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

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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/mm³ (IQR, 49–333 cells/mm³). Major PI resistance-associated mutations (RAMs) were demonstrated in 44 (51%) non-nucleoside reverse transcriptase inhibitor RAMs and 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/mm³ (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 prevalence 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.
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 Dolutegavir, 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/mm³), 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/mm³ (IQR, 49–333 cells/mm³), 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>
<thead>
<tr>
<th>Parameter</th>
<th>All Patients (n = 86)</th>
<th>No PI Mutation (n = 42)</th>
<th>Any PI Mutation (n = 44)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (IQR), y</td>
<td>27.7 (19.7–42.3)</td>
<td>21.2 (18.0–38.3)</td>
<td>37.4 (25.9–46.9)</td>
<td>.004</td>
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<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>41 (47.7)</td>
<td>22 (52.4)</td>
<td>19 (43.2)</td>
<td>.729</td>
</tr>
<tr>
<td>Marital status, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>47 (54.7)</td>
<td>32 (76.2)</td>
<td>15 (34.1)</td>
<td>.001</td>
</tr>
<tr>
<td>Married</td>
<td>30 (34.9)</td>
<td>7 (11.7)</td>
<td>23 (52.3)</td>
<td></td>
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<tr>
<td>Widowed</td>
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<td>3 (7.1)</td>
<td>4 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>2 (2.3)</td>
<td>0 (0)</td>
<td>2 (4.6)</td>
<td></td>
</tr>
<tr>
<td>Level of Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>12 (14)</td>
<td>9 (21.4)</td>
<td>3 (6.8)</td>
<td>.006</td>
</tr>
<tr>
<td>Primary</td>
<td>17 (19.7)</td>
<td>12 (28.6)</td>
<td>5 (11.4)</td>
<td></td>
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<tr>
<td>Secondary</td>
<td>43 (50)</td>
<td>18 (42.9)</td>
<td>25 (56.8)</td>
<td></td>
</tr>
<tr>
<td>Tertiary</td>
<td>14 (16.3)</td>
<td>3 (7.1)</td>
<td>11 (25)</td>
<td></td>
</tr>
<tr>
<td>CD4 count, median (IQR), cell/mm³</td>
<td>201 (49–333)</td>
<td>243 (132–379)</td>
<td>97 (22–277)</td>
<td>.032</td>
</tr>
<tr>
<td>HIV RNA, median (IQR), copies/mL</td>
<td>65,210 (8728–208,920)</td>
<td>37,238 (4620–147,592)</td>
<td>79,362 (20,376–254,612)</td>
<td>.039</td>
</tr>
<tr>
<td>Duration of ART (IQR), y</td>
<td>7.7 (5.3–9.4)</td>
<td>7.3 (5.0–9.4)</td>
<td>7.9 (6.1–9.5)</td>
<td>.388</td>
</tr>
<tr>
<td>2nd-line ART duration (IQR), y</td>
<td>2.6 (1.6–4.9)</td>
<td>2.4 (1.6–4.7)</td>
<td>2.6 (1.7–5.2)</td>
<td>.318</td>
</tr>
<tr>
<td>No. of ART drugs received, median (IQR) at GRT</td>
<td>6 (6–7)</td>
<td>6.5 (6–8)</td>
<td>6 (5–7)</td>
<td>.081</td>
</tr>
</tbody>
</table>

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

**Figure 2.** Distribution of specific HIV drug resistance mutations. Abbreviation: HIVDR, HIV drug resistance.
The 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, 21 (25%) to efavirenz, 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/mm³ (odds ratio, 3.67 cells/mm³; 95% confidence interval [CI], 1.43–9.43 cells/mm³) 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/mm³ (IQR, 28–252.5 cells/mm³) and a median HIV viral load of 57774 copies/mL (IQR, 18809–215624 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/mm³ (IQR, 187.5–381 cells/mm³). 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.

![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.](https://academic.oup.com/ofid/article-abstract/5/2/ofy005/4835575/4835575/15283343d3a8326013f33668dd0e7d7a)
among patients referred for second-line failure and geno-
typed 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 treat-
ment. 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.

**DISCUSSION**

Among patients referred for second-line failure and geno-
typed after 6 weeks of aggressive adherence support, 14% had
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**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 first-line 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 roll-out 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 M184V, 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

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