Short communication

HIV drug resistance mutations among patients failing second-line antiretroviral therapy in Rwanda

Jean d'Amour Ndahimana[1†], David J Riedel[2†], Ribakare Muhayimpundu[1], Sabin Nsanzimana[1], Gad Niyibizi[1], Emmanuel Mutaganzwa[3], Augustin Mulindabigwi[1], Cyprien Baribwira[4], Athanase Kiromera[4], Linda L Jagodzinski[5], Sheila A Peel[5], Robert R Redfield[2]*

[1]HIV/AIDS Division, Rwanda Biomedical Center, Kigali, Rwanda
[2]Institute of Human Virology and Division of Infectious Diseases, University of Maryland School of Medicine, Baltimore, MD, USA
[3]Reference Laboratory, Rwanda Biomedical Center, Kigali, Rwanda
[4]University of Maryland School of Medicine – Institute of Human Virology, Kigali, Rwanda
[5]Walter Reed Army Institute of Research, Silver Spring, MD, USA

*Corresponding author e-mail: driedel@ihv.umaryland.edu
†These authors contributed equally to this work

Background: Studies of patients failing second-line antiretroviral therapy (ART) in resource-limited settings (RLS) are few. Evidence suggests most patients who appear to be virologically failing do so not due to drug resistance but to poor adherence, which, if properly addressed, could allow continued use of less expensive first- and second-line regimens. Drug resistant mutations (DRMs) were characterized among patients virologically failing second-line ART in Rwanda.

Methods: A total of 128 adult patients receiving second-line ART for at least 6 months were invited to participate; 74 agreed and had HIV-1 viral load (VL) measured. Resistance genotypes were conducted in patients with virological failure (VF; that is, VL ≥1,000 copies/ml).

Results: In total, 35 patients met the criteria for VF. The median time on lopinavir/ritonavir-based second-line ART was 2.7 years. Of 30 successful resistance genotype analyses, 13 (43%) had ≥1 nucleoside reverse transcriptase inhibitor (NRTI) mutation, 18 (60%) had at least 1 non-NRTI mutation and 5 (17%) had at least 1 major protease inhibitor mutation. Eleven (37%) had virus without significant mutations that would be fully sensitive to first-line ART; 12 (40%) had DRM to first-line ART but sensitive to second-line ART. Only 7 patients (23%) demonstrated a DRM profile requiring third-line ART.

Conclusions: Among 30 genotyped samples of patients with VF on second-line ART, more than one-third had no significant DRMs, implicating poor adherence as the primary cause of VF. The majority of patients (77%) would not have required third-line ART. These findings reinforce the need for intensive adherence assessment and counselling for patients who appear to be failing second-line ART in RLS.

Introduction

Rwanda has successfully scaled-up first-line antiretroviral therapy (ART) [1]. However, as in other resource-limited settings (RLS), viral load (VL) monitoring during the initial scale-up period was not routinely available. Patients failing first-line ART regimens were at risk of acquisition and accumulation of HIV drug resistance mutations (DRMs) that could compromise effectiveness of second-line ART regimens [2–4].

The number of patients on second-line ART in Rwanda has increased substantially, from 388 in 2006 to 2,275 at the end of March 2012. About 4% of all patients on ART in Rwanda are taking second-line regimens [5], similar to other RLS, where the proportion of patients taking second-line ART is about 3% [6].

Studies of patients receiving second-line ART in low- and middle-income countries have shown rates of virological failure ranging from 22% to 38% [6–9]. Only a few studies have investigated emergence of HIV DRMs among patients failing second-line ART in RLS [10–12].

As third-line antiretroviral agents become increasingly available in RLS, HIV resistance genotyping will be an important tool to assess ART options.

However, when adherence is the reason for virological failure rather than resistance, it may be possible for patients to continue second-line ART regimens after enhanced adherence counselling instead of transitioning to expensive and more complex third-line regimens. This study was conducted to characterize DRMs for patients failing second-line ART regimens.

Methods

Study setting

According to the Rwanda 2011 National HIV Guidelines [13], eligibility criteria for initiation of second-line ART were virological failure (VL ≥1,000 copies/ml) with documented good adherence after at least 6 months of first-line ART. HIV VL was monitored every 12 months. HIV-1 resistance genotyping was not routinely available.

Before 2009, the standard ART regimen included two nucleoside reverse transcriptase inhibitiors (NRTIs; stavudine or zidovudine) plus lamivudine, combined with one non-nucleoside reverse transcriptase inhibitor (NNRTI; nevirapine or efavirenz). Tenofovir was recommended as the first NRTI option for first-line ART in 2009. The second-line regimen included lopinavir/ritonavir plus two NRTIs. A third-line ART regimen including etravirine, darunavir/ritonavir and raltegravir (with or without tenofovir/lamivudine) became available in Rwanda in 2012.

Study design

The national electronic reporting tool (Tracnet) was used to identify all health facilities in Rwanda reporting patients on second-line ART as of 31 March 2012. All patients prescribed second-line ART with documented VL ≥1,000 copies/ml in the preceding 12 months were contacted and invited to participate (patients <15 years and those on second-line ART for <6 months were excluded). A questionnaire (including socio-demographic information) and adherence assessment for past 30 days (visual analogue scale) were administered. VL determinations were by the Roche Cobas Ampliprep and Cobas TaqMan 96 (CAP/CTM; Roche Molecular Diagnostics, Pleasanton, CA, USA) with kit HI2CAP at the National Reference Laboratory, Kigali, Rwanda.

Samples with VL ≥1,000 copies/ml were analysed for the presence of DRMs using the TRUGENE HIV-1 Genotyping Assay on the OpenGene DNA system (Siemens HealthCare, Malvern, PA, USA) at the HIV Diagnostics and Reference Laboratory, US Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, USA. ART resistance mutations and drug susceptibility were generated with the FDA-approved TRUGENE HIV-1 software Guidelines Rules 17.0. HIV-1 subtype determination was based upon phylogenetic tree analysis of the pro/rt sequences (MegAlign, DNASTAR, Inc., Madison, WI, USA; HIV BLAST and phylogenetic tree tools. Protease and reverse transcriptase sequences were submitted to GenBank having accession numbers KT982483-KT982533.

Statistical analyses

All data were entered into EpiData version 2.0 (Odense, Denmark). Descriptive statistics were analyzed using SPSS version 16.0 (IBM, Chicago, IL, USA).

Ethical considerations

Informed consent was obtained from subjects prior to participation in the study. The protocol was approved by the National Health Research Committee and Rwanda National Ethical Committee.

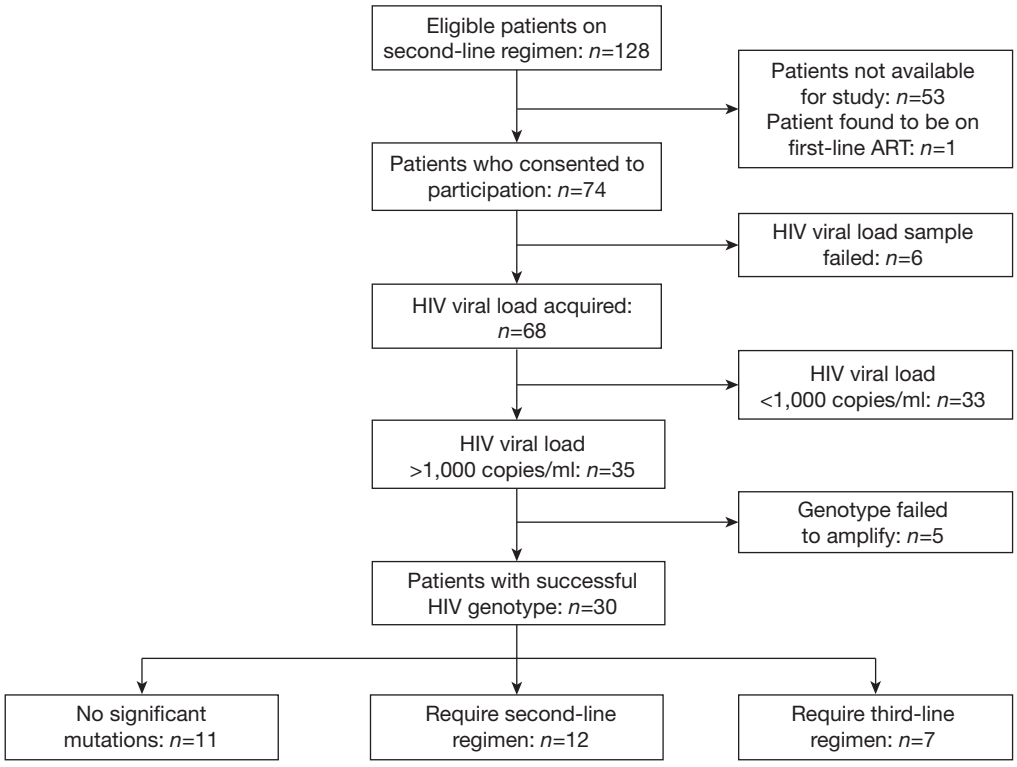
Results

A total of 74 patients participated in the study (Figure 1). The median age was 40.5 years (IQR 30–50), and 69% were female (Table 1). The median $CD4^+$ T-cell count at data collection was 170 cells/mm^3 (IQR 75–320), and the median VL was 1,230 copies/ml (IQR 82–57,525). The median total time on ART was 6.9 years (IQR 4.8–8.1). The overall mean and median adherence in the preceding 30 days was 88% and 100%, respectively. Reported adherence was not associated with VL suppression (OR=0.5 [0.2–1.6]; *P*=0.4).

The majority of patients (90%) had been treated with first-line ART-containing thymidine analogues (Table 1): stavudine (51%) and zidovudine (38%). All participants initiated second-line ART with lopinavir/ritonavir. The NRTI backbone for second-line therapy consisted of tenofovir (40%), abacavir/didanosine (16%), zidovudine (16%) and abacavir (15%), each with lamivudine. The median time on second-line ART at the time of DRM analysis was 2.6 years (IQR 1.5–3.8).

Among 68 VL determinations, 13 (19%) were virologically suppressed (<50 copies/ml), 29 (43%) had VL <500 copies/ml and 35 (51%) were in virological failure with VL >1,000 copies/ml. Genotypes were successfully performed on 30 samples: 21 (70%) sequences monophyletically clustered with subtype A1, 8 (27%) were subtype C and 1 (3%) was subtype D. Among the 30 genotypes (Table 2), 10 (33%) contained one or more thymidine analogue mutations (TAMs). Mutations M41L, D67N and T215Y/F/V were the most common and were found in 6 (20%),

Figure 1. Study flow diagram



ART, antiretroviral therapy.

6 (20%) and 9 (30%) of the genotypes, respectively. Three or more TAMs were present in 6 genotypes (20%). Nine (30%) genotypes detected a M184V mutation. The K65R mutation was not detected. A majority of genotypes detected significant NNRTI mutations (60%), most commonly the K103N/S (27%) and Y181C/V/Y (20%). Five (17%) genotypes detected at least one major protease inhibitor (PI) mutation: M46I (13%), I54V (17%), V82A/V (13%) and I84V (6%).

DRM analysis demonstrated that among 30 patients, 19 (63%) exhibited resistance to at least one ART. NNRTI resistance was most common (60%), followed by NRTI (30%) and PI (17%). NRTI resistance profiles were 30% to lamivudine, 27% to zidovudine, 27% to abacavir and 23% to tenofovir. In contrast, 17% of profiles indicated reduced susceptibility to lopinavir and atazanavir, while darunavir retained activity in all cases of major PI mutations. Reported adherence was not associated with DRMs.

Review of the full resistance profile for each patient determined that 11 had no significant mutations (that is, a first-line regimen would be effective assuming no archived or undetected mutations). Second-line regimens would be required for 12 (40%) while 7 (23%) would require a third-line regimen. Of the 11 patients with VLs >100,000 copies/ml, 1 patient had significant DRMs requiring a third-line regimen.

Discussion

This study was the first to identify and analyse DRMs in HIV-infected patients failing second-line ART regimens in Rwanda. Little data exists for DRMs in patients failing second-line ART in Africa [4,6].

Table 1. Characteristics of 74 patients receiving second-line antiretroviral therapy

Characteristic	Value
Median age, years (IQR)	40.5 (30–50)
Female sex, *n* (%)	51 (69)
Median duration between HIV diagnosis and first-line ART initiation, years (IQR)[a]	1.6 (0.2–5.3)
Median duration of first-line ART, years (IQR)	3.7 (2.0–5.2)
First-line ART before change to second-line[a]	
d4T + 3TC + NVP, *n* (%)	29 (43)
AZT + 3TC + NVP, *n* (%)	19 (28)
d4T + 3TC + EFV, *n* (%)	5 (7)
AZT + 3TC + EFV, *n* (%)	5 (7)
TDF + 3TC + NVP, *n* (%)	5 (7)
AZT + 3TC + IDV, *n* (%)	2 (3)
TDF + 3TC + EFV, *n* (%)	1 (1)
ABC + 3TC + NVP, *n* (%)	1 (1)
d4T + ABC + EFV, *n* (%)	1 (1)
Median $CD4^+$ T-cell count at second-line start, cells/mm^3 (IQR)	149 (57–288)
Median HIV viral load at second-line start, copies/ml (IQR)	64,150 (6,762–286,250)
Median duration of second-line ART, years (IQR)	2.6 (1.5–3.8)
Second-line ART at data collection	
TDF + 3TC + LOP/r, *n* (%)	30 (40)
ABC + ddI + LOP/r, *n* (%)	12 (16)
AZT + 3TC + LOP/r, *n* (%)	12 (16)
ABC + 3TC + LOP/r, *n* (%)	11 (15)
d4T + 3TC + LOP/r, *n* (%)	3 (4)
TDF + ddI + LOP/r, *n* (%)	3 (4)
TDF + ABC + LOP/r, *n* (%)	1 (1)
ddI + 3TC + LOP/r, *n* (%)	1 (1)
AZT + 3TC + ddI + LOP/r, *n* (%)	1 (1)
Median total duration on ART (both first- and second-line), years (IQR)	6.9 (4.8–8.1)
Median $CD4^+$ T-cell count at data collection, cells/mm^3 (IQR)	170 (75–320)
Median HIV viral load at data collection, copies/ml (IQR)[a]	1,230 (82–57,525)
Median adherence evaluation by visual analogue scale	100%

[a]Missing data: duration between HIV diagnosis and first-line antiretroviral therapy (ART): 2; first-line ART regimen: 6; viral load at data collection: 6. ABC, abacavir; AZT, zidovudine; ddI, didanosine; d4T, stavudine; EFV, efavirenz; IDV, indinavir; LOP/r, lopinavir/ritonavir; NVP, nevirapine; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine.

Of patients with successful genotype analyses, more than one-third demonstrated no evidence of resistance, while 30% had DRMs to at least one class of ARVs. Resistance profiles of a minority of patients demonstrated DRMs requiring a switch to a third-line ART regimen.

Among patients with successful genotypes, 36% had pan-sensitive virus (that is, no significant DRMs detected); thus, virological failure in these patients was most likely secondary to poor adherence to ART. This finding was unanticipated since these patients had been prescribed two separate ART regimens with a median total ART duration of nearly 7 years. Our findings are similar to other second-line ART failure studies that underscore adherence as the main reason for ART failure [7,8], but contrast from first-line failure studies where 80–90% of patients have NRTI and/or NNRTI resistance mutations at the time of failure [14–18]. While it is possible that some genotypes failed to detect archived or low-frequency mutations, the absence of resistance among most patients with VL >100,000 copies/ml suggests that drug pressure was negligible in these patients. Such results reinforce the crucial need to emphasize life-long treatment adherence in national programmes among RLS in order to avoid costly and unnecessary switches to second- or third-line ART regimens.

At the time of this study, the annual cost for the preferred first-line regimen of tenofovir/lamivudine/efavirenz in Rwanda was $161.45 (Table 3) which was less than half the cost of the second-line regimen of zidovudine/lamivudine/lopinavir/ritonavir ($418.78). A third-line regimen costs 18× more than the preferred first-line regimen and increases the pill burden 10-fold. As this study revealed that only a

Table 2. Drug resistance mutations and their distribution by the three main antiretroviral classes among 30 genotypes

NRTIs		NNRTIs		PIs	
Mutation	Frequency, *n* (%)	Mutation	Frequency, *n* (%)	Mutation	Frequency, *n* (%)
None	17 (57)	**None**	12 (40)	**None**[a]	25 (83)
M41L	6 (20)	A98A/G	3 (10)	L10F/I/L/V	10 (33)
E44D	4 (13)	K101E/N	3 (10)	I13V	4 (13)
D67N	6 (20)	**K103N/S**	8 (27)	K20I/R	6 (20)
T69N	3 (10)	V106I	2 (7)	L33F	2 (7)
K70R	3 (10)	V108I	1 (3)	E35D	9 (30)
V75M	4 (13)	E138A/E/Q	3 (10)	M36I	28 (93)
V118I/M/V	4 (13)	V179I	7 (23)	**M46I**	4 (13)
M184V	9 (30)	**Y181C/V/Y**	6 (20)	**I54V**	5 (17)
L210W	3 (10)	**Y188L**	1 (3)	L63T	1 (3)
T215F/V/Y	9 (30)	V189I	1 (3)	A71V	1 (3)
K219E/K/Q	4 (13)	**G190A**	4 (13)	T74P/S	2 (7)
		H221H/Y	3 (10)	**L76V**	2 (7)
		P225H	2 (7)	**V82A/V**	4 (13)
		K238K/T	2 (7)	**I84V**	2 (7)
		T369I/T/V	2 (7)	L89M	9 (30)

[a]No major protease inhibitor (PI) mutations. Major mutations in bold type. NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor.

Table 3. Comparison of antiretroviral regimen pill burden and costs for first-, second- and third-line in Rwanda

ART line	Regimen	Pill burden (daily total)	Schedule	Annual cost
First	TDF + 3TC + EFV	1	1 daily	$161.45
First	AZT + 3TC + NVP	2	1 twice daily	$117.04
Second	AZT + 3TC + LOP/r	6	3 twice daily	$418.78
Second	TDF + 3TC + LOP/r	5	3 in AM; 2 in PM	$390.06
Third	ETV + DRV/r + RAL	10	5 twice daily	$2,912.60
Third	TDF + 3TC + ETV + DRV/r + RAL	11	6 in AM; 5 in PM	$2,984.60

ART, antiretroviral therapy; AZT, zidovudine; DRV/r, darunavir/ritonavir; EFV, efavirenz; ETV, etravirine; LOP/r, lopinavir/ritonavir; NVP, nevirapine; RAL, raltegravir; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine.

minority of patients failing second-line regimens truly required shifting to a third-line regimen due to resistance, it is imperative that national programmes in RLS focus on adherence as a critical component of both scale-up and long-term maintenance of ART programmes. Modelling [19] has predicted that a public health approach to third-line therapy is unaffordable, and even among the patients in this small sample, avoiding unnecessary switches to second- and third-line regimens could save $80,000 per year (30 patients on first-line versus third-line, $4,843 versus $87,378).

This study had several limitations in addition to the relatively small sample size. Patients included in this study were selected by convenience among sites with multiple patients receiving second-line ART – the results may not be representative of all patients on second-line ART in Rwanda. Additionally, since the predominant circulating HIV subtype in Rwanda is A and VL monitoring in the national programme is relatively frequent, mutational patterns may not be generalizable to RLS with other HIV subtypes or less frequent VL monitoring. As some patients started TAM-based first-line regimens while others were taking abacavir- or tenofovir-based regimens, the pattern of resistance after second-line failure may also have been different. The standard genotyping assays used in this protocol may not have identified all resistance mutations present in individual patients due to minority variants or archived mutations, which may have underestimated the presence of some mutations. Lastly, the cost analysis was exploratory and was not a formal cost-effectiveness analysis.

In conclusion, among 30 patients failing second-line ART regimens in Rwanda, resistance profiles of only a quarter indicated a requirement for salvage (third-line) ART. The majority of patients with virological failure had no genotypic evidence of resistance to currently prescribed ART, emphasizing the critical nature of continued adherence counselling for patients in RLS who appear to be failing prescribed ART regimens.

Acknowledgements

The authors are grateful to the participants in this study, the health-care providers who invited patients and arranged the place for interview, the Rwanda Biomedical Center for facilitating all financial and administrative procedures. We would also like to thank Neil Gupta (Partners in Health, Kigali, Rwanda) who helped read and revise an early version of this manuscript.

These data were presented in part at the *7th International HIV Research Conference* in Kigali, Rwanda on 4 December 2014.

This study was financially supported by Great Lakes Initiatives (GLIA). The HIV genotyping work was supported by a cooperative agreement (W81XWH-11-2-0174) between the Henry M Jackson Foundation for the Advancement of Military Medicine, Inc. and the US Department of Defense (DOD).

The views and opinions expressed are those of the authors and should not be construed as official or representing the positions of the Department of Army or Defense.

Study conception and design: JdN, DJR, RM, SN and RRR. Data acquisition: JdN, GN and AM. Laboratory analysis: EM, LLJ and SAP. Data analysis and interpretation: all. Drafting the manuscript: JdN and DJR. Critical revisions: all. Approval of the final manuscript: all.

Disclosure statement

The authors declare no competing interests.

References

1. Nsanzimana S, Ruton H, Lowrance DW, *et al*. Cell phone-based and internet-based monitoring and evaluation of the National Antiretroviral Treatment Program during rapid scale-up in Rwanda: TRACnet, 2004-2010. *J Acquir Immune Defic Syndr* 2012; **59:**e17–e23.
2. Art-Linc of IeDEA Study Group, Keiser O, Tweya H, *et al*. Switching to second-line antiretroviral therapy in resource-limited settings: comparison of programmes with and without viral load monitoring. *AIDS* 2009; **23:**1867–1874.
3. Sungkanuparph S, Win MM, Kiertiburanakul S, Phonrat B, Maek-a-nantawat W. HIV-1 drug resistance at virological failure versus immunological failure among patients failing first-line antiretroviral therapy in a resource-limited setting. *Int J STD AIDS* 2012; **23:**316–318.
4. Osinusi-Adekanmbi O, Stafford K, Ukpaka A, *et al*. Long-term outcome of second-line antiretroviral therapy in resource-limited settings. *J Int Assoc Provid AIDS Care* 2014; **13:**366–371.
5. Rwanda Biomedical Center. TRACnet monthly report on key indicators. Kigali, Rwanda 2014.
6. Hosseinipour MC, Gupta RK, Van Zyl G, Eron JJ, Nachega JB. Emergence of HIV drug resistance during first- and second-line antiretroviral therapy in resource-limited settings. *J Infect Dis* 2013; **207 Suppl 2:**S49–S56.
7. Ajose O, Mookerjee S, Mills EJ, Boulle A, Ford N. Treatment outcomes of patients on second-line antiretroviral therapy in resource-limited settings: a systematic review and meta-analysis. *AIDS* 2012; **26:**929–938.
8. Murphy RA, Sunpath H, Castilla C, *et al*. Second-line antiretroviral therapy: long-term outcomes in South Africa. *J Acquir Immune Defic Syndr* 2012; **61:**158–163.
9. Schoffelen AF, Wensing AM, Tempelman HA, Geelen SP, Hoepelman AI, Barth RE. Sustained virological response on second-line antiretroviral therapy following virological failure in HIV-infected patients in rural South Africa. *PLoS ONE* 2013; **8:**e58526.
10. 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:**1679–1687.
11. 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:**2943–2948.
12. Saravanan S, Vidya M, Balakrishnan P, *et al*. Viremia and HIV-1 drug resistance mutations among patients receiving second-line highly active antiretroviral therapy in Chennai, Southern India. *Clin Infect Dis* 2012; **54:**995–1000.
13. Ministry of Health, Republic of Rwanda. National Guidelines for Comprehensive Care of People Living with HIV in Rwanda, 2011. (Accessed 15 July 2015.) Available from www.rbc.gov.rw/IMG/pdf/national_guidelines_for_comprehensive_care_of_people_living_with_hiv_in_rwanda.pdf
14. Hosseinipour MC, van Oosterhout JJ, Weigel R, *et al*. The public health approach to identify antiretroviral therapy failure: high-level nucleoside reverse transcriptase inhibitor resistance among Malawians failing first-line antiretroviral therapy. *AIDS* 2009; **23:**1127–1134.
15. Sungkanuparph S, Manosuthi W, Kiertiburanakul S, Piyavong B, Chumpathat N, Chantratita W. Options for a second-line antiretroviral regimen for HIV type 1-infected patients whose initial regimen of a fixed-dose combination of stavudine, lamivudine, and nevirapine fails. *Clin Infect Dis* 2007; **44:**447–452.
16. Cozzi-Lepri A, Phillips AN, Martinez-Picado J, *et al*. 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:**687–697.
17. Hamers RL, Sigaloff KC, Wensing AM, *et al*. Patterns of HIV-1 drug resistance after first-line antiretroviral therapy (ART) failure in 6 sub-Saharan African countries: implications for second-line ART strategies. *Clin Infect Dis* 2012; **54:**1660–1669.

18. Hanson DL, Adje-Toure C, Talla-Nzussouo N, *et al*. HIV type 1 drug resistance in adults receiving highly active antiretroviral therapy in Abidjan, Cote d'Ivoire. *AIDS Res Hum Retroviruses* 2009; **25:**489–495.

19. 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:**1083–1093.

Accepted 4 October 2015; published online 12 November 2015