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HIV-1 drug resistance testing at second-line regimen failure in Arua, Uganda: avoiding unnecessary switch to an empiric third-line

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Abstract

Objectives. The number of patients on second-line antiretroviral therapy is growing, but data on HIV drug resistance patterns at failure in resource-constrained settings are scarce. We aimed to describe drug resistance and investigate the factors associated with extensive resistance to nucleoside/nucleotide reverse transcriptase inhibitors (NRTI), in patients failing second-line therapy in the HIV outpatient clinic at Arua Regional Referral Hospital, Uganda.

Methods: We included patients who failed on second-line therapy (two consecutive viral loads ≥1000 copies/mm^3 by SAMBA-1 point-of-care test) and who had a drug resistance test performed between September 2014 and March 2017. Logistic regression was used to investigate factors associated with NRTI genotypic sensitivity score (GSS) ≤1.

Results: 78 patients were included: 42% female, median age 31 years and median time of 29 months on second-line therapy. Among 70 cases with drug resistance test results, predominant subtypes were A (47%) and D (40%); 18.5% had ≥1 major protease inhibitor mutation; 82.8% had ≥1 NRTI mutation and 38.5% had extensive NRTI resistance (NRTI GSS ≤1). A nadir CD4 count ≤100/ml was associated with NRTI GSS ≤1 (OR 4.2, 95%CI [1.3-15.1]). Thirty (42.8%) patients were switched to third-line therapy, composed of integrase inhibitor and protease inhibitor (60% darunavir/r) +/-NRTI. A follow-up viral load was available for 19 third-line patients at 12 months: 84.2% were undetectable.

Conclusions: Our study highlights the need for access to drug resistance tests to avoid unnecessary

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switches to third-line therapy, but also for access to third-line drugs, in particular integrase inhibitors. Low nadir CD4 count might be an indicator of third-line drug requirement for patients failing second-line therapy.

Keywords: second-line antiretroviral therapy; drug resistance; HIV-1; Uganda

Introduction

As of June 2017, approximately 20.9 million people living with HIV were on antiretroviral therapy (ART) worldwide [1], and up to 25 million are expected to receive ART in 2020, of whom 0.5 to 3 million would be on a second-line regimen [2]. Prevalence of boosted protease inhibitor-based second-line virological failure in observational studies in sub-Saharan Africa ranges from 10 to 38% [3-6] and was 19% and 14% at 48 and 144 weeks respectively in two experimental studies [7,8]. Factors commonly associated with second-line failure are low CD4 count, high viral load and high genotypic sensitivity score for nucleoside/nucleotide reverse transcriptase inhibitors (NRTI GSS) at first-line failure, tuberculosis treatment while on second-line and poor adherence of adults and children [9-14]. Genotypic resistance to the NRTIs included in the second-line therapy might not increase the risk of failure [15,16].

Although HIV drug resistance mutations (DRM) associated with first-line failure in resource-constrained settings have been well described, with mutations conferring high-level resistance to first-generation non-nucleoside reverse transcriptase inhibitors (NNRTI) and the M184V mutation conferring high-level resistance to lamivudine occurring in more than 75% [17,18], data on HIV drug resistance associated with second-line regimen failure are still scarce. In resource-constrained countries, where drug resistance tests (DRT) are commonly not available, WHO recommends a combination of darunavir/ritonavir and one HIV integrase inhibitor as “empirical” third-line ART [19]. However, access to these drugs is still insufficient in low-resource countries.

We aimed to describe the drug resistance mutations and resistance level, to identify factors associated with extensive resistance to NRTIs and to describe the DRT-adapted regimens and their virological outcome in patients failing second-line in the HIV clinic supported by *Médecins Sans Frontières* (MSF) at Arua Regional Referral Hospital (ARRH) in Uganda.

Methods

Setting and clinical procedure

In 2001 MSF set up the HIV outpatient clinic at ARRH in collaboration with the Ugandan Ministry of

Health (MOH) and in 2013 implemented routine viral load monitoring with the point-of-care viral load (POC VL) semi-quantitative test SAMBA-1, which defines plasma specimen as "detectable" or "undetectable" around the cut-off of 1000 copies/mL within 2 hours [20].

Since 2014, the year of the hand-over of the clinic to MOH, MSF supports the clinic with access to genotypic DRT and to third-line ART. In 2016, around 6500 patients on ART were followed, of whom 730 were on a second-line regimen (one ritonavir-boosted protease inhibitor (PI) combined with two NRTIs). Patients on a second-line regimen with two consecutive detectable VL 6 months apart despite enhanced adherence counselling are diagnosed with second-line virological failure (VF) and should receive a DRT, performed on frozen plasma at the Uganda Virus Research Institute (UVRI) in Entebbe as previously described [21]. Ideally, DRT blood collection is done on the same day as the second detectable VL. If, according to DRT results, VF is considered to be mainly due to poor adherence, the same second-line regimen is continued or simplified (by replacing the PI with once-per-day boosted-atazanavir with a lower pill burden). Otherwise treatment is adapted based on DRT results by an "optimized second-line" (replacing NRTI(s) and/or PI based on resistance profile), or by switching to a third-line regimen (definition in our study: one integrase inhibitor combined with one boosted PI, possibly in combination with etravirine and/or NRTIs).

Study design and data collection

This study was a retrospective analysis of routine clinical, immunological and virological data of patients on second-line ART followed in ARRH, who were diagnosed with VF and underwent a DRT between September 2014 and March 2017. Clinical and demographic data were obtained from the patient's file. The date of viral load tests, the date DRT blood samples were received at the UVRI laboratory and the date of report of DRT results to the clinic were collected. The nadir CD4 count was defined as the historically lowest CD4 count available in the patient file since ART initiation. Resistance mutations and interpretation are reported using the Stanford HIV Drug Resistance Database version 8.5 (last updated April 2018). Genotypic sensitivity score (GSS) was calculated by assigning a value to each ARV of interest, based on the 5 resistance levels: susceptible (=1), potential low-level resistance (=0.75), low-level resistance (=0.5), intermediate resistance (=0.25), high-level resistance (=0). The NRTI GSS was a combined score for lamivudine, abacavir, didanosine, stavudine, tenofovir and zidovudine with a maximum possible score of 6. Extensive resistance to NRTI was defined as NRTI GSS ≤1. The specific GSS (sGSS) was a combined score for all ARVs in the second-line regimen at failure as well as for the regimen after DRT-informed treatment change. Treatment failure was defined as second-line VF with sGSS <2. Viral sequences are available in GenBank accession numbers MH423509-MH423577.

Statistical analysis

Participant characteristics were summarized using frequencies, median and interquartile range [IQR]. HIV subtypes were grouped into D or "other" (A, C, recombinant), second-line regimen was categorized as "typical" (boosted PI in combination with zidovudine or tenofovir + lamivudine or emtricitabine) or "atypical" (PI with other NRTI). To assess factors associated with extensive resistance to NRTI (NRTI GSS ≤1), logistic regression was used to estimate odds ratio (ORs) and 95% confidence intervals (CI). Variables associated with NRTI GSS ≤1 with a p-value <0.2 in univariate analysis were selected for inclusion in the multivariate model. Then, a back-step selection procedure was used to keep variables with a p-value <0.05. Age and time on second-line were kept in the final model as they were *a priori*-specified confounders. In a sensitivity analysis, all children under 5 years at the date of ART initiation were excluded. Analyses were done using R software.

Ethical approval

The study was approved by the Mbarara University of Science and Technology Research Ethics Committee and by the Uganda National Council for Science and Technology.

Results

Demographic and clinical characteristics

Seventy-eight patients were included (Table 1). The sex ratio was 1.3, median age was 31.5 years [IQR 15.0-40.2], and median time on second-line was 29 months [IQR 17.7-52.9]. Median body mass index at failure (n=45 adults) was 19.8 kg/m^2 [IQR 17.6-21.8] and median Middle Upper Arm Circumference (n=21 children aged 10 to 18 years) was 21 cm [IQR 18.5-23.0]. The current ART regimen included lopinavir/r (79%) or atazanavir/r (21%). Most frequent NRTI combinations were tenofovir with lamivudine (58%), abacavir with lamivudine (29%), and zidovudine with lamivudine (9%). CD4 counts since ART initiation were available for 76 of 78 patients. The median nadir CD4 count was 77/mm^3 [IQR 32-192], recorded in 37 cases at ART initiation and in 39 cases after a median time on ART of 65 months [IQR 38-90].

Resistance mutations and reduced drug susceptibility associated with second-line ART failure

DRT results were available for 70 patients, HIV RNA amplification failed in 8 cases. HIV subtypes were A (n=33, 47.1%), D (n=28, 40.0%), recombinants (n=8, 11.4%) and C (n=1). At least one major PI mutation was found in 13 patients (18.5%) and 10 patients (14.2%) had more than one. Most frequent major PI mutations were M46I/L (n=11, 15.7%), V82A/S (n=9, 12.8%), I54V (n=7, 10%)

(Figure 1). Fifty-eight patients (82.8%) had at least one NRTI mutation; their median number of mutations was 2 [IQR 1-5]. Thymidine Analogue Mutations (TAMs) were present in 37 cases (52.8%) and K65R was present in 1 patient. Fifty-five patients (78.5%) had at least one NNRTI mutation. Six patients (12%) had wild type virus.

The median sGSS at second-line failure was 1.5 [IQR 1-2] and it was <2 in 39 cases (55.7%). The median NRTI GSS was 2.5 [IQR 0.25-4.25] and 27 cases (38.5%) had extensive resistance to NRTI (NRTI GSS ≤1). Decreased susceptibility to atazanavir/r and lopinavir/r was observed in 13 cases (18.5%) and was associated with extensive NRTI resistance in all cases but one. Cross-resistance to darunavir/r (no high-level) was observed in 6 cases (8.5%). Decreased susceptibility to etravirine was observed in 39 cases (55.7%), with intermediate or high-level resistance in 25 cases (35.7%) (Figure 2).

Factors associated with extensive resistance to NRTI (NRTI GSS ≤1) at second-line ART failure

In the univariate analysis, NRTI GSS≤1 was associated with a nadir CD4 count ≤100/mm^3 (p=0.002) and with a time on 2nd-line ≤48 months (p=0.04). In multivariate analysis, after adjustment on age and time on second-line, the association between NRTI GSS≤1 and nadir CD4 count ≤100/mm^3 remained significant (OR=4.2, 95%CI [1.3-15.1]) (Table 2). In a sensitivity analysis, after exclusion of 10 patients who were under 5 years at ART initiation, the association was still significant (OR=4.4, 95%IC [1.24-18.6], p=0.027).

DRT-informed regimen and time between events in the management of second-line failure

Thirty-seven among 70 patients with DRT results (52.8%) were maintained on second-line regimen: in 29 cases, the same regimen was continued (patients on TDF, 3TC and atazanavir/r with high-level resistance to lamivudine only) or “simplified” (mostly by replacing lopinavir/r with atazanavir/r), and in 8 cases, second-line ART was “optimized” (in 2 cases, lopinavir/r was replaced with darunavir/r and in 6 cases, abacavir was replaced with tenofovir). Three patients were switched back to first-line-type regimens because DRT showed no mutation and the switch to second-line was retrospectively considered inappropriate. Thirty patients (42.8%) were switched to a third-line regimen; they had multi-NRTI resistance (including the 27 cases with NRTI GSS ≤1) associated or not with resistance to PI. Third-line regimens were composed of an integrase inhibitor (raltegravir in 21 cases, dolutegravir in 9 cases) associated with a PI (darunavir/r in 18 cases, atazanavir/r in 12 cases) and most with NRTI (25 cases) (Table 3). The sGSS of third-line regimen was >2 in all cases but one.

Of note, in the 8 cases with VF for whom the genotyping reaction failed, the regimen was not changed; all but one had at least one undetectable VL thereafter.

The median time between the first SAMBA-detectable VL test (suspicion of second-line failure) and the day the DRT sample was received at the UVRI laboratory was 11.4 months [IQR 8.2-16.1], and was not significantly different among patients with at least one major PI mutation (14.3 months) *versus* patients without any major PI mutation (11 months). The median time between confirmed second-line ART failure and treatment adaptation was 11.9 months [IQR 8.3-17.5]: the median time between second detectable VL and receipt of the sample for DRT was 4.4 months [IQR 2.1-7.6], the median time between reception of the sample and report of the results was 0.9 months [0.5-1.5], and the median time between the report and treatment adaptation was 4.7 months [IQR 2.4-8.9].

Follow-up viral load after treatment adaptation

Among the 30 patients switched to third-line ART, 20 were actively followed for more than 6 months. Among those 20 patients, 19 had a month 6 VL test (done after a median time of 6.5 months [IQR 5.6-7.9] on third-line ART) and 15 (78.9%) were undetectable. Reasons why 10 patients were not followed at 6 months were (i) recent switch (n=2), (ii) lost to follow-up (n=5), (iii) death (n=1) and (iv) transfer-out (n=2). Nineteen patients were actively followed for more than 12 months; all of them had a month 12 VL available (median time 12.5 months [IQR 11.7-15.4]) and 16 (84.2%) were undetectable, including one patient with detectable VL at 6 months.

Of the 40 patients not switched to third-line ART, 31 were actively followed for more than 6 months. 27 had a VL test at around 6 months after treatment adaptation or after DRT report (if treatment was not changed) and 19 (70.3%) had undetectable VL. Reasons why 9 patients were not followed at 6 months were (i) lost to follow-up (n=8) and (ii) transfer-out (n=1). Thirty patients were actively followed for more than 12 months, of whom 22 had a VL test at around 12 months available and 16 (72.2%) had undetectable VL.

Discussion

This retrospective study is one of the first reporting DRT results at second-line ART failure in an A- and D- predominant HIV-1 subtypes-infected patient cohort. Worldwide, 48% of infections are caused by subtype C, which is largely predominant in eastern and southern Africa, and only 12% by subtype A and 2% by subtype D [22]. In a previous cross-sectional study conducted in Uganda that assessed 36 patients failing second-line ART, HIV subtype distribution was similar to that in our study, major PI mutation was observed in 19.4% of cases (18.5% in our study) and the 3 most prevalent DRM were the same: V82A, I54V and M46I/L [23]. The reported rate of major PI mutations at second-line failure is very variable: 3% and 21.9% in two clinical trials and 64% in one observational study [10,6,11]. There is no evidence that subtype would have an impact on the rate,

but it may have an impact on spectra of PI mutations. *In vitro*, mutation I54V seems to emerge more frequently in subtype A than in subtype C virus selected with lopinavir [24]. Moreover, it has been suggested that subtype would impact spectra of NRTI mutations and subtype C has been shown to be a predictor of K65R mutation in patients treated with ART containing tenofovir compared to subtype B [25]. K65R was only found in 1 case in our cohort, supporting previous observations that subtypes A and D infections seem to facilitate less likely emergence of this mutation [26,27]. In addition, the previously predominant use of thymidine analogue-based first-line ART (D4T or AZT) in our setting and accumulation of TAMs may have reduced the risk of acquisition of K65R in our second-line patients [28].

Thirty-nine (55.7%) patients diagnosed with VF by Samba POC VL included in our study had sGSS <2 (i.e VF potentially due to drug resistance) and 30 (42.8%) were switched to third-line ART because of extensive resistance to NRTI associated or not with resistance to PI. These results highlight the critical importance of ongoing poor adherence as main factor of second-line VF, as well as the need of DRT to identify the smaller but considerable group with treatment failure. The 8 cases of VF with failed resistance genotyping reaction might be explained by a low-level viremia (near 1000 copies/ml) at follow up test. Also viral re-suppression at the time of blood sample collection is likely (the sample for DRT was not taken the same day as the second SAMBA VL test) given the virological outcome of these 8 patients: 7 had undetectable VL thereafter, without any treatment change and enhanced adherence counselling. Taken together, our results show a low positive predictive value of two consecutive VL >1000 copies/ml for identification of "treatment failure" (failure due to HIV drug resistance). In other words, without DRT, more than 60% of patients with VF included would have been unnecessarily switched to third-line ART. Recent studies also reported a significant proportion of second-line failure without major resistance to the current regimen [6,26,29] and poor adherence was indicated as a major factor underlying VF [10,30].

In Arua clinic, DRT also allowed clinicians to choose atazanavir/r as PI of the third-line ART in 40% of cases, which is a fixed dose combination and has better availability in resource-constrained countries than darunavir/r. The 2016 Ugandan guidelines indicate to switch patients with persistent VF and PI resistance to a third-line regimen composed of boosted darunavir, an integrase inhibitor, possibly in combination with NRTIs [31]. However, no clear guidance nor terminology is given regarding regimen adaptation for virological failure without PI DRM but with extensive NRTI resistance. In our study, extensive resistance to NRTI without resistance to PI was frequent and resistance to PI but not to NRTI was exceptional (1 case). This can be explained by the known high genetic barrier of PI, and its short half-life, which decreases suboptimal drug exposure in case of poor adherence [32]. Previous studies have shown that efficacy of darunavir/r (plus an optimized

background regimen) in highly ART-experienced patients was superior to other PIs, but all patients included had ≥1 primary PI mutation [33] and up to 63% of them were resistant to all current available PIs [34], which is not the case in our patients. High-level or intermediate resistance to 2nd-generation NNRTI etravirine was present in more than one third of cases in our study, further highlighting the need of the integrase inhibitor class drugs for salvage regimens.

For patients included in our analysis, time between confirmed diagnosis of VF and adaptation of treatment was quite long (median time 11.9 months). This time is a period of potential accumulation of resistance mutation and transmission of resistant HIV strains. The time between first detectable VL (suspicion of failure) and DRT sample reception in the laboratory was not associated with the presence of major PI mutation in our study, but it has been shown that a long duration of non-suppressive second-line may increase this risk [35]. The high work load at the clinic led to difficulties to follow the recommended procedure which was to use SAMBA POC VL results to collect blood samples for DRT on the same day as the second detectable VL. This procedure has since been implemented. Furthermore, considerable difficulties with third-line drug supply contributed to delays in switching.

Low CD4 count and high viral load have been associated with a higher number of resistance mutations to NRTIs at first-line failure [17,18]. To our knowledge, our study is the first to suggest that a nadir CD4 ≤100/mm^3 would be a predictor of extensive NRTI resistance at second-line failure and therefore of the need for third-line ART. Quantitative viral load was not available in our study and low nadir CD4 count might be linked with high plasmatic viral load before initiation of ART or at failure. We only had absolute nadir CD4 count and not relative count (ratio CD4/total lymphocytes). It might induce a measurement bias because there were 10 patients who started ART before they were 5 years old, and it is well-known that in under-fives the absolute CD4 count is normally much higher than in adults. A cut-off at 100/mm^3 might underestimate the level of immunosuppression in these children. However, the sensitivity analysis, which excluded all children under 5 years at the start of ART, showed the same significant association.

The rate of undetectable VL after 6 and 12 months on third-line ART in our sample (78.9% and 84.2% respectively) is difficult to interpret because of the limited number of patients. In a recent study including 152 South-African patients in the private health sector treated with third-line regimen (mostly with darunavir/r and in 38% with integrase inhibitor), Kaplan Meier estimation of the proportion of patients with VL below 400 copies/ml at 1000 days was 92.2%, not taking into account death and loss to follow-up as competing risks of failure [36]. Another study including 54 children and adolescents in Thailand found a viral suppression rate of 70% at 48 weeks, mainly due to poor adherence [37]. The relatively low rate of VL suppression among the non-third line regimen

group in our sample also does not allow much interpretation, but might be due to poor adherence.

The advantage of our study is the availability of data that are not always routinely available in comparable settings: we report on the real-life use of routine POC VL and DRT for the management of 2nd-line failure. The main limitation is that, due to the retrospective design, we could only analyse available DRT data. Some patients failing second-line ART might not have been identified as VF and are thus not included in this analysis. However, a first analysis on the VL testing cascade in the Arua hospital over a 3-year period showed good coverage and good follow-up. Among 9305 patients on ART and eligible for testing, 78% received a VL test; 24% had VL >1000 copies/ml and 70% of those received a follow-up VL test within a median time of 6 months [38]. Furthermore, the study design did not allow a direct assessment of treatment adherence and might also have compromised the completeness of the nadir CD4 count data which was collected from patients' files.

In conclusion, this study highlights the critical need of access to DRT in order to differentiate failure with major HIV drug resistance from failure simply due to poor adherence and therefore to avoid unnecessary switch to third-line ART regimens. Treatment failure was often due to extensive resistance to NRTI rather than resistance to PI; access to new class integrase inhibitor is therefore crucial. On the other hand, many VF did not require treatment change, highlighting the importance of adherence support. A nadir CD4 below 100/mm^3 was associated with extensive resistance to NRTI and might be an indicator of third-line ART requirement, but other studies should be performed to confirm our results and to investigate on others predictors of extensive NRTI resistance at second-line ART virological failure.

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Figure legends

Figure1. Frequency of main HIV resistance mutations at second-line failure (N= 70).
*protease inhibitor ** nucleoside/nucleotide reverse transcriptase inhibitor *** non-nucleoside reverse transcriptase inhibitor

Figure 2. Level of resistance according to HIV drug at second-line failure (N=70).
ATZ: atazanavir; DRV: darunavir; FPV: fosamprenavir ; IDV: indinavir ; LPV: lopinavir ; NFV: nelfinavir ; SQV: saquinavir; TPV: tipranavir; r: ritonavir; 3TC: lamivudine; ABC: abacavir; AZT: zidovudine; D4T: stavudine; DDI: didanosine; TDF: tenofovir; EFV: efavirenz; ETV: etravirine; NVP: nevirapine; RPV: rilpivirine

Table 1. Demographic and clinical characteristics of patients at second-line failure (N=78)

Characteristic	n (%)
Age [a]	31.5 [15-47.2]
Female	33 (42.3)
Country of residence	
DRC [b]	11 (14.4)
Uganda	65 (85.6)
WHO clinical stage (n=73)	
1	54 (74.0)
2	14 (19.2)
3	5 (6.8)
Body Mass Index (adults, n=45)	
< 17	8 (17.7)
≥ 17	37 (82.3)
MUAC [c] (children aged 10-18, n=21)	
< 18,5	3 (14.3)
≥ 18,5	18 (85.7)

Time on ART (years) [a]	8.1 [6.8-10]
Time on second-line ART (months) [a]	29 [17.7-52.9]
Second-line regimen[d] (n=76)	
TDF+3TC+LPV/r	30 (39.4)
ABC+3TC+LPV/r	20 (26.3)
TDF+3TC+ATV/r	14 (18.4)
AZT+3TC+LPV/r	7 (9.2)
other	5 (6.5)
CD4 count /mm^3 (n=43)	271 [113-523]

[a] Quantitative variables are expressed with median [IQR] [b]Democratic Republic of Congo

[c] Middle Upper Arm Circumference (cm) [d]TDF: tenofovir, 3TC: lamivudine, LPV:lopinavir, ABC:abacavir, ATV:atazanavir, r:ritonavir

Table 2. Factors associated with NRTI GSS[a] ≤1 at second-line failure (N=70)

		NRTI GSS≤1	Univariate analysis			Multivariate analysis		
	N[b]	n (%)	OR	95%CI	p	OR	95%CI	p
Age								
<20 years	29	8 (27.6)	1		.12	1		
≥20 years	39	18 (46.1)	2.20	0.8-6.5		1.61	0.5-5.3	.42
Sex								
Male	39	13 (33.3)	1		.31	-		
Female	31	14 (45.1)	1.60	0.6-4.4		-		
Address								
Uganda	58	21 (36.2)	1		.41	-		
DRC[c]	10	5 (50.0)	1.76	0.4-7.0		-		
Time on 2nd-line								
>48 months	21	4 (19.0)	1		.04	1		.15
≤48 months	46	21 (45.6)	3.57	1.1-13.8		2.62	0,7-10.9	
WHO Clinical stage								
=1	49	18 (36.7)	1		.68	-		

>1	19	8 (42.1)	1.25	0.4-3.6		-		
HIV subtype								
Others	42	15 (35.7)	1		.51	-		
D	28	12 (42.8)	1.38	0.5-3.7		-		
Nadir CD4 count								
>100/mm^3	30	5 (16.6)	1		.002	1		.02
≤100/mm^3	39	21 (53.8)	5.80	1.9-20		4.2	1.3-15.1	
Delay before DRT[d]								
≤18 months	55	21 (38.0)	1		.72	-		
>18 months	9	4 (44.0)	1.29	0.3-5.4		-		
2nd-line regimen								
Atypical	25	9 (36.0)	1		.74	-		
Typical	45	18 (40.0)	1.18	0.4-3.3		-		

[a] Genotypic sensitivity score for nucleoside/nucleotide reverse transcriptase inhibitors

[b] Data available for analysis

[c] Democratic Republic of Congo

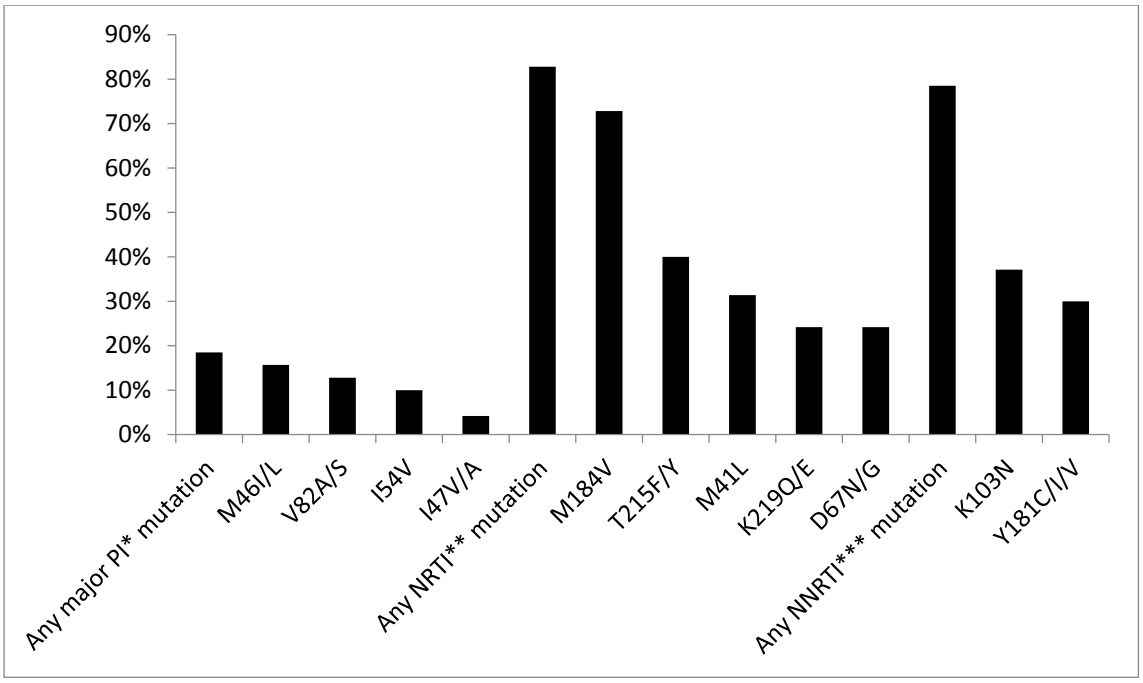
[d] Time between first detectable viral load and drug resistance test

Table 3. Drug resistance test-informed regimen (N=70)

	n (%)
First-line	3 (4.3)
Second-line	37 (52.8)
Continued/simplified 2nd-line	29 (41.4)
Optimized 2nd-line	8 (11.4)
Third-line	30 (42.8)
Darunavir/r + integrase inhibitor + etravirine +/- NRTI[a]	7 (10.0)
Darunavir/r + integrase inhibitor +/- NRTI	11 (15.7)
Atazanavir/r + integrase inhibitor +/- NRTI	12 (17.1)

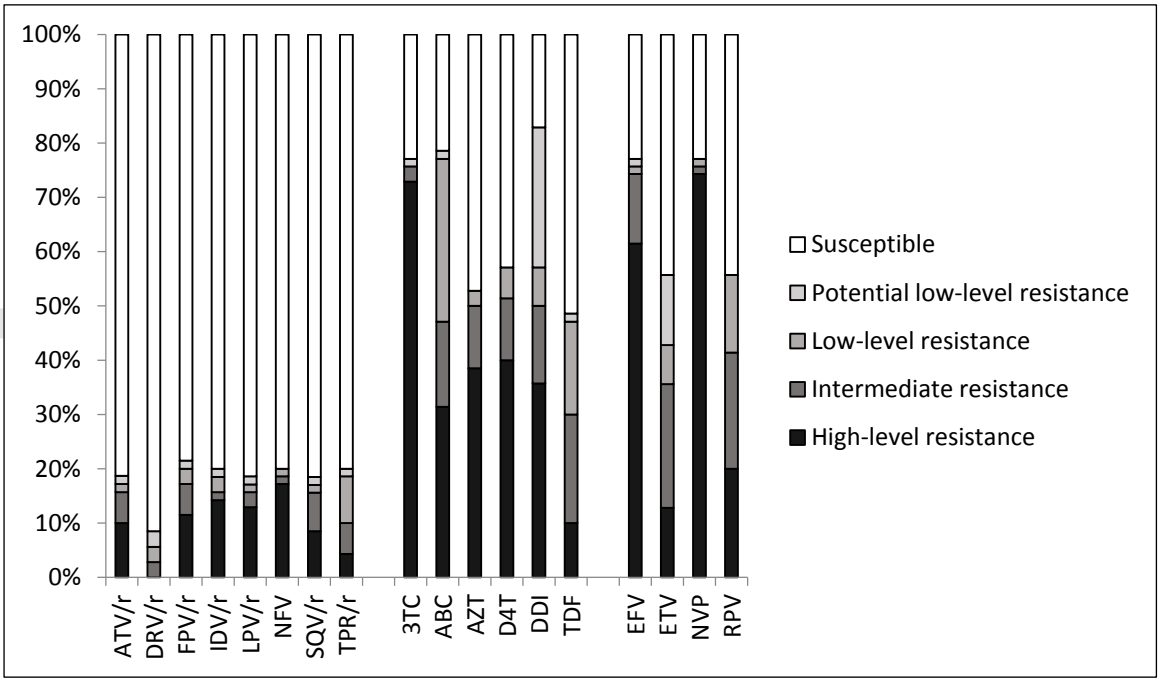
[a] nucleoside/nucleotide reverse transcriptase inhibitors

Figure1. Frequency of main HIV resistance mutations at second-line failure (N= 70)



***protease inhibitor** ** **nucleoside/nucleotide reverse transcriptase inhibitor** *** **non-nucleoside reverse transcriptase inhibitor**

Figure 2. Level of resistance according to HIV drug at second-line failure (N=70)



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