HIV-1 Drug Resistance and Third-Line Therapy Outcomes in Patients Failing Second-Line Therapy in Zimbabwe

Chimbetete, Cleophas; Katzenstein, David; Shamu, Tinei; Spoerri, Adrian; Estill, Janne Anton Markus; Egger, Matthias; Keiser, Olivia

How to cite

CHIMBETETE, Cleophas et al. HIV-1 Drug Resistance and Third-Line Therapy Outcomes in Patients Failing Second-Line Therapy in Zimbabwe. In: Open Forum Infectious Diseases, 2018, vol. 5, n° 2. doi: 10.1093/ofid/ofy005

This publication URL: https://archive-ouverte.unige.ch//unige:102017

Publication DOI: 10.1093/ofid/ofy005

Open Forum Infectious Diseases

MAJOR ARTICLE

HIV-1 Drug Resistance and Third-Line Therapy Outcomes in Patients Failing Second-Line Therapy in Zimbabwe

Cleophas Chimbetete,[1,2] David Katzenstein,[3] Tinei Shamu,[2] Adrian Spoerri,[4] Janne Estill,[1,4,5] Matthias Egger,[4] and Olivia Keiser[1,4]

[1]Institute of Global Health, University of Geneva, Geneva, Switzerland; [2]Newlands Clinic, Harare, Zimbabwe; [3]School of Medicine, University of Stanford, Stanford, California; [4]Institute of Social and Preventive Medicine (ISPM), University of Bern, Bern, Switzerland; [5]Institute of Mathematical Statistics and Actuarial Science, University of Bern, Bern, Switzerland

Objectives. To analyze the patterns and risk factors of HIV drug resistance mutations among patients failing second-line treatment and to describe early treatment responses to recommended third-line antiretroviral therapy (ART) in a national referral HIV clinic in Zimbabwe.

Methods. Patients on boosted protease inhibitor (PI) regimens for more than 6 months with treatment failure confirmed by 2 viral load (VL) tests >1000 copies/mL were genotyped, and susceptibility to available antiretroviral drugs was estimated by the Stanford HIVdb program. Risk factors for major PI resistance were assessed by logistic regression. Third-line treatment was provided as Darunavir/r, Raltegravir, or Dolutegravir and Zidovudine, Abacavir Lamivudine, or Tenofovir.

Results. Genotypes were performed on 86 patients who had good adherence to treatment. The median duration of first- and second-line ART was 3.8 years (interquartile range [IQR], 2.3–5.1) and 2.6 years (IQR, 1.6–4.9), respectively. The median HIV viral load and CD4 cell count were 65 210 copies/mL (IQR, 8728–208 920 copies/mL) and 201 cells/mm^3 (IQR, 49–333 cells/mm^3). Major PI resistance-associated mutations (RAMs) were demonstrated in 44 (51%) non-nucleoside reverse transcriptase inhibitor RAMs in 72 patients (83%) and nucleoside reverse transcriptase inhibitors RAMs in 62 patients (72%). PI resistance was associated with age >24 years (P = .003) and CD4 cell count <200 cells/mm^3 (P = .007). In multivariable analysis, only age >24 years was significantly associated (adjusted odds ratio, 4.75; 95% confidence interval, 1.69–13.38; P = .003) with major PI mutations. Third-line DRV/r- and InSTI-based therapy achieved virologic suppression in 29/36 patients (81%) after 6 months.

Conclusions. The prevelance of PI mutations was high. Adolescents and young adults had a lower risk of acquiring major PI resistance mutations, possibly due to poor adherence to ART. Third-line treatment with a regimen of Darunavir/r, Raltegravir/Dolutegravir, and optimized nucleoside reverse transcriptase inhibitors was effective.

Keywords. HIV-1 drug resistance; second-line therapy; third-line ART outcomes; Zimbabwe.

The many benefits of combination antiretroviral therapy (ART) may be compromised by virologic failure and drug resistance [1]. ART programs in countries hard hit by the HIV pandemic in Sub-Saharan Africa are facing increasing virologic failure of firstline ART and high levels of drug resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs) [2]. The emergence of resistance to ART is a consequence of expanded access to treatment and longer duration of ART exposure. To maintain the benefits of ART, international guidelines recommend switching to second-line, boosted protease inhibitor (PI)–based ART to maintain virologic suppression [3]. Routine HIV viral load monitoring is essential for the early diagnosis of ART treatment failure [4]. In contrast to patients failing firstline NNRTI- and nucleoside reverse transcriptase inhibitor (NRTI)–based ART, the majority of patients failing with a PI-based second-line ART regimen do not acquire major PI resistance-associated mutations [5, 6].

As more people with suboptimal adherence are on ART, the number of patients failing first- and second-line ART regimens is increasing, and an increase in multiclass drug resistance is expected [7]. Ongoing success of ART programs will require an understanding of the emergence and patterns of HIV drug resistance among individuals in whom treatment has failed. Virologic failure occurs for multiple reasons, including suboptimal adherence and drug intolerance/toxicity leading to drug resistance. After second-line failure, evidence of multiclass resistance following exposure to boosted PI regimens requires treatment with at least 2 fully active antiretroviral drugs to suppress viremia, reduce the transmission of resistant virus, and optimize the effectiveness of third-line ART. Several factors, such as the duration of PI use and viral load, have been identified as risk factors for developing PI resistance mutations [8]. Modeling provides evidence that genotyping to optimize third-line ART is more cost-effective than switching patients failing

Received 10 October 2017; editorial decision 22 December 2017; accepted 23 January 2018.
Correspondence: C. Chimbetete, MBChB, MPH, 56 Enterprise Road, Harare, Zimbabwe (docchimbetete@gmail.com).

Open Forum Infectious Diseases®

DOI: 10.1093/ofid/ofy005

second-line to third-line based only on virologic failure [9]. In the Zimbabwe National ART program, boosted darunavir, recycling available nucleoside/nucleotide reverse transcriptase inhibitors, and an integrase strand transfer inhibitor (InSTI) are provided, as recommended by the World Health Organization (WHO) [3]. However, identification of multiclass drug resistance and eligibility for third-line therapy requires genotypic resistance testing (GRT). For patients who have developed multiclass resistance to NNRTI, NRTIs, and boosted PIs, InSTIs have been approved as a new class of ART with a high barrier to resistance and an exceptional safety profile [10]. While recently approved in Botswana for firstline treatment, InSTIs are currently reserved for patients with evidence of multiclass resistance in most resource-limited countries, including Zimbabwe. Darunavir, a second-generation protease inhibitor, has been shown to be effective against HIV resistant to Atazanavir and Lopinavir, and hence is a useful third-line option [11, 12]. Third-line treatment should include new drugs with a minimum risk of cross-resistance to previously used regimens that are available in resource-limited settings [13].

In this study, we analyzed the patterns of HIV drug resistance mutations among patients failing second-line treatment and the risk factors for acquiring major PI resistance. We further describe early treatment responses to recommended third-line ART in an HIV clinic in Zimbabwe. Our broad aim was to inform planning for third-line ART programs in sub-Saharan Africa.

MATERIALS AND METHODS

Study Setting and ART Treatment Guidelines

Newlands Clinic is an HIV treatment center in Harare, Zimbabwe, that is a national referral site for patients who are supposed to start third-line ART treatment after second-line virologic failure. Firstline regimens comprise 2 nucleoside/NRTIs—among them tenofovir (TDF), zidovudine (ZDV), abacavir (ABC), and lamivudine (3TC), and an NNRTI, either efavirenz (EFV) or nevirapine (NVP). Until 2013, stavudine (D4T) was part of the national firstline ART regimen. Second-line regimens include 2 NRTIs and a ritonavir-boosted PI, either atazanavir or lopinavir. The NRTIs used in second-line are 3TC and AZT or TDF or ABC, depending on what the patient received for firstline treatment. Protease inhibitor monotherapy is not part of national guidelines, and none of the patients studied received it. National guidelines recommend a change to a third-line regimen if virologic failure (2 consecutive HIV RNA tests >1000 copies/mL) occurs after at least 6 months of therapy and adherence is estimated to be greater than 95% by pill counts and/or pharmacy refill records [14]. National guidelines recommend that patients on second-line ART have at least 1 viral load test done per year. Patients with an elevated VL (>1000 copies/mL) must have a repeat test done 3 months after adherence support. At Newlands Clinic only, patients who are deemed adherent (assessed by pill counts) after an intensive adherence support program and who have acquired major PI resistance mutations are commenced on third-line ART. Third-line regimens consist of Darunavir/ritonavir, Raltegravir or Dolutegravir, and an (optimized) NRTI based on GRT.

Enhanced Adherence Support Program

All patients suspected of second-line ART failure, that is, patients who had a VL >1000 copies/mL, were enrolled in a 6-week enhanced adherence support program before GRT between August 1, 2013, and July 31, 2016. Patients who had elevated viral loads met in support groups of 8–10 participants once weekly for approximately 2.5 hours. Group cognitive behavioral counseling was aimed at discussion of HIV and ART, the identification of barriers and challenges to adherence, and the strengthening of medication adherence. These meetings were facilitated by trained counselors. There were separate groups for participants age 24 years and younger and for those older than age 24 years. All patients had the VL test repeated after the adherence support program. HIV-1 viral load was measured by the Roche COBAS Ampliprep/COBAS Taqman HIV-1 Test, version 2.0.

ART Resistance Testing

GRT was done for patients suspected of second-line failure with a confirmed viral load >1000 copies/mL after 6 weeks of enhanced adherence support, and had good adherence, as per national guidelines. GRT was not done in patients who still had confirmed poor adherence after adherence support. Patients with poor adherence continued to receive adherence support, and repeated viral load tests were done every 3 months. Plasma viral RNA was extracted, reverse transcribed, and 1.3 kb of the HIV-1 protease and reverse transcriptase genes was amplified as described by Manasa [15]. Amplicons were sequenced at MCLab Molecular Cloning Laboratories (http://www.mclab.com), San Francisco, California. The chromatograms were assembled using Geneious software, version 8 (http://www.geneious.com) [16], and consensus sequences were analyzed using the Stanford University HIV Drug Resistance Database's HIVdb program, version 8.3 (https://hivdb.stanford.edu/hivdb/by-sequences) [17]. The estimated level of resistance to ART was determined by the genotypic susceptibility scores (GSS) associated with each of the drug resistance mutations. The estimated level of resistance was calculated as follows: susceptible (total score 0–9), potential low-level resistance (total score 10–14), low-level resistance (total score 15–29), intermediate resistance (total score 30–59), and high-level resistance (total score of 60 and above).

Data Analysis

We analyzed the clinical data that were routinely collected for each patient. We used univariable and multivariable logistic regression to study the association between explanatory variables with the development of at least 1 major PI resistance-associated mutation (RAM). We included the following explanatory variables: age (≤24 and >24 years according to the WHO, which

defines adolescents and young adults as people aged ≤24 years [18]), HIV RNA (≤100 000 and >100 000 copies/mL), sex, CD4 cell count (≤200 and >200 cells/mm^3), and duration of second-line therapy (≤2 and >2 years). All variables were retained in multivariable logistic regression regardless of association in univariable analysis. Statistical tests were 2-sided, with a significance level of .05. There were no missing values. All statistical analyses were performed in Stata, version 13.0 (StataCorp, College Station, TX).

Ethics

The study was approved by the Medical Research Council of Zimbabwe (approval No. MRCZ/A/1336). Patients provided written informed consent before being enrolled into the enhanced adherence support program.

RESULTS

A total of 186 participants received adherence support for second-line failure, 61 achieved postadherence support viral loads of less than 1000 copies/mL, 3 were lost to follow-up, 1 was transferred out, and 35 did not meet clinical criteria for genotyping due to confirmed poor adherence.

Of the 86 who were genotyped, 41 (48%) were female. Thirty-six (42%) had initiated firstline ART at Newlands Clinic and had been switched to a second-line regimen after failing first-line ART, and 50 patients (58%) were referred to Newlands Clinic, receiving second-line ART. The median age at genotyping was 27.7 years (IQR, 19.7–42.3 years). The median HIV viral load and CD4 cell count at the time of genotyping were 65 210 copies/mL (IQR, 8728–208 920 copies/mL) and 201 cells/mm^3 (IQR, 49–333 cells/mm^3), respectively. Participants had received firstline ART for a median of 3.8 years (IQR, 2.3–5.1 years) and second-line ART for a median of 2.6 years (IQR, 1.6–4.9 years). Participants had received a median of 6 (IQR, 6–7) antiretroviral medicines for first- and second-line ART. Table 1 summarizes participant demographic and clinical characteristics at the time of GRT. There were differences in education level (P = .006), CD4 cell counts (P = .032), HIV viral load (P = .039), and marital status (P = .001) between patients who had PI RAMs and those without. Only 2 participants received ART for the prevention of mother-to-child transmission; both had received single-dose nevirapine.

Drug Resistance–Associated Mutations

Sanger sequencing was successful for all 86 patients. All patients had subtype C virus. Wild-type virus was found in 12 (14%) participants, and 74 (86%) had mutant virus. Most (n = 72, 83%) had at least 1 NNRTI RAM, as summarized in Figure 1. The most common NNRTI mutation was K103N (n = 30, 35%), followed by Y181C (n = 26, 32%) and G190 (n = 24, 28%).

Sixty-two participants (72%) had at least 1 NRTI RAM. The distribution of major NRTI mutations is summarized in Figures 1 and 2. Two-thirds had the NRTI mutation M184V (n = 58, 67%), followed by the thymidine analogue mutations T215Y (n = 31, 36%) and D67N (n = 31, 36%). Overall, 13 (15%) patients had the K65R mutation, which confers high-level resistance to Tenofovir. All 13 patients with the K65R mutation had been exposed to TDF for either first- or second-line ART.

Table 1. Sociodemographic, Clinical, and Biological Characteristics of Study Population With HIV-1 Sequences (n = 86) Comparing Those With and Without PI Mutations

Parameter	All Patients (n = 86)	No PI Mutation (n = 42)	Any PI Mutation (n = 44)	*P* Value
Median age (IQR), y	27.7 (19.7–42.3)	21.2 (18.0–38.3)	37.4 (25.9–46.9)	.004
Gender, n (%)				
Female	41 (47.7)	22 (52.4)	19 (43.2)	.729
Marital status, n (%)				
Single	47 (54.7)	32 (76.2)	15 (34.1)	.001
Married	30 (34.9)	7 (11.7)	23 (52.3)	
Widowed	7 (8.1)	3 (7.1)	4 (9.1)	
Divorced	2 (2.3)	0 (0)	2 (4.6)	
Level of Education				
None	12 (14)	9 (21.4)	3 (6.8)	.006
Primary	17 (19.7)	12 (28.6)	5 (11.4)	
Secondary	43 (50)	18 (42.9)	25 (56.8)	
Tertiary	14 (16.3)	3 (7.1)	11 (25)	
Clinical				
CD4 count, median (IQR), cell/mm^3	201 (49–333)	243 (132–379)	97 (22–277)	.032
HIV RNA, median (IQR), copies/mL	65 210 (8728–208 920)	37 238 (4620–147 592)	79 362 (20 376–254 612)	.039
Duration of ART (IQR), y	7.7 (5.3–9.4)	7.3 (5.0–9.4)	7.9 (6.1–9.5)	.388
2nd-line ART duration (IQR), y	2.6 (1.6–4.9)	2.4 (1.6–4.7)	2.6 (1.7–5.2)	.318
No. of ART drugs received, median (IQR) at GRT	6 (6–7)	6.5 (6–8)	6 (5–7)	.081

Abbreviations: GRT, genotypic resistance testing; PI, protease inhibitor; RAM, resistance-associated mutation.

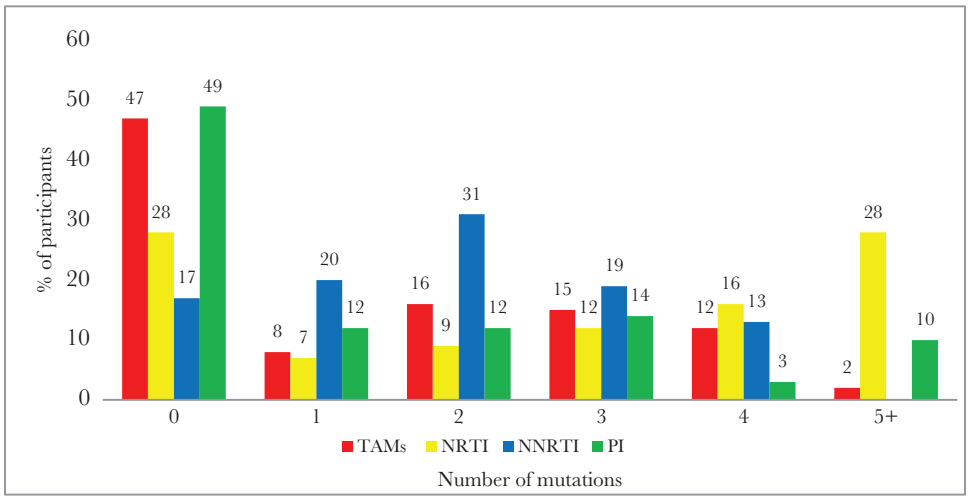

Figure 1. Distribution of HIV drug resistance mutations (n = 86). Abbreviations: NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TAM, thymidine analogue mutation.

At the time of GRT, of the patients with the K65R mutation, 4 were receiving TDF/3TC, 2 AZT/3TC, and 7 ABC/3TC, as the NRTI backbone for second-line treatment. The multidrug NRTI drug resistance mutations T69Ins and Q151M were present in 7 (8%) and 4 (5%) participants, respectively. Overall, 46 (54%) had at least 1 thymidine analogue mutation (TAM), and 25 (30%) had 3 or more TAMs. Figure 1 highlights the observed frequency of TAMs among the participants; 66 (76.7%) participants had pathway 2 TAMs [19].

PI RAMs were present in 50 (58%) participants, and major PI RAMs were present in 44 (51%) participants. Figures 1 and 2 summarize the frequency of major PI mutations and the

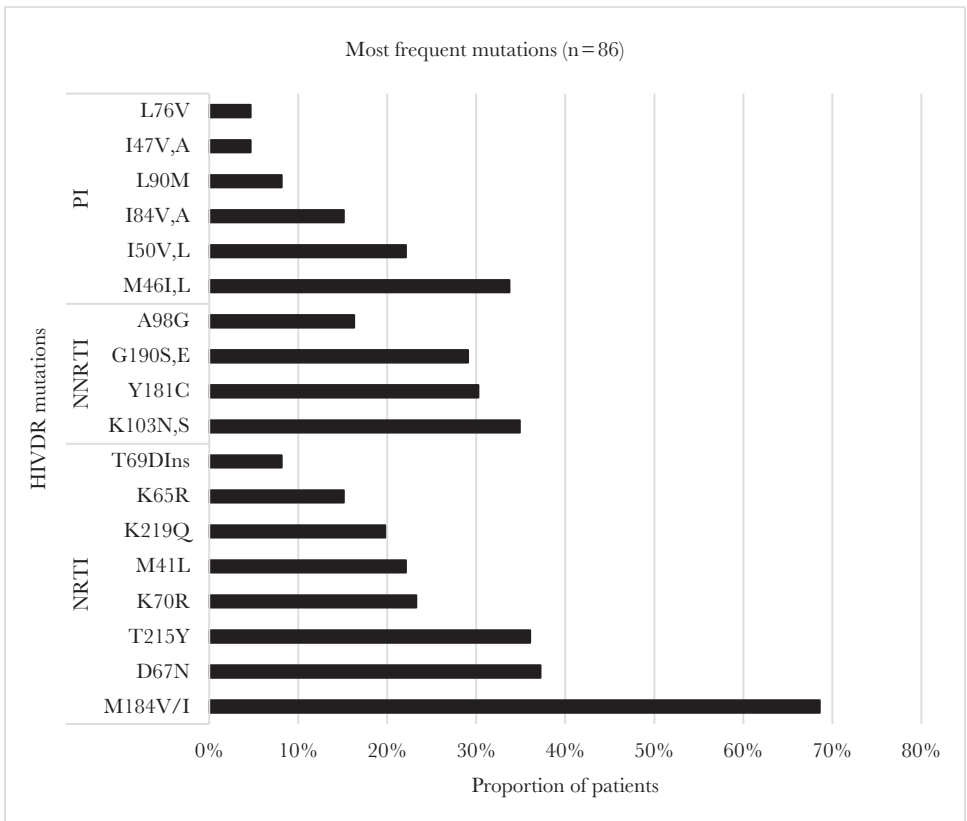

Figure 2. Distribution of specific HIV drug resistance mutations. Abbreviation: HIVDR, HIV drug resistance.

number of major PI mutations per patient. The most common PI mutation was M46I (n = 28, 33%), followed by I50V (n = 18, 21%) and V82A (n = 18, 21%). A total of 24 participants (28%) had at least 3 major PI RAMs.

Figure 3 highlights the results of estimated resistance for potential third-line ART drugs. The HIV drug resistance interpretation for PI showed that 40 (48%) participants had a virus susceptible to atazanavir, 62 (74%) were fully susceptible to darunavir, and 44 (52%) were fully susceptible to lopinavir. For NNRTI, full susceptibility was predicted for 18 (21%) to nevirapine, 19 (23%) to efavirenz, 21 (25%) to etravirine, and 19 (22%) to rilpivirine. For the NRTIs, full susceptibility to Lamivudine/Emtricitabine was noted in 26 (31%), to Abacavir or Didanosine was noted in 25 (30%), to Zidovudine was noted in 44 (51%), and to Tenofovir was noted in 35 (42%).

Risk Factors for PI Drug Resistance Mutations

Table 2 summarizes the risk factors for developing PI mutations. In univariable analysis, participants who had a CD4 cell count of <200 cells/mm^3 (odds ratio, 3.67 cells/mm^3; 95% confidence interval [CI], 1.43–9.43 cells/mm^3) were more likely to have major PI mutations. Age >24 years was independently associated with the risk of having major PI mutations in multivariable analysis (adjusted odds ratio, 4.75 years; 95% CI, 1.69–13.38 years). HIV viral load and CD4 cell count were not independently associated with the risk of having major PI mutations; neither was the duration of receiving PI-based second-line ART.

Early Third-Line Outcomes

The decision to switch to third-line ART was based on the presence of major PI RAMs conferring resistance to Atazanavir and Lopinavir. A total of 36 patients (19 females and 17 males) were commenced on a third-line ART regimen of darunavir, raltegravir, and optimized NRTI. Two patients with PI mutations continued on second-line (1 had very poor adherence due to psychiatric illness, and the other was receiving palliative care for disseminated cancer of the cervix), and 6 patients died before commencing third-line ART. Figure 4 highlights the outcomes of the patients who received third-line therapy. The median age of patients at commencement of third-line therapy was 41 years (IQR, 30–47.5 years). Patients were severely immunosuppressed with a median CD4 cell count of 147.5 cells/mm^3 (IQR, 28–252.5 cells/mm^3) and a median HIV viral load of 57 774 copies/mL (IQR, 18 809–215 624 copies/mL) at commencement of third-line therapy. At the time of analysis among participants commenced on third-line ART, none had been lost to follow-up and 2 had died, 1 due to chronic renal failure (diagnosed while the participant was on firstline therapy) and 1 due to acute alcohol-induced pancreatitis.

At week 24 on third-line therapy, the median CD4 cell count increased from 147.5 to 251.5 cells/mm^3 (IQR, 187.5–381 cells/mm^3). At week 24 on third-line therapy, 29/36 (81%) participants achieved viral suppression of <50 copies/mL, 5/36 (14%) patients had VL between 50 and 1000 copies/mL and 1/36 (3%) had died. One, a 17-year-old adolescent, had a week 24 VL of 2244 copies/mL and has been receiving adherence support, and to date he has not managed to achieve virological suppression. There were no reported discontinuations due to toxicity of any of the third-line medicines.

Among the 39 patients who had no PI mutations and continued on second-line ART with ongoing adherence support, only 8 achieved virological suppression 24 weeks after HIV drug resistance testing. Two participants were recommenced on firstline (because they had wild-type virus), and both achieved virological suppression after 24 weeks of firstline ART.

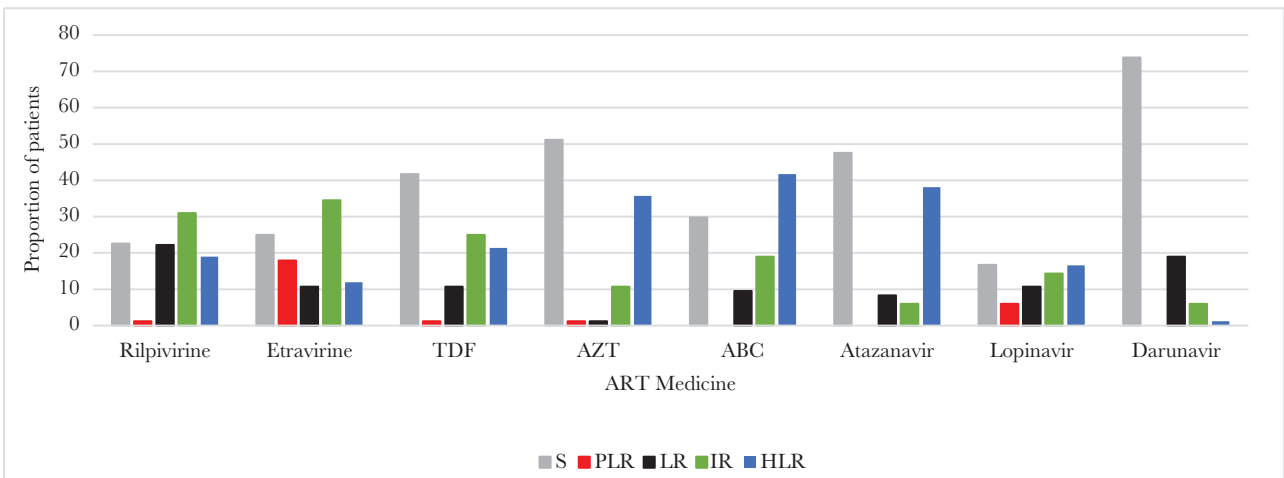

Figure 3. HIV drug resistance interpretation. Abbreviations: ABC, Abacavir; AZT, Zidovudine; HLR, high-level resistance; IR, intermediate resistance; LR, low-level resistance; PLR, potential low-level resistance; S, susceptible; TDF, Tenofovir.

Table 2. Risk Factors for Major Protease Inhibitor Resistance Mutations

	Univariable		Multivariable	
Risk Factor	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
VL > 100 000 copies/mL	2.04 (0.84–4.92)	.114	2.14 (0.75–6.12)	.155
2nd-line duration > 2 y	1.31 (0.5–3.14)	.541	1.65 (0.61–4.50)	.327
Age > 24 y	4.11 (1.62–10.43)	.003	4.75 (1.69–13.34)	.003
CD4 < 200 cells/mm^3	3.67 (1.43–9.43)	.007	2.53 (0.90–7.15)	.079

Abbreviations: CI, confidence interval; OR, odds ratio; VL, viral load.

DISCUSSION

Among patients referred for second-line failure and genotyped after 6 weeks of aggressive adherence support, 14% had wild-type virus, suggesting very low adherence, and 86% had mutant virus. Among those with drug resistance mutations, all had 1 or more NNRTI and/or NRTI mutations and 44/86 (51%) had major PI resistance mutations. Younger patients (<24 years), were less likely to have acquired major PI drug resistance mutations upon failing PI-based second-line treatment. Viral load and immunological status at resistance testing were not independently associated with major PI RAMs. Early third-line treatment outcomes were excellent, with 30/36 patients achieving viral loads <50 copies/mL at 24 weeks.

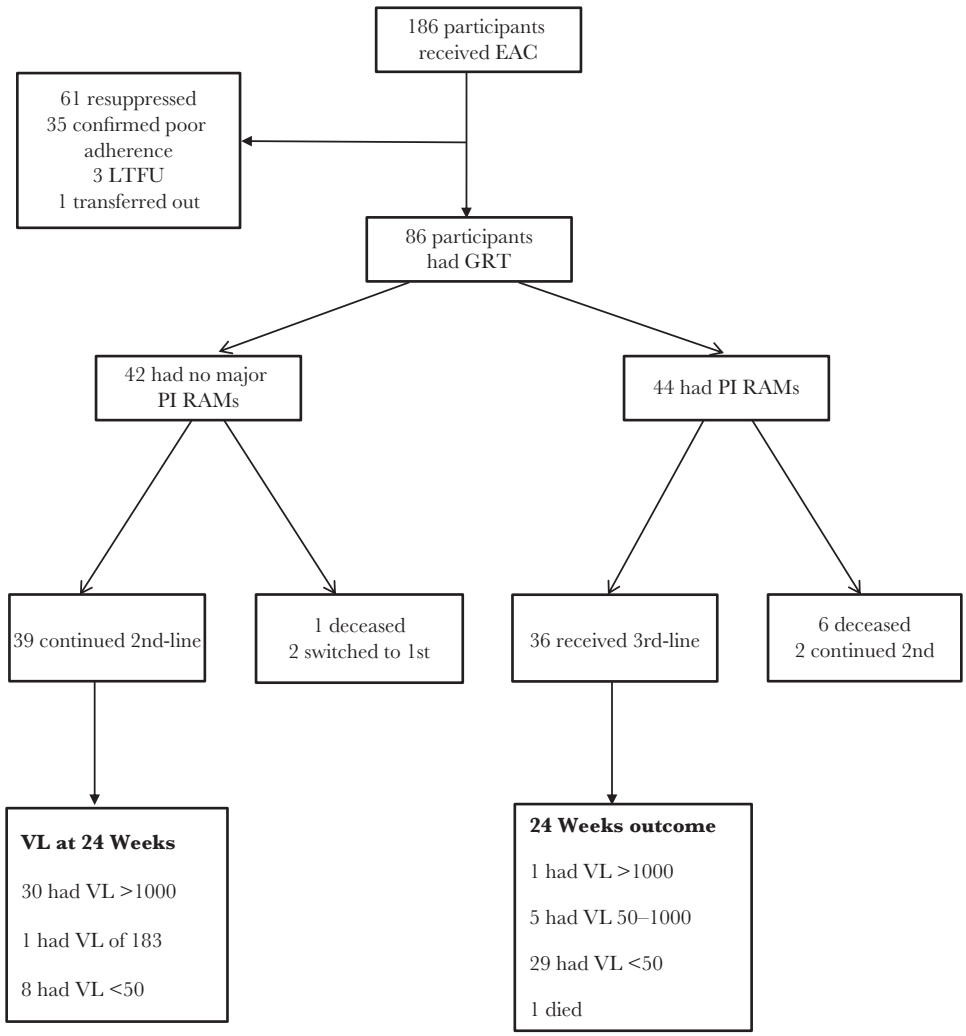

Figure 4. Outcomes of patients failing second-line antiretroviral therapy who received genotypic resistance testing. Abbreviations: EAC, enhanced adherence counseling; GRT, genotypic resistance testing; LTFU, loss to follow-up; PI, protease inhibitor; RAM, resistance-associated mutation; VL, viral load.

This study has some limitations. Our sample size was small. However, as resistance data after second-line failure are scarce, we believe that these results are important to clinicians looking after patients failing second-line ART. Population-based sequencing, used in this analysis, is not able to detect minority resistant viral strains, thus potentially underestimating resistance [20]. Moreover, the durability of suppression on third-line therapy was only established for 6 months, and longer follow-up is essential. Lack of resistance patterns after firstline failure was also a limitation.

Patterns of DRM demonstrate that despite interventions to improve adherence, almost half of the patients who had GRT done did not acquire major PI resistance-associated mutations. A total of 61 (33%) patients out of the original 186 resuppressed after adherence support, highlighting that they had a virus susceptible to second-line ART. This provides evidence that virologic failure is likely due to poor adherence, leading to reduced drug exposure. A number of studies from resource-limited settings have reported low rates of PI resistance after failure of second-line ART [5, 6, 21], often attributing this finding to poor medication adherence. The presence of major protease inhibitor mutations at the time of second-line failure ranges from 0% to 50% [22]. A recent national survey in Kenya reported a 25% prevalence of PI mutations among patients failing second-line ART [23]. The high prevalence of PI resistance in our cohort can be attributed to possible selection bias. Only patients with reported good adherence (after at least 6 weeks of enhanced adherence counseling) had a GRT done, that is, only 86 out of the original 186. The association of younger age and PI resistance is consistent with findings from similar studies in South Africa and the United Kingdom [5, 24, 25]. These studies show that second-line failure in young people is often due to poor adherence rather than development of PI RAMs. Age may provide an explanation for some of the patient-level, regimen-specific, and structural factors associated with the absence of PI mutations and reduced adherence to second-line ART [26–29]. Social and structural obstacles to adherence can include inaccessible clinic location or lack of access to transportation, work/child care responsibilities, and low health care provider to patient ratio as a consequence of the rapid growth in ART rollout programs [30, 31]. Optimizing treatment adherence and retention at all stages in the cascade of HIV care is critical to the prevention of resistance.

As expected in Africa, the predominant NRTI mutation observed in our cohort was MI84V, which confers high-level resistance to Lamivudine and Emtricitabine [32]. The observed prevalence of TAMs was high, the commonest being T215Y and D67N. TAMs are known to accumulate in patients who remain on a failing ART regimen due to delays in detecting treatment failure [33]. Patients may have been failing on second-line ART for a long time before enrolling in the adherence program, but either viral loads were not done prior to that time or the data were not provided. It is important for HIV treatment programs to offer routine viral load testing to enable early diagnosis of treatment failure and hence prevent accumulation of TAMs. TAMs have the potential to confer resistance to all drugs in the NRTI class.

Despite the absence of NNRTI exposure during second-line ART, the virus from almost 84% of patients had at least 1 major NNRTI resistance mutation, and the virus from 40% had a mutation at the K103N codon, strongly associated with resistance to nevirapine and efavirenz. The persistence of NNRTI resistance is consistent with genotypic analyses of 2 South African cohorts that failed PI-based ART [5, 25] and precludes recycling of firstline NNRTI drugs in third-line therapy. The high levels of NNRTI resistance mutations may indicate extensive drug resistance including NRTI mutations prior to the onset of second-line therapy.

The need for evidence regarding the implementation of third-line ART in resource-limited settings has been recognized by the WHO [3]. Our data demonstrate the effectiveness of third-line ART in a cohort of patients who are infected with HIV subtype C. The majority of patients achieved virologic suppression on regimens including darunavir/ritonavir, raltegravir, and NRTIs, suggesting that this can be used as a standardized third-line regimen in Zimbabwe. Data on the treatment outcomes of third-line ART are still very scarce in sub-Saharan Africa; however, 2 other reports have provided evidence of effectiveness [34, 35]. In a small Indian cohort, early treatment outcomes showed excellent effectiveness of third-line ART [36]. Although the small cohort size limits wider assumptions of efficacy, the preliminary outcomes suggest that third-line therapy can be effectively implemented in a resource-limited setting with excellent rates of virologic suppression. Furthermore, our results support the use of darunavir/ritonavir and an InSTI backbone for third-line ART, as recommended by the WHO [3].

CONCLUSIONS

Prevalence of RAMs was high among participants failing second-line ART. However, only half of these participants had major PI RAMs, which necessitate the switch to third-line treatment. The presence of major PI RAMs was significantly associated with an increase in age. Younger participants were more likely to fail second-line treatment due to poor adherence rather than development of PI resistance. GRT is essential to identify those with triple class resistance, and those who require third-line therapy to regain and sustain virologic suppression. A Darunavir/r, Integrase strand transfer inhibitor and optimized NRTI (based on GRT) regimen was effective in achieving virologic suppression in early follow-up. Our results show that third-line regimens for patients with multidrug-resistant HIV in Africa are likely to be effective.

Acknowledgments

Financial support. The authors acknowledge Ruedi Luethy for his support of the study. Research reported in this publication was supported by the Ruedi Luethy Foundation and the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award number U01AI069924 (PIs: Egger and Davies). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. O. Keiser and J. Estill were supported by a professorship grant from the Swiss National Science Foundation (grant No. 163878).

Potential conflicts of interest. All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Clavel F, Hance AJ. HIV drug resistance. N Engl J Med **2004**; 350:1023–35.
2. Hamers RL, Wallis CL, Kityo C, et al; and PharmAccess African Studies to Evaluate Resistance (PASER). HIV-1 drug resistance in antiretroviral-naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study. Lancet Infect Dis **2011**; 11:750–9.
3. World Health Organization. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection. Geneva: WHO; **2016**.
4. Haas AD, Keiser O, Balestre E, et al; IeDEA southern Africa, east Africa, and west Africa. Monitoring and switching of first-line antiretroviral therapy in adult treatment cohorts in sub-Saharan Africa: collaborative analysis. Lancet HIV **2015**; 2:e271–8.
5. Wallis CL, Mellors JW, Venter WD, et al. Protease inhibitor resistance is uncommon in HIV-1 subtype C infected patients on failing second-line lopinavir/r-containing antiretroviral therapy in South Africa. AIDS Res Treat **2011**; 2011:769627.
6. Levison JH, Orrell C, Gallien S, et al. Virologic failure of protease inhibitor-based second-line antiretroviral therapy without resistance in a large HIV treatment program in South Africa. PLoS One **2012**; 7:e32144.
7. Cambiano V, Bertagnolio S, Jordan MR, et al. Predicted levels of HIV drug resistance: potential impact of expanding diagnosis, retention, and eligibility criteria for antiretroviral therapy initiation. AIDS **2014**; 28(Suppl 1):S15–23.
8. Rossouw TM, Feucht UD, Melikian G, et al. Factors associated with the development of drug resistance mutations in HIV-1 infected children failing protease inhibitor-based antiretroviral therapy in South Africa. PLoS One **2015**; 10:e0133452.
9. Lorenzana SB, Hughes MD, Grinsztejn B, et al. Genotype assays and third-line ART in resource-limited settings: a simulation and cost-effectiveness analysis of a planned clinical trial. AIDS **2012**; 26:1083–93.
10. Wong E, Trustman N, Yalong A. HIV pharmacotherapy. J Am Acad Physician Assist **2016**; 29:36–40.
11. Clotet B, Bellos N, Molina J-M, et al. Lefebvre, and POWER 1 and 2 study groups. Efficacy and safety of darunavir-ritonavir at week 48 in treatment-experienced patients with HIV-1 infection in POWER 1 and 2: a pooled subgroup analysis of data from two randomised trials. Lancet **2007**; 369:1169–78.
12. Saravanan S, Madhavan V, Balakrishnan P, et al. Darunavir is a good third-line antiretroviral agent for HIV type 1-infected patients failing second-line protease inhibitor-based regimens in South India. AIDS Res Hum Retroviruses **2013**; 29:630–2.
13. Maiga AI, Fofana DB, Cisse M, et al. Characterization of HIV-1 antiretroviral drug resistance after second-line treatment failure in Mali, a limited-resources setting. J Antimicrob Chemother **2012**; 67:2943–8.
14. National Medicine and Therapeutics Policy Advisory Committee; The AIDS and TB Directorate Ministry of Health and Child Care. Guidelines for Antiretroviral Therapy for the Prevention and Treatment of HIV in Zimbabwe. Zimbabwe: Ministry of Health; **2013**.
15. Manasa J, Danaviah S, Pillay S, et al. An affordable HIV-1 drug resistance monitoring method for resource limited settings. J Vis Exp **2014; (85)**.
16. Kearse M, Moir R, Wilson A, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics **2012**; 28:1647–9.
17. Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. Clin Infect Dis **2006**; 42:1608–18.
18. World Health Organization. HIV and Adolescents: Guidance for HIV Testing and Counselling and Care for Adolescents Living With HIV. Geneva: WHO; **2015**.
19. 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.
20. Kozal MJ, Chiarella J, St John EP, et al. Prevalence of low-level HIV-1 variants with reverse transcriptase mutation K65R and the effect of antiretroviral drug exposure on variant levels. Antivir Ther **2011**; 16:925–9.
21. El-Khatib Z, Ekstrom AM, Ledwaba J, et al. Viremia and drug resistance among HIV-1 patients on antiretroviral treatment: a cross-sectional study in Soweto, South Africa. AIDS **2010**; 24:1679–87.
22. Hosseinipour MC, Gupta RK, Van Zyl G, et al. Emergence of HIV drug resistance during first- and second-line antiretroviral therapy in resource-limited settings. J Infect Dis **2013**; 207(Suppl 2):S49–56.
23. London A, April E, Mukundi I, et al. Emergence of untreatable, multidrug-resistant HIV-1 in patients failing second-line therapy in Kenya. AIDS **2017;** 31:1494–8.
24. Fox MP, Berhanu R, Steegen K, et al. Intensive adherence counselling for HIV-infected individuals failing second-line antiretroviral therapy in Johannesburg, South Africa. Trop Med Int Health **2016**; 21:1131–7.
25. El Bouzidi K, White E, Mbisa JL, et al. Protease mutations emerging on darunavir in protease inhibitor-naïve and experienced patients in the UK. J Int AIDS Soc **2014**; 17:19739.
26. Chesney MA. Factors affecting adherence to antiretroviral therapy. Clin Infect Dis **2000**; 30(Suppl 2):S171–6.
27. Letta S, Demissie A, Oljira L, Dessie Y. Factors associated with adherence to antiretroviral therapy (ART) among adult people living with HIV and attending their clinical care, Eastern Ethiopia. BMC Int Health Hum Rights **2015**; 15:33.
28. Mekonnen Aychiluhem MA, Aychiluhem M, Ayana M, Jara D. Level of ART adherence and associated factors among HIV sero- positive adult on highly active antiretroviral therapy in debre markos referral hospital, Northwest Ethiopia. J Antivir Antiretrovir **2014**; 6:1–7.
29. Pahari S, Roy S, Mandal A, et al. Adherence to anti-retroviral therapy & factors associated with it: A community based cross-sectional study from West Bengal, India. Indian J Med Res **2015**; 142:301–10.
30. Mekuria LA, Prins JM, Yalew AW, et al. Sub-optimal adherence to combination anti-retroviral therapy and its associated factors according to self-report, clinician-recorded and pharmacy-refill assessment methods among HIV-infected adults in Addis Ababa. AIDS Care **2017**; 29:428–35.
31. Geng EH, Bangsberg DR, Musinguzi N, et al. Understanding reasons for and outcomes of patients lost to follow-up in antiretroviral therapy programs in Africa through a sampling-based approach. J Acquir Immune Defic Syndr **2010**; 53:405–11.
32. Ssemwanga D, Lihana RW, Ugoji C, et al. Update on HIV-1 acquired and transmitted drug resistance in Africa. AIDS Rev **2015**; 17:3–20.
33. Cozzi-Lepri A, Phillips AN, Martinez-Picado J, et al; EuroSIDA Study Group. Rate of accumulation of thymidine analogue mutations in patients continuing to receive virologically failing regimens containing zidovudine or stavudine: implications for antiretroviral therapy programs in resource-limited settings. J Infect Dis **2009**; 200:687–97.
34. Meintjes G, Dunn L, Coetsee M, et al. Third-line antiretroviral therapy in Africa: effectiveness in a Southern African retrospective cohort study. AIDS Res Ther **2015**; 12:39.
35. Rawizza HE, Chaplin B, Meloni ST, et al. Accumulation of protease mutations among patients failing second-line antiretroviral therapy and response to salvage therapy in Nigeria. PLoS One **2013**; 8:e73582.
36. Khan S, Das M, Andries A, et al. Second-line failure and first experience with third-line antiretroviral therapy in Mumbai, India. Glob Health Action **2014**; 7.