1.

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Contributors

R.L.H., M.S., C.L.W., W.S.S., M.V.V., R.S., T.F.R.D.W. designed the study. M.S., M.L., R.V.H. acquired the data. C.L.W., W.S.S. performed the laboratory testing. R.L.H., T.F.R.D.W. analyzed and interpreted the data. R.L.H. wrote the first draft of the article. C.L.W., R.S., A.M.J.W., T.F.R.D.W. critically revised the article for important intellectual content. All authors contributed to subsequent drafts and reviewed and approved the final article.

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