Research Letter

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

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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 harbouring 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.

To date, nearly half a million HIV-1 infected patients in sub-Saharan Africa have been switched to second-line antiretroviral therapy (ART), based on boosted protease inhibitors (bPIs), after first-line failure [1]. With scale-up of viral-load testing, the number is forecast 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 programme 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 more than 1000 cps/ml or two consecutive pVL more than 1000 cps/ml after intensive adherence counselling. 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 less than 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 and darunavir-ritonavir) [6] and current [INSTI and darunavir-ritonavir+1 or two nucleoside reverse transcriptase inhibitors (NRTIs)] [7] WHO recommendations using the z-test. Factors associated with intermediate to high-level protease inhibitor resistance were assessed using multivariable logistic regression analyses. The study was approved by the scientific and ethics committees of the Kenya Medical Research Institute.

One hundred and twenty-three out of 126 viral isolates had a successful genotype and were included in the analysis. The median age was 24 (IQR 10–36) years, median $CD4^+$ cell count was 114.5 [interquartile range (IQR) 24–251] cells/μl and mean viral load was 4.8 (SD 0.1) $\log_{10}$ 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. One hundred and sixteen (97%) patients were on lopinavir-ritonavir, with the most common NRTI-backbone being tenofovir and lamivudine (35%), followed by abacavir and lamivudine (23%), abacavir and didanosine (11%) and zidovudine and lamivudine (11%).

Sixty-three percent of patients had at least one NRTI resistance mutation, predominantly M184I/V (51%) and thymidine analogue mutations (TAMs) (37%). Thirty-two percent of patients had at least one major protease inhibitor resistance mutation with a median number of 3 (range 1–5), most frequently M46I/L (24%), I54V (22%) and V82A/T/F/S (20%). Twenty-four percent of patients had triple-class (NNRTI, NRTI and protease inhibitor) resistance, 34% had no NRTI or protease inhibitor mutations, 18% had wild-type virus.

Twenty-seven percent 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 all patients, including 94% of those with PI resistance, had exhausted all first-line and second-line drug options available in Kenya (Fig. 1).

Patients with protease inhibitor resistance were more likely to have at least two TAMs [odds ratio (OR) 15.1, 95% confidence interval (95% CI) 5.3–42.9], but

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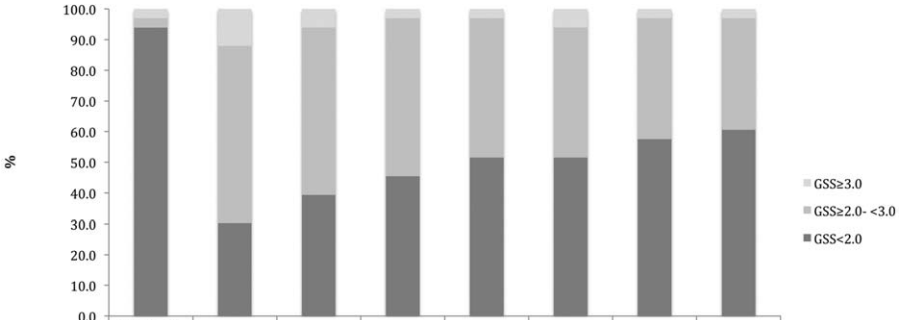

	Any 1st or 2nd line	ETR	3TC+ZDV	TDF+3TC	3TC/FTC	ZDV	TDF	ABC
GSS≥3.0	3.0	12.1	6.1	3.0	3.0	6.1	3.0	3.0
GSS≥2.0- <3.0	3.0	57.6	54.5	51.5	45.5	42.4	39.4	36.4
GSS<2.0	93.9	30.3	39.4	45.5	51.5	51.5	57.6	60.6

Fig. 1. Predicted antiretroviral susceptibility to available WHO-recommended first, second and potential third-line regimens among patients resistant to protease-inhibitor second-line treatment in Kenya. First-line, NNRTIs + 2NRTIs; second-line, PIs + 2 NRTIs. The calculations for GSS in third-line include the core drugs INSTI and darunavir-ritonavir and the third agent as either etravirine (second-generation NNRTI) or single or dual NRTI regimens as indicated in the x-axis. 3TC, lamivudine; ABC, abacavir; ETR, etravirine; FTC, emtricitabine; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; TDF, tenofovir; ZDV, zidovudine.

associations with duration of treatment, sex, age, $CD4^+$ cell count and pVL were nonsignificant.

Predicted probability for having GSS more than 2 was highest if third-line regimens of darunavir-ritonavir along with INSTI included etravirine as the third-agent (0.70). If etravirine was replaced with an NRTI-backbone, the probabilities of GSS more than 2 were somewhat (although not statistically significantly) lower for dual NRTIs (zidovudine and lamivudine (0.61, $P = 0.219$), tenofovir and 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, $P = 0.013$), abacavir (0.39, $P = 0.007$) (Fig. 1).

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 programme 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 [8–10]. These data indicate an urgent need for increasing access to third-line drugs, that is 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-line and second-line regimens [11]. Sustainability is thus a challenge for ART programmes 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 nearly 40% of all ART resources [12]. Ongoing negotiations with pharmaceutical companies for production of generic third-line options may potentially lead to price reductions in the near future [13].

About two-thirds of the participants did not have protease inhibitor resistance mutations, which concurs with previous studies [10,14,15]. Possible explanations include complete nonadherence, hence no resistance mutations are selected in the absence of drugs; the characteristic short-mutant selection window for protease inhibitors, attributed to the rapid fall in the inhibitory concentration during nonadherence [16]; and mediation of protease inhibitor resistance by mutations outside the protease gene, specifically in the *gag* [17] and *env* genes [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 protease inhibitor 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 along with darunavir/ritonavir was highest if it included etravirine as the third agent instead of a single NRTI, but was comparable with inclusion of two 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 underestimated 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 programme. However, with the inclusion of routine viral-load tests and HIV drug resistance testing for second-line failures in recent guidelines [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.

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