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Chapter 9

Patterns of HIV-1 drug resistance after first-line antiretroviral therapy failure in six sub-Saharan African countries: implications for second-line ART strategies

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ABSTRACT

Background

HIV-1 drug resistance may limit the benefits of combination antiretroviral therapy (ART). This cohort study examined patterns of drug-resistance mutations (DRMs) in individuals with virological failure on first-line ART at 13 clinical sites in six African countries, and predicted their impact on second-line drug susceptibility.

Methods

A total of 2588 HIV-1 infected antiretroviral-naïve individuals initiated ART consisting of different nucleoside reverse transcriptase inhibitor (NRTI) backbones (zidovudine, stavudine, tenofovir or abacavir, plus lamivudine or emtricitabine) with either efavirenz or nevirapine. Population sequencing after 12 months of ART was retrospectively performed if HIV-RNA >1000 copies/mL. The 2010 IAS-USA list was used to score major DRMs. Stanford algorithm was used to predict drug susceptibility.

Results

HIV-1 sequences were generated for 142 participants who virologically failed ART, of whom 70% carried ≥1 DRM and 49% had dual-class resistance, with an average of 2.4 DRMs per sequence (range 1–8). The most common DRMs were M184V (53.5%), K103N (28.9%), G190A (14.1%), Y181C (15.5%). Thymidine analogue mutations were present in 8.5%. K65R was frequently selected by stavudine (15.0%) or tenofovir (27.7%). Among participants with ≥1 DRM, the predicted HIV-1 susceptibility was reduced in 93% for efavirenz/nevirapine, in 81% for lamivudine/emtricitabine, in 59% for etravirine/rilpivirine, in 27% for tenofovir, in 18% for stavudine, and in 10% for zidovudine.

Conclusions

Early failure detection limited the accumulation of drug-resistance. After stavudine failure in African populations, zidovudine rather than tenofovir may be preferred in second-line ART. Strategies to prevent HIV-1 resistance are a global priority.

INTRODUCTION

The rapid scale-up of access to combination antiretroviral therapy (ART) for HIV-1 infected persons in sub-Saharan Africa during the past decade, through a WHO-recommended public-health approach [1], has dramatically reduced HIV-related mortality [2]. However, the widespread use of HIV clinical staging and, if available, CD4 cell counts to diagnose ART failure in resource-limited settings, rather than routine virological monitoring, is associated with the accumulation of HIV-1 drug-resistance mutations [3-5], which may limit subsequent drug options and constitutes a source for onward transmission. Studies from the region have reported high levels of drug resistance in individuals with prolonged first-line ART failure, including complex nucleoside reverse transcriptase inhibitor (NRTI) resistance profiles, such as K65R, Q151M and thymidine analogue mutations (TAMs), in addition to highly prevalent M184V and non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations [4-8].

Most reports on HIV-1 drug resistance deal with subtype B infections in developed countries. There is limited knowledge of resistance pathways in the different HIV-1 non-B subtypes and their clinical relevance [9], despite the fact that more than 90% of HIV-1 infections globally belong to non-B subtype variants [10].

The PASER-M study is a prospective cohort of HIV-1 infected individuals from 13 clinical sites in 6 sub-Saharan African countries who initiated first-line ART including different NRTI backbones (zidovudine [37%], tenofovir [34%], stavudine [27%] or abacavir [3%]) and NNRTIs (efavirenz [60%] or nevirapine [40%]), in accordance with national guidelines [11]. The present study assessed HIV-1 DRMs in participants with virological failure of different first-line regimens, and predicted viral drug susceptibility to gain insight about the optimal strategies for second-line therapy.

METHODS

Study population

The PASER-M study includes clinical sites in Kenya (2), Nigeria (1), South Africa (3), Uganda (3), Zambia (3), and Zimbabwe (1) [11]. We previously reported the participants' baseline resistance profiles [12], and the effect of pre-treatment drug resistance on the immunological and virological patient outcomes after the first year of ART [13].

For the present analysis, individuals were included if they were aged ≥18 years with HIV-1 infection and had initiated standard first-line NNRTI-based ART in accordance

with national guidelines- that is, advanced immunodeficiency (CD4 cell count < 200 cells/μL) or advanced HIV disease (WHO clinical stage 3 or 4) (13). Individuals who were previously exposed to any antiretroviral drugs for prevention and/or treatment, were excluded. Other exclusion criteria were pregnancy at study screening, or –screened in Nigeria only– HIV-2 co-infection.

Participants provided written informed consent at enrollment. The study protocol was approved by the appropriate national and local research ethics committees at all collaborating sites and the Academic Medical Center of the University of Amsterdam in The Netherlands.

Data collection

Participants were followed-up in accordance with local standard-of-care guidelines. A single-drug substitution, due to toxicity or intolerance, was not considered a regimen switch. Plasma was collected at the baseline visit and after 12 months of ART (time window, 11-15 months), and stored for retrospective assessment of HIV RNA and genotypic drug resistance. Virologic failure was defined as a plasma HIV RNA value of ≥400 copies/mL. Participants who were switched to second-line ART earlier than month 12 (owing to locally diagnosed ART failure) were not included in the month 12 summary statistics.

Virological analysis

All virological testing was conducted at 1 of 2 reference laboratories in South Africa or Uganda, as previously described [12]. Briefly, HIV RNA was determined using NucliSens EasyQ real-time assay, version 2.0 (bioMérieux, Lyon, France) or COBAS Ampliprep/COBAS Taqman assay (Roche, Branchburg, New Jersey, USA). Population-based genotyping of HIV-1 protease and reverse transcriptase was undertaken in specimens with HIV RNA >1000 copies/mL, using in-house sequencing methods. HIV-1 subtypes were inferred from the *pol* sequences using the STAR algorithm [14] and, if required, the REGA tool (version 2.0) [15]. Genotypic drug resistance was defined as the presence of ≥1 major amino acid substitution included in the International Antiviral Society-USA mutation list of December 2010 [16]. Drug-classes considered were NRTIs, NNRTIs and protease inhibitors (PIs). HIV-1 drug susceptibility for each participant was predicted using the Stanford algorithm, version 6.1.0 [17], and was categorized as susceptible, potential low-level resistance, low-level resistance, intermediate resistance, or high-level resistance. All sequences have been deposited in GenBank (accession numbers JQ480157-JQ480298).

Statistical analysis

Group comparisons for categorical data were done using chi-square or Fisher's exact test, and for continuous data using Kruskal-Wallis test, as appropriate. Mantel-Haenszel

method was used to compute weighted odds ratios (OR) that adjusted for a single confounding factor. Reported p-values are two-sided and p<0.05 was considered statistically significant. All analyses were performed using Stata version 11 (StataCorp, Texas, USA).

RESULTS

Study population and clinical outcomes

Between March 2007 and September 2009, 2588 participants were enrolled. Table 1 shows the baseline characteristics of all participants (n=2588), and of those with a resistance test result by month 12 (n=142). Participants were initiated on ART regimens containing either zidovudine (964 [37.2%]), tenofovir (867 [33.5%]), stavudine (691 [26.7%]), or abacavir (66 [2.6%]), combined with either efavirenz (1543 [59.6%]) or nevirapine (1045 [40.4%]). Baseline resistance was detected in 140 (5.4%) participants; the proportions did not differ between the initial regimens.

Table 1. Baseline characteristics of participants

Variable	Baseline	Month 12			p-value [a]
	Overall (n=2588)	Overall (n=142)	No resistance (n=42)	Resistance (n=100)	
Sex					0.13
Women	1482 (57.3)	61 (43.0)	14 (33.3)	47 (47.0)	
Men	1106 (42.7)	81 (57.0)	28 (66.7)	53 (53.0)	
Age at initiation, mean years (SD)	38.0 (9.0)	36.0 (8.5)	36.7 (7.9)	35.7 (8.8)	0.43
Country					0.79
Zambia	555 (21.5)	28 (19.7)	9 (21.4)	19 (19.0)	
South Africa	593 (22.9)	26 (18.3)	9 (21.4)	17 (17.0)	
Uganda	602 (23.3)	38 (26.8)	8 (19.1)	30 (30.0)	
Kenya	425 (16.4)	22 (15.5)	7 (16.7)	15 (15.0)	
Zimbabwe	211 (8.2)	12 (8.5)	3 (7.1)	9 (9.0)	
Nigeria	17 (25.8)	16 (11.3)	6 (14.3)	10 (10.0)	
WHO clinical stage at initiation					0.51
1 or 2	1015 (39.2)	52 (36.6)	17 (40.5)	35 (35.0)	
3 or 4	1573 (60.8)	90 (63.4)	25 (59.5)	65 (65.0)	
Baseline HIV-1 drug-resistance [b]	140 (5.4) [c]	15 (10.6)	1 (2.4)	14 (14.0)	0.10
Initial ART regimen					0.78
Efavirenz-based	1543 (59.6)	69 (48.6)	21 (50.0)	48 (48.0)	
Tenofovir-containing	720 (27.8)	33 (23.4)	10 (23.8)	23 (23.0)	

Table 1 (continued)

Variable	Baseline	Month 12			p-value [a]
	Overall (n=2588)	Overall (n=142)	No resistance (n=42)	Resistance (n=100)	
Stavudine-containing	430 (16.6)	11 (7.8)	5 (11.9)	6 (6.0)	
Zidovudine-containing	342 (13.2)	23 (16.2)	6 (14.3)	17 (17.0)	
Abacavir-containing	51 (2.0)	2 (1.4)	0 (0.0)	2 (2.0)	
Nevirapine-based	1045 (40.4)	73 (51.4)	21 (50.0)	52 (52.0)	
Zidovudine-containing	622 (24.0)	48 (33.8)	13 (31.0)	35 (35.0)	
Stavudine-containing	261 (10.1)	9 (6.3)	4 (9.5)	5 (5.0)	
Tenofovir-containing	147 (5.7)	14 (9.9)	3 (7.1)	11 (11.0)	
Abacavir-containing	15 (0.6)	2 (1.4)	1 (2.4)	1 (1.0)	
CD4 cell count, median cells/μL (IQR)	133 (62-204) [d]	120 (52-198	157 (136)	108 (127.5)	0.002
Plasma HIV RNA, median $\log_{10}$ c/mL (IQR)	5.00 (4.38-5.59) [e]	5.23 (4.53-5.67)	5.18 (1.31)	5.24 (.99)	0.19
HIV-1 subtypes [f]					0.14
A	611 (24.9)	37 (26.1)	14 (33.3)	23 (23.0)	
C	1329 (54.2)	69 (48.6)	22 (52.4)	47 (47.0)	
D	276 (11.3)	19 (13.4)	1 (2.4)	18 (18.0)	
CRF02_AG	116 (4.7)	6 (4.2)	2 (4.8)	4 (4.0)	
Other	118 (4.8)	11 (7.8)	3 (7.1)	8 (8.0)	

Baseline characteristics of all participants at baseline (n=2588) and of those who had a genotypic resistance test results by month 12 (n=142). Data are no. (%) unless stated otherwise. [a] P values for comparison between no resistance vs resistance outcome groups. [b] Defined as at least 1 drug-resistance mutation of the 2010 International Antiviral Society–USA list. [c] Data available for n=2442. [d] Data available for n=2578. [e] Data available for n=2564. [f] Data available for n=2450. ART, antiretroviral therapy; HIV-1, human immunodeficiency virus type 1; IQR, interquartile range; SD, standard deviation; WHO, World Health Organization.

A total of 2132 (82.4%) participants were retained in care up to 12 months of follow-up, of whom 2128 (82.2%) were still on first-line ART and 4 (0.2%) were switched to second-line therapy earlier than month 12 due to locally diagnosed ART failure. The remaining 456 (17.6%) participants were not retained, because they died (190 [7.3%]), were lost to follow-up (200 [7.7%]), transferred out (61 [2.4%]), or discontinued ART (5 [0.2%]) (figure 1).

HIV RNA by month 12 was assessed for 94.6% (2014 of 2128) of participants who were still on first-line ART. For all who initiated first-line ART, 70.3% (1820 of 2588; 95%CI 68.5-72.1) achieved viral suppression, and for those who had a 12-month HIV RNA result 90.4% (1820 of 2014; 95%CI 89.0-91.6) achieved viral suppression (figure 1).

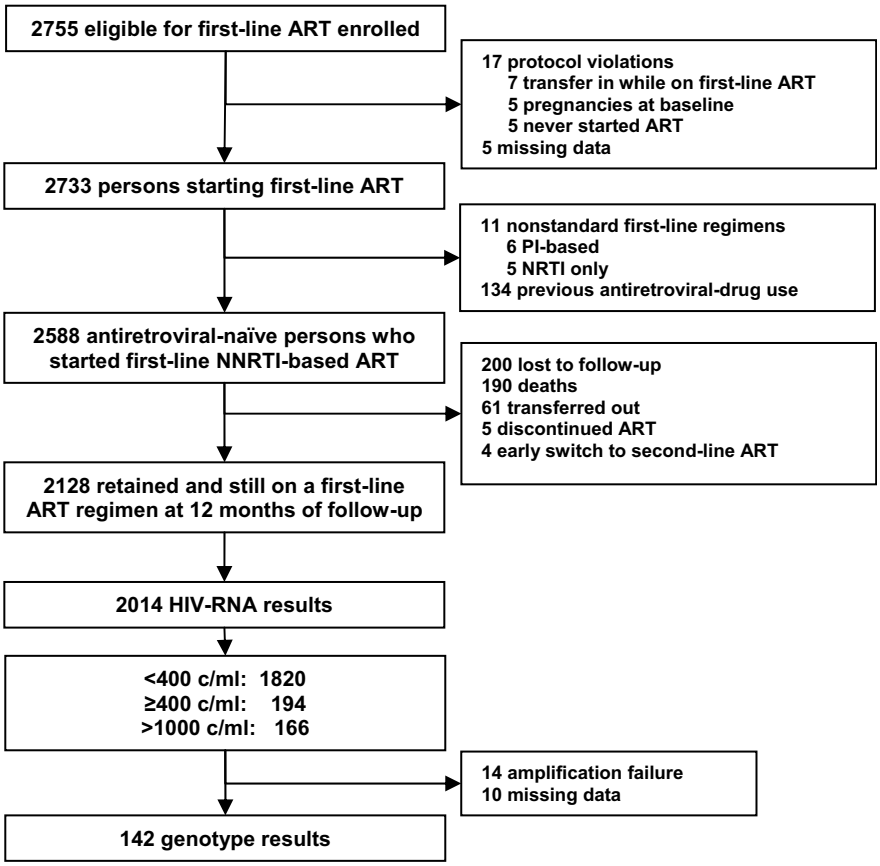

Figure 1. Study profile

ART, antiretroviral therapy; PI, protease inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-NRTI.

Drug-resistance mutations by drug regimen

Of the 166 participants with HIV RNA >1000 copies/mL by month 12, sequence results were available for 142 (85.5%); 14 specimens failed to amplify, and results were missing for 10 specimens. One hundred (70.4%, 95%CI 62.2-77.8) sequences harboured ≥1 DRM and 42 (29.6%) did not harbour any DRMs. The average number of DRMs (any class) per sequence was 2.4, with a range of 1–8. Detected DRMs were associated with NRTIs (82 [57.8%]), NNRTIs (86 [60.6%]) (figure 2). Dual-class resistance to NRTIs and NNRTIs was detected in 69 (48.6%) participants. Combinations of DRMs included M184V and NNRTI (64 [45.1%]), M184V and TAMs (10 [7.6%]), TAMs and NNRTI (8 [5.6%]), and M184V, TAMs, and NNRTI (7 [4.9%]) (figure 2). In 2 (1.4%) participants, DRMs known to be associated with PIs were observed (both tipranavir-associated Q58E); no triple-class resistance was detected. Exclusion of the 16 (9.6%) participants who had ≥1 single-drug substitutions

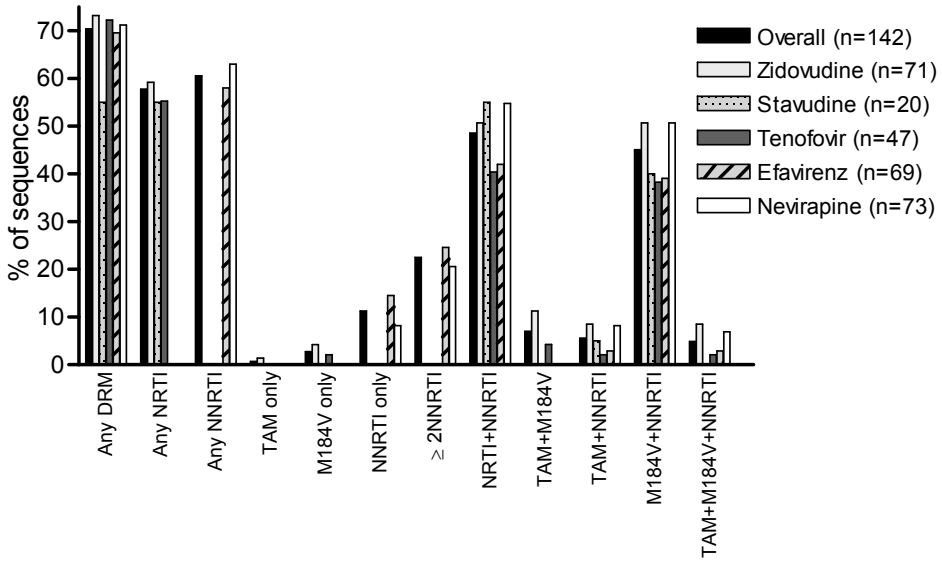

Figure 2. Frequencies of any drug resistance according to drug class and permutations by nucleoside reverse transcriptase inhibitor (NRTI) and by non-nucleoside reverse transcriptase inhibitor (NNRTI).
Figure shows major International Antiviral Society–USA drug-resistance mutations associated with NRTIs and NNRTIs in participants experiencing virological failure. The legend includes the number of patients exposed to each antiretroviral drug. DRM, drug-resistance mutation; TAM, thymidine analogue mutation.

during the first-year of ART did not significantly change the frequencies of the DRMs (not shown).

Of the NRTI-associated DRMs (figure 3), M184V was the most frequent (76 [53.5%]), followed by K65R (17 [12.0%]) and any TAM (12 [8.5%]). In 9 (6.3%) participants, ≥2 TAMs were detected and in 3 (2.1%) participants, ≥3 TAMs were detected. TAM-1 mutations included M41L and T215 F/Y (L210W not observed); TAM-2 mutations included D67N, K219E and K70R (K219Q not observed). In participants who failed regimens containing zidovudine, stavudine or tenofovir, ≥1 TAM was detected in 12.7% (9 of 71), 5.0% (1 of 20), and 4.3% (4 of 47), respectively; K65R in 0% (0 of 71), 15.0% (3 of 20) and 27.7% (13 of 47), respectively. Q151M was not detected.

Of the NNRTI-associated DRMs (figure 3), K103N (41 [28.9%]) was the most frequent, followed by Y181C (22 [15.5%]) and G190A (20 [14.1%]). In participants failing an efavirenz-based or nevirapine-based regimen, NNRTI-associated DRMs were detected in 58.0% (40 of 69) and 63.0% (46 of 73), respectively (p=0.5); K103N in 33.3% (23 of 69) and 24.7% (18 of 73), respectively (p=0.3); Y181C in 7.3% (5 of 69) and 23.3% (17 of 73) participants, respectively (p=0.008); and V106M in 20.3% (14 of 69) and 1.4% (1 of 73), respectively (p<0.001). V106A only occurred after nevirapine exposure. Sixteen (11.3%) participants

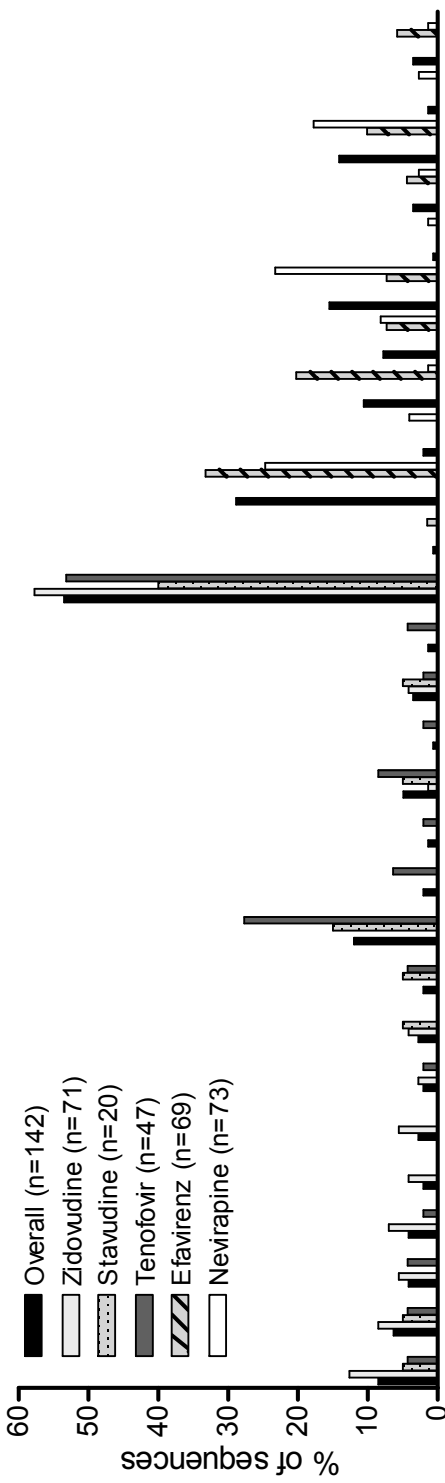

Figure 3. Frequencies of individual drug-resistance mutations by nucleoside reverse transcriptase inhibitor (NRTI) and by non-nucleoside reverse transcriptase inhibitor (NNRTI).

Figure shows major International Antiviral Society–USA drug-resistance mutations associated with NRTIs and NNRTIs in participants experiencing virological failure. The legend includes the number of patients exposed to each antiretroviral drug. TAM, thymidine analogue mutation.

harbored only DRMs associated with NNRTIs, and 33 (22.5%) participants harbored ≥2 NNRTI-associated DRMs. K103N occurred as the only NNRTI mutation in 23 of 41 participants (56.1%), and occurred in combination with other NNRTI mutations in 18 participants (with Y181C [7], P225H [3], G190A [2], V106M [1], M230L [1], V106M+Y181C [1], Y181C+G190A [1], P225H+M230L [1], L100I+P225H [1]).

Compared with participants with virological failure by month 12 who did not harbour any DRM, those with ≥1 DRM had a marginally lower median HIV RNA (4.33 vs 4.71 $\log_{10}$ copies/mL; p=0.04); this difference was least pronounced for NNRTI resistance (4.30 vs. 4.55 $\log_{10}$ copies/mL, p=0.05) and most pronounced for M184V (4.21 vs. 4.59 $\log_{10}$ copies/mL, p=0.02).

Of the 142 participants with a genotype result by month 12, 135 had a baseline genotype result (7 baseline specimens not collected); of these, 14 (10.4%) participants harboured ≥1 DRM before start of ART. Of the DRMs detected by month 12, 96% were newly acquired during the first year of ART.

HIV-1 subtype diversity

Among the 142 participants who had a resistance test result by month 12, HIV-1 subtype C was most commonly identified, followed by A, D, A/G recombinant, and other subtypes/recombinants (table 1). The subtype distribution did not differ significantly between those with or without DRMs (p=0.14). The DRMs according to subtype are shown in figure 4. K65R was more frequent in subtype C (12 [17.4%]) than non-C (5 [6.9%]) (p=0.05). After adjusting for differential tenofovir and stavudine exposure, the association with subtype was not significant: OR for C vs non-C (by Mantel-Haenszel method) 1.27; 95%CI 0.41-3.93; p=0.68).

Compared with subtype C (13 [18.8%]), K103N occurred more frequently in D (10 [52.6%]; p=0.003) and A (13 [35.1%]; p=0.06), but did not differ between A and D (p=0.21). After adjusting for differential efavirenz and nevirapine exposure, K103N remained significantly associated with subtype: OR for C vs non-C (by Mantel Haenszel method) 0.33; 95%CI 0.15-0.75; p=0.005, and OR for D vs non-D 3.40; 95%CI 1.21-9.58; p=0.014. Frequencies of other DRMs, except for V106M, which was exclusively observed in subtype C, did not differ between HIV-1 subtypes (figure 4).

Predicted genotypic drug susceptibility

Of the viruses harboring ≥1 DRM (n=100), the predicted HIV-1 susceptibility to lamivudine and emtricitabine was reduced in the majority (81%), due to the high frequency of M184V (figure 5). For the other NRTIs, reduced HIV-1 susceptibility was predicted for

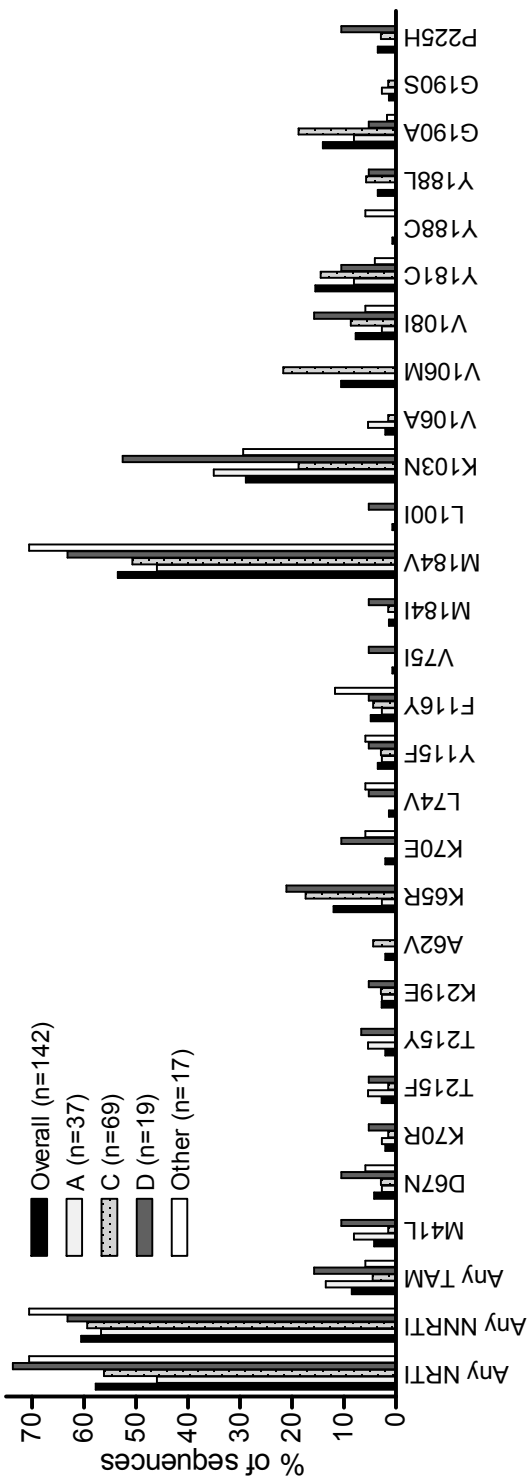

Figure 4. Frequencies of individual drug-resistance mutations by human immunodeficiency virus type 1 (HIV-1) subtype.
Figure shows major International Antiviral Society–USA drug-resistance mutations associated with nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the participants experiencing virological failure. The legend includes the number of patients infected with each HIV-1 subtype. TAM, thymidine analogue mutation.

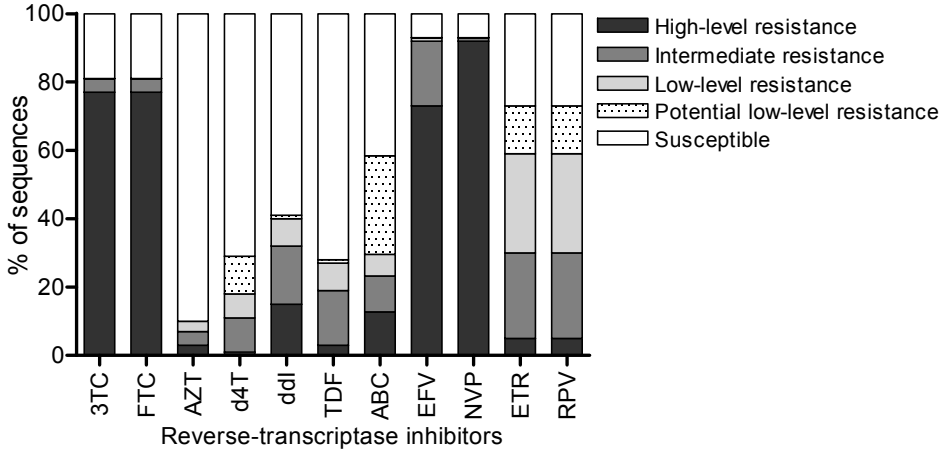

Figure 5. Predicted viral susceptibility to reverse transcriptase inhibitors.
Figure includes the viruses that harbored ≥1 drug-resistance mutation (n= 100). Genotypic drug susceptibility was predicted using the Stanford HIVdb algorithm (version 6.1.0). 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; d4T, stavudine; ddI, didanosine; EFV, efavirenz; ETR, etravirine; FTC, emtricitabine; NVP, nevirapine; RPV, rilpivirine; TDF, tenofovir.

abacavir in 42% of sequences, didanosine in 40%, tenofovir in 27%, stavudine in 18%, and zidovudine in 10%. For the NNRTIs, reduced HIV-1 susceptibility was frequently predicted for the first-generation drugs efavirenz and nevirapine (93%) and less frequently for the second-generation drugs etravirine and rilpivirine (59%). High-level resistance was most frequently predicted for nevirapine (92%), followed by lamivudine/ emtricitabine (77%) and efavirenz (73%), but was uncommon for etravirine and rilpivirine (5%). Reduced HIV-1 susceptibility was not predicted for any of the ritonavir-boosted PIs.

DISCUSSION

This multi-country cohort study examined HIV-1 resistance mutation patterns in African patients with virological failure on different first-line ART regimens, and predicted their impact on second-line drug susceptibility. After the first year of ART, 70% of those with virological failure had a virus with ≥1 DRM, and dual-class resistance was observed in 49%. Nearly all (96%) DRMs detected by month 12 were newly acquired in the first year of ART. Previous studies from the region have reported higher frequencies (83-93% of those with virological failure) and complexity of DRM patterns in persons with prolonged ART failure, in the absence of viral load monitoring [4-8]. By contrast, in the present study, routine viral load testing after 12 months of ART enabled the relatively early detection of virological ART failure, which, to some extent, may have prevented the accumulation of resistance. Nonetheless, observed resistance patterns for this cohort were more

extensive than for cohorts that received intensive virological monitoring in South Africa [18] and resource-rich countries [19, 20]. Therefore, our data underscore the importance of implementing routine viral load monitoring in ART programs in sub-Saharan Africa to prevent drug-resistance accumulation [3-5]. The presence of DRMs was associated with lower HIV RNA values, which results from the reduced fitness of resistant variants.

In patients who failed a tenofovir-containing regimen, the K65R mutation, which is tenofovir's signature mutation, was commonly observed. Notably, K65R, rather than TAMs, was also frequently selected after stavudine failure. This finding concurs with previous clinical studies that reported high rates of K65R in subtype C–infected Africans who failed stavudine-containing regimens [4, 5, 7], and contrasts with the low frequencies of K65R observed in subtype-B-infected individuals from developed countries [21, 22]. K65R and TAMs represent antagonistic pathways of NRTI resistance [23], and K65R confers cross-resistance to all NRTIs except zidovudine.

Several mechanistic studies have demonstrated *in vitro* that the K65R mutation, which confers broad cross-resistance to the NRTI class, develops more readily in HIV-1 subtype C, the predominant subtype in sub-Saharan Africa, than in subtype B [24-29]. In our cohort, which included only non-B subtypes, K65R was detected more frequently in subtype C than in subtypes A or D. However, after adjusting for differential stavudine and tenofovir use across the subtypes, the association was not statistically significant. This discrepancy with the published literature is most likely explained by the limited statistical power of the present study to assess the effects of subtype on the rates of individual DRMs. Nonetheless, overall, our data support the conclusion that K65R is frequently selected after stavudine or tenofovir use in African populations receiving first-line ART. Therefore, from a virological perspective, zidovudine rather than tenofovir (as currently recommended by the WHO guidelines [30]), may be preferred in second-line therapy for non-B subtype infected patients who fail a stavudine-containing first-line regimen in the absence of stringent viral load and resistance monitoring. This scenario would warrant enhanced toxicity monitoring, particularly for zidovudine-associated anemia [31].

The WHO advises that stavudine be phased out of first-line regimens due to its serious adverse effects, to be replaced with either tenofovir or zidovudine [30]. Dose reduction (from 40 mg to 30 mg, and even 15-20 mg per day) has been suggested to limit the mitochondrial toxicities from stavudine [32]. However, suboptimal dosing of stavudine might in theory result in even more frequent emergence of K65R, particularly when used as part of NNRTI-based regimens. Emerging K65R will compromise future NRTI backbones, and clinical trials are ongoing to establish the effectiveness of ("simplified") PI-based second-line therapy without an effective NRTI-backbone [33].

For second-line ART, WHO recommends a ritonavir-boosted PI (preferably lopinavir or atazanavir), with a dual backbone of 2 new or recycled NRTIs, that is, tenofovir plus lamivudine/emtricitabine (after use of stavudine or zidovudine in first-line), or zidovudine plus lamivudine (after use of tenofovir in first-line) [30]. In our cohort, HIV-1 susceptibility to all PIs was preserved for all participants; zidovudine and tenofovir susceptibility was preserved in the majority of patients who failed first-line ART (90% and 73%, respectively). This means that the majority of patients who are switched empirically to second-line PI-based therapy will receive at least two active drugs. Preliminary studies in Africa have indeed suggested high chance of viral re-suppression with empirically prescribed second-line regimens [34-38].

The second-generation NNRTIs rilpivirine or etravirine are being considered as second-line drugs in resource-limited countries in patients previously exposed to efavirenz or nevirapine, because of their different resistance profiles and suggested high genetic barrier. Susceptibility to the second-generation NNRTIs is preserved in viruses containing the K103N mutation, preferentially selected by efavirenz, but susceptibility is reduced in the presence of Y181C, preferentially selected by nevirapine [39]. In our cohort, viral susceptibility to the second-generation NNRTIs was already reduced in 59% of participants who had ≥1 DRM. Given that NRTI and NNRTI resistance mutations rapidly accumulate in the absence of viral load monitoring [4, 5], second-line regimens based on 2 NRTIs and a second-generation NNRTI are therefore unlikely to be effective. Instead, use of second-generation NNRTIs as part of first-line ART in resource-limited countries merits further evaluation.

In our cohort, K103N occurred more frequently in subtype D infections than in subtype C infections. By contrast, previous studies in women and infants after the use of single-dose nevirapine to prevent vertical HIV-1 transmission have suggested that K103N accumulates faster in subtype C than D, and faster in D than A [40-42]. Additional studies are needed to elucidate the virological mechanisms for different subtypes.

This study has some limitations. Population-based sequencing is not able to detect minority resistant viral strains, thus potentially underestimating resistance [43]. The lack of frequent serial blood sampling after ART initiation precluded a more detailed analysis of the evolution of DRMs after ART initiation.

The number of HIV-1 infected people accessing ART in resource-limited countries is projected to increase further in view of the goal of universal access [2] and, particularly, the earlier start of ART [44] and emerging treatment-for-prevention [45]. Before widely implementing such strategies, mathematical modelling as well as empirical data are

urgently needed to investigate their acceptability, adherence as well as the possible consequences for the emergence and spread of drug resistance.

In conclusion, regular viral load monitoring aimed at early failure detection may limit the accumulation of HIV-1 drug-resistance. The strengthening of national HIV treatment programs through robust supply chains, routine viral load monitoring and improved access to alternative drug regimens in sub-Saharan Africa to prevent drug resistance, is an urgent priority. Zidovudine rather than tenofovir may be the preferred second-line NRTI after first-line stavudine failure. The high frequencies of Y181C and accumulated NNRTI mutations limit the effectiveness of second-generation NNRTIs for use in second-line ART.

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Contributors

RLH, CLW, WS, TFRW and RS designed the study and developed the protocol. RLH and KCES set up the study and trained and supervised study workers. CK, MS, FC, MB, KM, MW, and AO established the cohort and supervised data collection. CLW, CK, and WS supervised the laboratory testing. RLH conceived and undertook the data analyses, and drafted the manuscript. KCES, AMW, CLW and RS helped interpret the data and reviewed the manuscript. All authors reviewed and approved the final version of the manuscript.

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