Emergence of untreatable, multidrug-resistant HIV-1 in patients failing second-line therapy in Kenya

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Running head: Second-line antiretroviral treatment resistance
Abstract

We performed a countrywide assessment of HIV drug resistance among 123 patients with virological failure on second-line antiretroviral therapy (ART) in Kenya. The percentage of patients harboring intermediate-to-high-level resistance was 27% for lopinavir-ritonavir, 24% for atazanavir-ritonavir and 7% for darunavir-ritonavir, and 25% had complete loss of activity to all available first- and second-line drugs. Overall, one in four patients failing second-line ART have completely exhausted available antiretrovirals in Kenya, highlighting the need for increased access to third-line drugs.

Keywords HIV-1; second-line antiretroviral therapy; drug resistance; resource-limited setting
To date, nearly half a million HIV-1-infected patients in sub-Saharan Africa have been switched to second-line ART, based on boosted-protease-inhibitors (bPI), after first-line failure\(^1\). With scale-up of viral-load testing, the number is forecasted to grow to 4-6 million by 2030, comprising 20% of all on ART\(^2\). Virological failure on second-line ART, mostly lopinavir-ritonavir-based, has been reported in up to 38% of patients after 3 years of treatment\(^3\). However, data on resistance are limited and access to third-line ART is restricted due to exorbitantly high drug costs. In a cross-sectional study in the national ART program in Kenya, we assessed HIV drug resistance among patients failing second-line bPI-based ART between June 2010 and December 2015.

Treatment failure was defined as either clinico-immunological failure with a single confirmatory plasma viral load (pVL) of >1,000 cps/ml or two consecutive pVL >1,000 cps/ml after intensive adherence counseling. We included plasma/DBS specimens sent to the WHO-designated KEMRI/CDC laboratory for HIV drug resistance testing from ART sites in western Kenya (2010-2012) and nationwide (2013-2015). Pol gene sequences were obtained using the CDC in-house genotyping assay\(^4\). We calculated the genotypic susceptible scores (GSS) as 1.00-0.75-0.50-0.25 and 0, based on the Stanford HIV drug resistance algorithm v7.0: for susceptible, potential low-level-, low-level-, intermediate-level- and high-level resistance, respectively\(^5\). Predicted efficacy to WHO-recommended first-, second- and third-line regimens was calculated as an arithmetic sum of the individual-drug GSS; GSS of <2 was considered as exhaustion to the available drug options. Integrase-inhibitor (INSTI)-based regimens were assigned a full susceptibility score due to their limited use in the region. We compared the predicted GSS for potential third-line regimens based on the previous (INSTI+etravirine+darunavir-ritonavir)\(^6\) and current (INSTI+darunavir-ritonavir+1 or 2 NRTIs)\(^7\) WHO recommendations using the z-test. Factors associated with intermediate to high-level PI-resistance were assessed using multivariable logistic regression analyses. The study was approved by the scientific and ethics committees of the Kenya Medical Research Institute.
123 of 126 viral isolates had a successful genotype and were included in the analysis. The median age was 24 (IQR 10-36) years, median CD4 count was 114.5 (IQR 24-251) cells/μL, and mean VL was 4.8 (SD 0.1) log10 cps/mL. The median time on ART was 6.4 years (IQR 4.3-8.1), including 3.1 years (IQR 1.9-4.6) on second-line. 116 (97%) patients were on lopinavir-ritonavir, with the most common NRTI-backbone being tenofovir+lamivudine (35%), followed by abacavir+lamivudine (23%), abacavir+didanosine (11%) and zidovudine+lamivudine (11%).

63% of patients had ≥1 NRTI resistance mutation, predominantly M184I/V (51%) and thymidine analogue mutations (TAMs) (37%). 32% of patients had ≥1 major PI resistance mutation with a median number of 3 (range 1-5), most frequently M46I/L (24%), I54V (22%) and V82A/T/F/S (20%). 24% of patients had triple-class (NNRTI, NRTI and PI) resistance, 34% had no NRTI or PI mutations, 18% had wild-type virus.

27% of patients had intermediate-to-high level resistance to lopinavir-ritonavir, 24% to atazanavir-ritonavir and 7% to darunavir-ritonavir. Cross-resistance to the second-generation NNRTIs was present in 46% of patients for rilpivirine and 36% for etravirine. Of note, 25% (31/123) of the patients had exhausted all first- and second-line drug options available in Kenya (Figure).

Patients with PI-resistance were more likely to have ≥2 TAMs (OR 15.1, 95%CI 5.3-42.9) but associations with duration of treatment, sex, age, CD4 and pVL were non-significant.

Predicted probability for having GSS>2 was highest if third-line regimens of darunavir-ritonavir plus INSTI included etravirine as the third-agent (0.70). If etravirine was replaced with an NRTI-backbone the probabilities of GSS>2 were somewhat (although not statistically significantly) lower for dual NRTIs (zidovudine+lamivudine (0.61, p=0.219), tenofovir+lamivudine (0.55, p=0.102),and significantly lower for a single NRTI (lamivudine/emtricitabine (0.48, p=0.04), zidovudine (0.48, p=0.04), tenofovir (0.42, p=0.01 3), abacavir (0.39, p=0.007)) (Figure).
This is among the first nationwide assessments of HIV drug resistance among patients failing second-line ART in sub-Saharan Africa. This study in the Kenyan national ART program suggests that about 27% of patients with second-line failure are in need of a switch to third-line therapy, with 25% demonstrating complete exhaustion of alternative first or second-line regimens. Few other observational studies in the African region have reported on ART exhaustion in 9-32% of patients failing second-line therapy\textsuperscript{8–10}. These data indicate an urgent need for increasing access to third-line drugs, i.e. INSTIs (raltegravir, dolutegravir) and darunavir/ritonavir.

WHO-recommended third-line drugs are prohibitively expensive with costs nearly 6-14 times higher than the current first- and second-line regimens\textsuperscript{11}. Sustainability is thus a challenge for ART programs in low and middle-income countries (LMICs), citing the case of Brazil where provision of third-line to about 5% of the patients accounts for \textasciitilde40% of all ART resources\textsuperscript{12}. Ongoing negotiations with pharmaceutical companies for production of generic third-line options may potentially lead to price reductions in the near future\textsuperscript{13}.

About two-thirds of the participants did not have PI resistance mutations, which concurs with previous studies\textsuperscript{10,14,15}. Possible explanations include: complete non-adherence hence no resistance mutations are selected in the absence of drugs; the characteristic short-mutant selection window for PIs, attributed to the rapid fall in the inhibitory concentration during non-adherence\textsuperscript{16}; and mediation of PI-resistance by mutations outside the protease gene, specifically in the \textit{gag}\textsuperscript{17} and \textit{env} genes\textsuperscript{18}. In this study, we neither assessed the influence of these mutations nor that of adherence, hence we are unable to ascertain the cause of treatment failure in patients without major PI resistance mutations.

Due to limited data in support of NRTI-sparing regimens, WHO guidelines recommend recycling of NRTIs in third-line therapy. In our study, however, the predicted response
for third-line regimens comprising INSTI plus darunavir/ritonavir was highest if it included etravirine as the third agent instead of a single NRTI, but was comparable with inclusion of 2 NRTIs in a four-drug combination. The low GSS of the NRTIs could be attributed to accumulation of TAMs, due to delayed switches. Optimal efficacy may thus depend on timely detection of failure and switch to third-line treatment.

Study limitation exists. We may have under-estimated the prevalence of second-line treatment failure as some ART sites may have been less vigilant, or lacked appropriate tools to timely identify these patients and confidently notify the national program. However, with the inclusion of routine viral-load tests and HIV drug resistance testing for second-line failures in recent guidelines\textsuperscript{19,20}, it is anticipated that patient identification will be significantly improved.

In conclusion, our study indicates that nearly one in four patients in Kenya failing second-line treatment has complete exhaustion to available antiretrovirals, emphasizing the need for increased access to third-line treatment in LMICs.

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Conflicts of interest

We declare that we have no conflicts of interest.
Reference list


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Genotypic sensitivity scores (GSS) calculated as 1.00-0.75-0.50-0.25 and 0, based on Stanford HIV drug resistance algorithm categories for susceptible, potential low-level-, low-level-, intermediate-level- and high-level resistance respectively; GSS for combined ART calculated as arithmetic sum of individual drugs. 3TC-lamivudine; ABC-Abacavir; AZT-Zidovudine; ETR-etravirine; FTC-emtricitabine; TDF-tenofovir. Any 1st and 2nd-line includes NNRTIs NVP or EFV (1st-line), PIs lopinavir-ritonavir or atazanavir-ritonavir (2nd-line) with NRTI backbone of 3TC or FTC plus either AZT, ABC or TDF. The calculations for GSS in third-line include the core drugs INSTI+darunavir-ritonavir and the third agent as either etravirine (2nd generation NNRTI) or single or dual NRTI regimens as indicated in the x-axis.