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HIV-1 drug resistance mutations among individuals with low-level viraemia while taking combination ART in Botswana

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Objectives: To assess whether a single instance of low-level viraemia (LLV) is associated with the presence of drug resistance mutations (DRMs) and predicts subsequent virological failure (VF) in adults receiving ART in 30 communities participating in the Botswana Combination Prevention Project.

Methods: A total of 6078 HIV-1 C *pol* sequences were generated and analysed using the Stanford HIV drug resistance database. LLV was defined as plasma VL = 51–999 copies/mL and VF was defined as plasma VL ≥ 1000 copies/mL.

Results: Among 6078 people with HIV (PWH), 4443 (73%) were on ART for at least 6 months. Of the 332 persons on ART with VL > 50 copies/mL, 175 (4%) had VL ≥ 1000 copies/mL and 157 (4%) had LLV at baseline. The prevalence of any DRM was 57 (36%) and 78 (45%) in persons with LLV and VL ≥ 1000 copies/mL, respectively. Major DRMs were found in 31 (20%) with LLV and 53 (30%) with VL ≥ 1000 copies/mL ($P = 0.04$). Among the 135 PWH with at least one DRM, 17% had NRTI-, 35% NNRTI-, 6% PI- and 3% INSTI-associated mutations. Among the 3596 participants who were followed up, 1709 (48%) were on ART for ≥6 months at entry and had at least one subsequent VL measurement (median 29 months), 43 (3%) of whom had LLV. The OR of experiencing VF in persons with LLV at entry was 36-fold higher than in the virally suppressed group.

Conclusions: A single LLV measurement while on ART strongly predicted the risk of future VF, suggesting the use of VL > 50 copies/mL as an indication for more intensive adherence support with more frequent VL monitoring.

Introduction

The development of HIV-1 drug resistance mutations (DRMs) and inadequate adherence to ART remain ongoing barriers to sustained HIV viral load (VL) suppression in people with HIV (PWH) on ART.[1] Resistance testing is important for preserving current regimens, choosing subsequent ART and predicting virological failure (VF). According to current WHO HIV treatment guidelines, VF is defined as two consecutive VLs of ≥1000 copies/mL after at least 6 months on ART.[2] The U.S. Department of Health and Human Services 2019 guidelines define VF as VL > 200 copies/mL[3] while the European AIDS Clinical Society (EACS) 2018 guidelines use a very strict threshold of VF as two consecutive VLs of >50 copies/mL after 6 months on treatment.[4] In Botswana, the HIV treatment guidelines use a VF threshold of two consecutive HIV VLs of >400 copies/mL after at least 6 months on ART.[5]

The use of different VF thresholds has led to different definitions of LLV, resulting in contradictory data on the clinical significance of LLV.[6] Several studies have found LLV to be associated with the emergence of MDR mutations[7–10] and periods of LLV are linked with immune activation and stable CD4+ T cell counts.[11–13] Furthermore, LLV strongly increases the risk of presenting with subsequent VF[14] and impairing future ART options.[15] Nevertheless, some studies have reported no association between very LLV (20–50 copies/mL) or LLV with negative immunological and virological consequences among individuals on ART.[16,17] Recent WHO guidelines recommend maintaining ART with continued enhanced adherence counselling and repeat VL testing after 3 months among individuals with VL = 50–1000 copies/mL. Furthermore, ART switch is recommended for those receiving NNRTI-based regimens based on the clinical considerations and no adherence concerns.[18] Currently, Botswana and WHO HIV treatment guidelines do not recommend drug resistance testing at LLV while the International Antiviral Society–USA panel recommend resistance testing at VL ≥ 200 copies/mL.[19] Most commercial assays require HIV plasma VL > 1000 copies/mL for increased success rate and accurate results.[20,21] However, there is increasing success of sequencing for lower plasma VL with in-house assays.[22] In addition, proviral (integrated) DNA is also very useful in LLV,[23] resulting in successful genotyping in >85% of persons with VL < 1000 copies/mL compared with 70% success with standard plasma HIV-1 RNA genotyping.[24–29] Proviral DNA can also reveal previous archived DRMs.[30]

In Botswana, HIV drug resistance testing is only recommended for patients with two consecutive HIV VLs of ≥1000 copies/mL after at least 6 months of ART. Persons with VL = 400–999 copies/mL are not eligible for drug resistance testing. There are scanty data from sub-Saharan Africa on the prevalence of DRMs in individuals with LLV, or on whether LLV increases the risk of future VF. We thus evaluated the prevalence of LLV (using different VL thresholds for defining LLV) at a single timepoint in PWH on ART in Botswana, to determine the proportion of individuals with DRMs, stratified by HIV VL group, and to determine whether a single instance of LLV predicts subsequent VF.

Methods

Study population

Blood samples were collected from all consenting persons with HIV (regardless of ART status) aged 16–64 years who were enrolled in the Botswana Combination Prevention Project (BCPP) from 2013 to 2018 in 30 communities across central, northern and southern parts of Botswana.[31] BCPP was a community-randomized trial evaluating whether a package of standard HIV prevention interventions could significantly reduce population-level HIV incidence over time, as previously described.[32] The BCPP impact evaluation survey enrolled adults (16 years or older, regardless of HIV or ART status) residing in a random sample of approximately 20% of households from all 30 communities at study entry (baseline household survey, BHS) into a longitudinal cohort (annual household survey, AHS) that was followed for 30 months, as described elsewhere.[33] In addition, in the 15 intervention communities, PWH who were receiving ART in local clinics were recruited to provide a one-time blood sample for HIV sequencing and metadata (but were not followed longitudinally). We included samples and data from all PWH in both the AHS cohort and from the clinics in this analysis.

Selection of study participants

HIV-1 RNA VL was quantified using Abbott Realtime HIV-1 assay on the automated *m2000rt*/*m2000sp* system (Abbott Laboratories, Wiesbaden, Germany) with detection limits of 40 to 10 million copies/mL (we excluded from these analyses 228 participants whose entry VL assay had a lower limit of detection of 400 copies/mL). In this analysis, we include PWH who were on ART for at least 6 months, who had VL measurement at the first BCPP study visit and had available HIV sequence. These participants were classified as having VF (VL ≥ 1000 copies/mL, as per the current WHO definition)[2] or LLV (VL = 51–999 copies/mL) or were virally suppressed (VL ≤ 50 copies/mL). LLV was further subcategorized into two groups: low LLV (51–400 copies/mL) and high LLV (401–999 copies/mL) (Figure 1).

A subset of participants who either had viral suppression or LLV at enrolment and who had at least one subsequent VL measurement (through participation in the longitudinal AHS cohort) were included in the further analysis for predictive factors for VF (Figure 2).

Ethics

Ethics approval for the BCPP study was obtained from the Botswana Health Research and Development Committee and the CDC's Institutional Review Boards (IRBs). BCPP study participants provided written informed consent and participants aged 16–17 years provided written informed assent with written permission from parents or guardians.

HIV viral sequences

In participants with VL > 50 copies/mL, HIV-1 C near-full length sequences were obtained by a long-range HIV genotyping technique from proviral DNA and viral plasma RNA, described elsewhere.[24] More than 99% of our sequences were generated using proviral DNA from 2013–18. Failed first-round PCR was repeated at an annealing temp of 58°C instead of 62°C to increase the success. The first-round amplicon was used as a template for next-generation sequencing (NGS) and carried out using Illumina platforms MiSeq and HiSeq[34] by the BioPolymers Facility at Harvard Medical School (Boston, USA; https:genome.med.harvard.edu/) in collaboration with the PANGEA HIV consortium at the Wellcome Trust Sanger Institute (Cambridge, UK; https://www.sanger.ac.uk).

HIV-1 C drug resistance analysis for participants with VL > 50 copies/mL

The HIV *pol* region was analysed for any DRMs associated with the highest levels of reduced susceptibility or those contributing to susceptibility to NRTIs, NNRTIs, PIs and integrase strand transfer inhibitors (INSTIs) according to the Stanford University HIV Drug Resistance Database.[35] Using the Stanford HIV database, HIV DRMs with the highest level of reduced susceptibility to ARVs were classified as major DRMs. Hypermutations were screened and adjusted for at the nucleotide-position level. We excluded HIV DRMs that are associated with hypermutations in the prevalence of any DRM.

Statistical analysis

Descriptive statistics are reported as median (IQR) for continuous variables and compared among three groups using Kruskal–Wallis tests. Categorical variables are reported as percentages and compared using the chi-squared test. The prevalence of DRMs for different VL groups was estimated, with 95% CI, using the binomial exact method and compared using a comparison of proportion test.

We evaluated the association between LLV at entry and subsequent VF (and between the presence of at least one DRM at entry and subsequent VF). Only the subset of participants who were on ART for at least 6 months at enrolment and had VL measurements at two or more

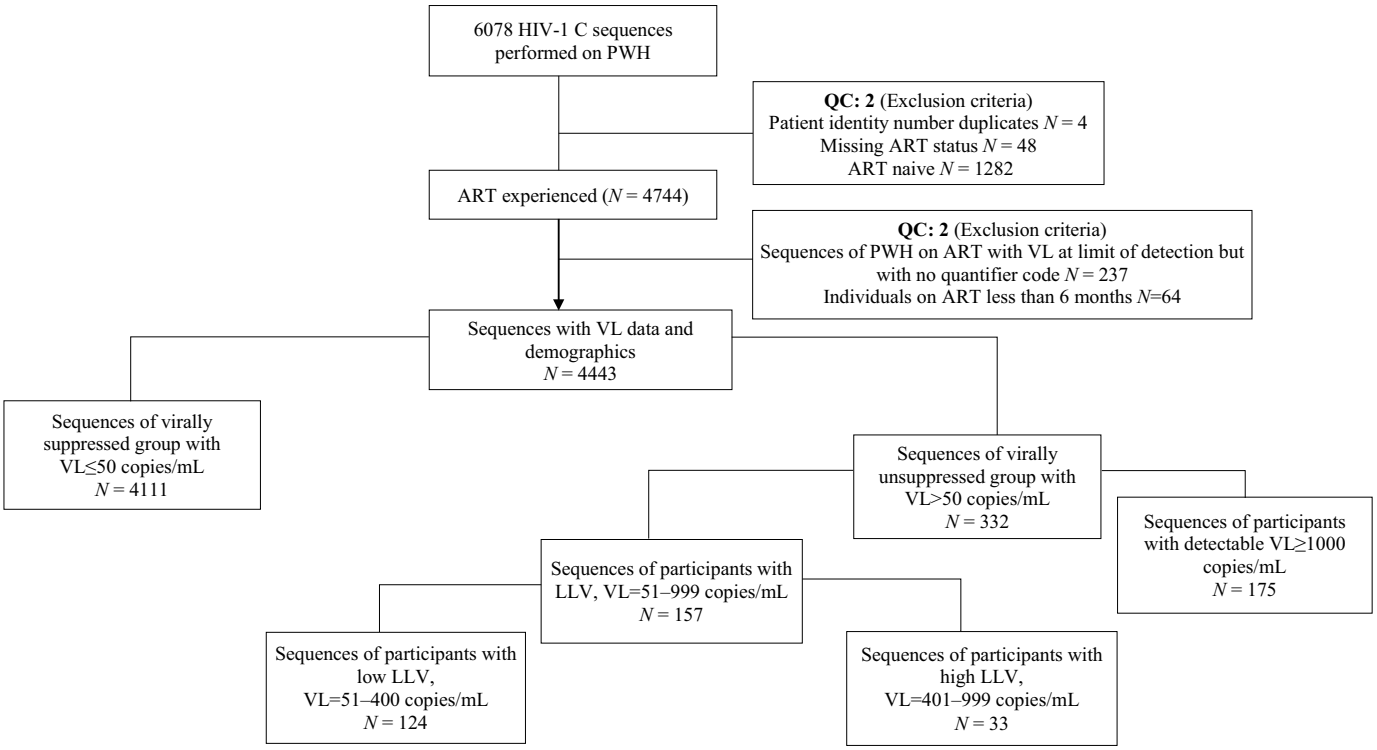

Figure 1. Flow chart showing how sequences were selected for drug resistance analysis. QC, quality control (not meeting any of inclusion criteria).

timepoints (at study enrolment and follow-up) were included in analyses of whether LLV predicts VF. Subsequent VF was defined as a single VL measurement of ≥1000 copies/mL at the end of the follow-up. We investigated the association between the following characteristics at entry and subsequent VF: VL group (suppressed VL versus LLV), LLV subgroups (low LLV and high LLV), the presence of major DRMs, ART regimen and duration on ART at the time of VF, sex and age. Both univariate and multivariate logistic regression models were performed. In the univariate analysis, all variables were adjusted for clustering by the community and all variables with $P < 0.2$ were included in the multivariate analysis. The LLV subgroup was excluded from multivariate analysis due to collinearity while those with major DRMs were excluded due to too few observations. Final multivariate models included VL group, age, sex and ART regimen and adjusted for clustering by the community. P values of <0.05 were considered statistically significant. All analyses were done using STATA version 15.

Results

The BCPP household survey enrolled 12 610 participants, 3596 of whom were HIV positive, 2901 (81%) of whom had HIV-1 sequence available. In addition, a total of 5022 PWH were enrolled from ART clinics to provide one-time blood draw, 3174 (63%) of whom had HIV-1 sequence available. Overall, 6078 HIV-1 sequences were available. In addition, 1709 PWH taking part in the AHS had a viral sequence result, at least one follow-up VL, and were on ART for at least 6 months at study entry and could hence be included in the analysis of subsequent VF.

Of the 6075 PWH who had HIV sequences available, 4443 (73%) were from persons on ART (for at least 6 months) at enrolment and were thus included in this analysis. The median age at enrolment was 41 years (IQR 35–49), 72% were female and the median duration of ART was 6.4 years (IQR 3.4–9.1). ART regimen was available for 4068 (92%), of whom 94% were on their initial regimen (60% on efavirenz-, 26.6% on nevirapine- and 7% on dolutegravir-based regimens, respectively) while 5% were on second-line therapy and 1% on salvage therapy. Among the 4443 participants on ART at enrolment who had VL data, 4111 (92%) were virally suppressed (VL ≤ 50 copies/mL) and 332 (8%) had detectable VLs of >50 copies/mL at entry. The study entry demographics of BCPP participants stratified by the VL group are shown in Table 1; female participants, older participants and those on ART for a longer period were more likely to have VLs of ≤50 copies/mL.

Prevalence of LLV and DRMs at baseline

Of the 332 participants with detectable VL (>50 copies/mL) on ART at entry, 157 (47%) had VL = 51–999 copies/mL and were categorized as having LLV. Within this LLV group, 124 (79%) had low LLV and 33 (21%) had high LLV.

One hundred and thirty-five (41%) of the 332 participants had at least one DRM. The prevalence and relative pattern of mutations were similar in the two VL groups (Table 2). Although a numerically higher proportion of individuals with VL ≥ 1000 copies/mL had at least one DRM (78; 45%) compared with the LLV group (57; 36%), we did not find statistically significant differences in the prevalence of DRMs between the two different VL groups overall, nor within drug class, with the exception of NRTI-associated DRMs.

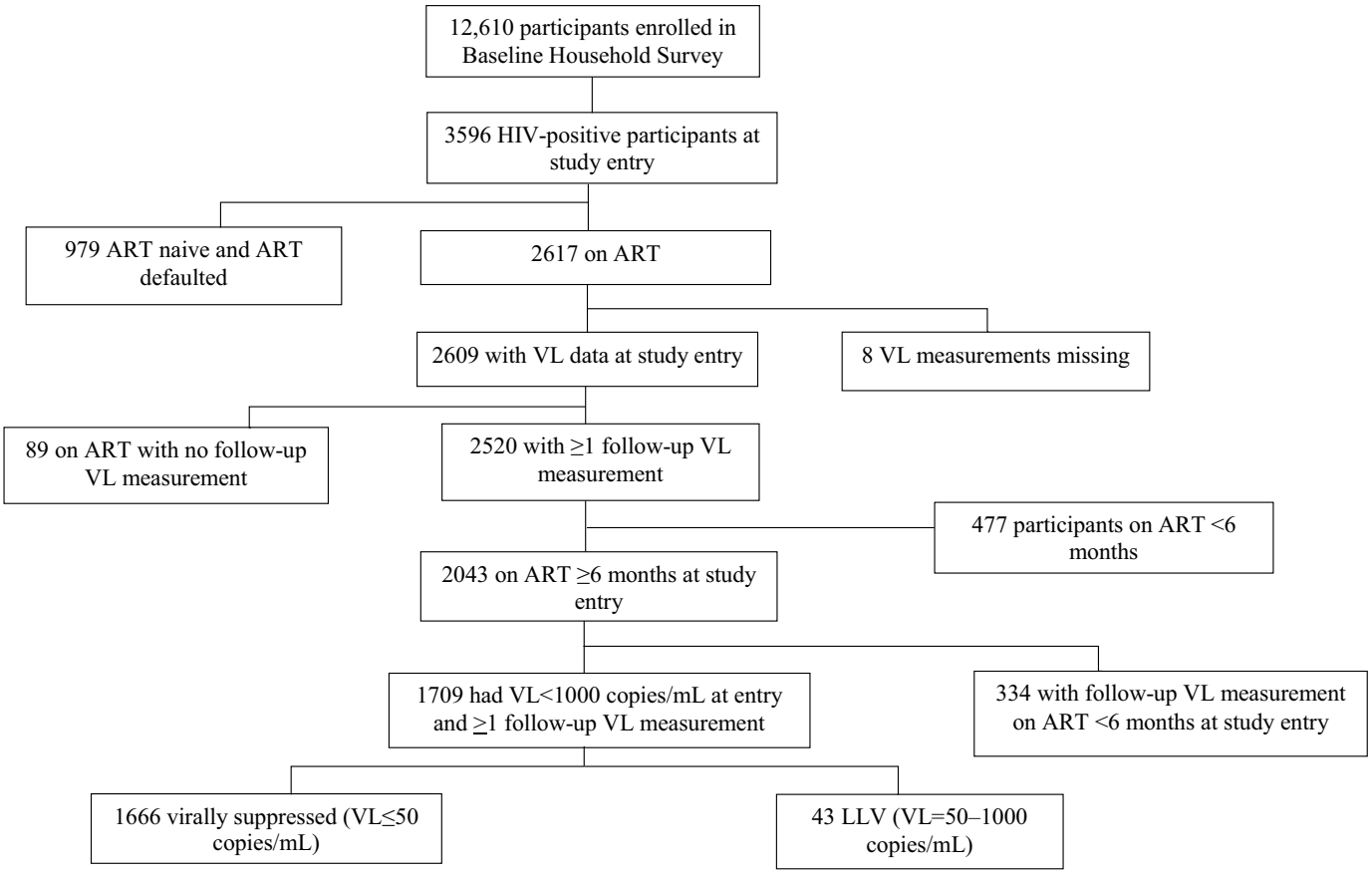

Figure 2. Flow chart showing how participants were selected for analysis of association of LLV at study entry with subsequent VF during the study follow-up.

Stratifying by LLV subgroup, the prevalence of any DRM was 43 (35%) and 14 (42%) in the low LLV and high LLV subgroups, respectively. In the low LLV subgroup, NNRTI-associated mutations (observed in 32% of persons with LLV) were most common, followed by NRTI-associated mutations (observed in 10%) while PI- and INSTI-associated mutations were found in 5% and 4%, respectively. In the high LLV subgroup, the prevalence of DRMs by class was 30% NNRTI, 12% NRTI, 3% PI and 6% INSTI (Table 3).

Eighty-four (25%) of 332 participants had at least one major DRM; 20% and 30% for LLV and VL ≥ 1000 groups, respectively (Table 4). Overall, the prevalence of at least one major DRM was significantly higher in the VL ≥ 1000 copies/mL group (30%) compared with the LLV group (30%); $P = 0.04$. The prevalence of at least one major DRM in each of the NNRTI and NRTI drug classes was significantly higher in the VL ≥ 1000 copies/mL group compared with the LLV group.

The predominant NRTI-associated mutations were M184I (4%) and M184 V (10%) (Figure 3). The NNRTI-associated mutations with prevalence of >2% were K103N (13%), E138A (11%), M230I (6%), Y181C (4%), A98G (3%) and G190A (2%). G173S (3%) was the most common PI-associated mutation. We didn't record any INSTI-associated mutations with overall prevalence of >2% among 332 participants with VL > 50 copies/mL, although D232N was found in 2/33 (6%) of participants with high LLV, while E138K was detected in 3/124 (2%) of participants with low LLV.

Of 135 participants with any DRM, 47% had one DRM, 19% had two DRMs, while 33% had at least three DRMs (Figure S1, available as Supplementary data at *JAC* Online). The proportions of PWH who had one DRM were 33 (58%) of 57 participants who had LLV and 31 (40%) of 78 individuals with VL ≥ 1000 copies/mL. The proportions of individuals with more than one DRM among LLV and VL ≥ 100 copies/mL groups was not statistically significant.

Predictive factors of VF

Among 3596 PWH in the BHS, 2617 were on ART and 2609 had baseline/enrolment VL measurement, of whom 97% (2520/2609) had at least one follow-up VL measurement and 81% (2043) were on ART for at least 6 months. A total of 84% (1709/2043) of those on ART for ≥6 months with VL < 1000 copies/mL at study entry had at least one follow-up VL measurement. Twelve (28%) of 43 persons with LLV at entry had subsequent VF compared with 12 (0.7%) of 1666 persons with VL ≤ 50 copies/mL at entry (unadjusted OR: 53; 95% CI: 19–150; $P < 0.001$; Table 5). In the adjusted analysis, being male, having LLV and being on second-line ART at enrolment were each associated with subsequent VF (Table 3). The adjusted OR of presenting with VF among individuals with LLV was 36 (95% CI: 10–137; $P < 0.001$). The OR of subsequent VF was 2.6 times higher among

Table 1. Baseline characteristics of included participants

Variable	Total $N=4443$	Suppressed $N=4111$	LLV $N=157$	VL ≥ 1000 copies/mL $N=175$	*P* value[a]
District ($N=4443$), *n* (%)					
Southern	1230 (28)	1121 (27)	50 (32)	59 (34)	0.1
Central	1805 (41)	1690 (41)	59 (38)	56 (32)	
Northern	1408 (32)	1300 (32)	48 (31)	60 (34)	
Sex ($N=4442$), *n* (%)		$N=4110$			
Male	1239 (28)	1126 (27)	53 (34)	60 (34)	0.03
Female	3203 (72)	2984 (73)	104 (66)	115 (66)	
Age at enrolment (years), median (IQR)	41 (35–49)	42 (35–49)	37 (31–45)	34 (26–42)	<0.001[b]
ART regimen at enrolment ($N=4068$), *n* (%)	$N=4068$	$N=3807$	$N=134$	$N=127$	<0.001
First line					
Second line	3807 (94)	3589 (94)	114 (85)	104 (82)	
Deep salvage	217 (5)	183 (5)	16 (12)	18 (14)	
	44 (1)	35 (1)	4 (3)	5 (4)	
ARV regimen at enrolment ($N=3940$), *n* (%)	$N=3940$	$N=3692$	$N=127$	$N=121$	
EFV-based					<0.001
NVP-based	2364 (60)	2226 (60)	76 (60)	62 (51)	
DTG-based	1049 (27)	1010 (27)	18 (14)	21 (17)	
LPV-based	289 (7)	257 (7)	14 (11)	18 (15)	
	238 (6)	199 (5)	19 (15)	20 (17)	
ART duration at enrolment (years), median (IQR)	6.4 (3.4–9.1)	6.5 (3.4–9.2)	6.5 (4.0–9.9)	5.0 (1.0–7.8)	<0.001[b]
VL (copies/mL), median (IQR)	40 (40–40)	40 (40–40)	145 (74–346)	19 574 (4219–65 319)	<0.001[b]

EFV, efavirenz; NVP, nevirapine; DTG, dolutegravir; LVP, lopinavir.
[a]*P* values were found using a chi-squared test for categorical variables and Kruskal–Wallis for continuous variables.
[b]These *P* values were obtained from Kruskal–Wallis.

those with high LLV compared with those in the low LLV group (95% CI: 1.0–7.3; $P=0.07$). The presence of major DRMs among participants with LLV at study enrolment did not increase the OR of VF.

Discussion

We found that 4% of a representative population-based cross-sectional sample of adults on ART for at least 6 months in Botswana had LLV (50–999 copies/mL). Persons with LLV were less likely to have at least one major DRM (archived or in RNA) than persons with VL ≥ 1000 copies/mL, although the presence of any DRM (major or minor) did not differ between groups. Persons on ART who had LLV were much more likely to experience subsequent VF than persons with HIV-1 RNA ≤ 50 copies/mL.

The prevalence of LLV that we found is on the lower end of LLV (5.3%–27%) reported in several other countries in Africa, including South Africa,[36] Kenya,[37] a multi-country African cohort (Uganda, Tanzania, Nigeria and Kenya)[38] and the PROMOTE longitudinal cohort (Uganda, Malawi, Zimbabwe and South Africa).[39] There are several possible explanations for the lower prevalence of LLV in our study. First, the ART programme in Botswana is quite mature and monitors VL in all patients on ART. We used one VL of 51–999 copies/mL to define LLV, while other studies generally used more than one VL. Currently, Botswana HIV treatment guidelines have no clear guidelines on the management of LLV. In high-income settings, the interventions for LLV include intensified interventions such as adherence counselling, DRM testing and empirically changing ARV drugs on a case-by-case basis, upon detection of VL > 50 copies/mL, with emphasis on persistent LLV,[40] suboptimal adherence to ART,[41–43] detection of DRMs[10] and subsequent VF.[44,45]

We did not find significant differences in the prevalence of at least one DRM (major or minor) by VL group. However, the prevalence of any major DRM was significantly higher in persons with VL ≥ 1000 copies/mL compared with those with LLV, as expected.[46–48] One third (36%) of individuals with LLV had at least one DRM, which is at the lower end of what has been reported in the literature (19%–72%).[37] The relative distribution of NNRTI-, NRTI-, PI- and INSTI-associated mutations stratified by VL was similar to that in persons with VL ≥ 1000 copies/mL, even though participants with DRM at LLV are not considered as failing ART in most resource-limited settings with annual VL monitoring.[49] Our findings highlight that considerable levels of DRM are detectable at VLs of 51–999 copies/mL, with higher rates of NNRTI- and NRTI-associated mutations.[9,10,50,51,52]

Overall, our BCPP participants harboured relatively frequent resistance towards lamivudine, emtricitabine, abacavir, nevirapine and efavirenz in sequences that were derived primarily from proviral DNA. Most of these DRMs were likely selected by prior and ongoing ART, e.g. with efavirenz/emtricitabine/tenofovir disoproxil fumarate and cabotegravir/nevirapine. Some DRMs may also be from archived transmitted resistance mutations.[53]

Table 2. Prevalence of HIV DRMs stratified by VL group at enrolment

Resistance measure	Total $N=332$	LLV (51–999 copies/mL) $N=157$	VL ≥ 1000 copies/mL $N=175$	*P* value
Any mutation, *n* (%)	135 (41) 95% CI: 35–46	57 (36) 95% CI: 29–44	78 (45) 95% CI: 37–52	0.09
Resistance category, *n* (%)				
NRTI-associated	55 (17)	17 (11)	38 (22)	0.007
NNRTI-associated	116 (35)	50 (32)	66 (38)	0.18
PI-associated	19 (6)	7 (4)	12 (7)	0.23
INSTI-associated	10 (3)	6 (4)	4 (2)	0.28

Numbers in the 'Any mutation' row are the number of participants with at least one mutation associated with any of the four drug classes (NNRTI, NRTI, PI and INSTI), Numbers in the 'Resistance category' rows are the number of participants with at least one mutation in any specific drug resistance class. Comparison of proportion test was used to test the difference in the prevalence of DRMs amongst the two VL groups. Proportions with 95% CI were estimated using the binomial exact method. Note that the sum of the number of persons with individual classes of mutations will exceed the number of persons with any mutation, as some people had more than one class of DRM.

Prior studies have reported accumulation of DRMs with persistent LLV[1,37,42,50,54,55] and the potential for negative outcomes among persons remaining on non-suppressing therapy with low-level viral replication.[43,45] These studies were in the era preceding dolutegravir-based ART when regimens with lower barriers to resistance were used. The impact of LLV in patients on dolutegravir-based ART remains unexplored, especially in resource-limited settings, but recent data indicate that patients on dolutegravir-based ART tend to have DRMs at low HIV VLs.[56–58]

We investigated the association between LLV and VF, age, gender, ART regimen and duration on ART among the subset of participants with follow-up VL data available. As in previous studies, being male and being on second-line ART were associated with VF.[59–61] The risk of subsequent VF in participants with baseline LLV was 36-fold higher than in persons without baseline LLV in multivariate analysis, consistent with studies from South Africa[36] and Canada.[43] We used a single VL measurement to define LLV and a single VL result at the end of the study to define VF. We therefore could not differentiate LLV from viral blips nor identify participants who shifted from one VL group to another, although prior studies have also found viral blips to be associated with VF.[62–64] The use of a single measurement of VL to define LLV was strongly associated with VF, starting with a lower range of LLV (51–200 copies/mL) in the HIV-1 C cohort in South Africa with 79% of LLV cases identified without confirmatory VL.[36] Similarly, a study in China found that a single VL measurement above 400 copies/mL was associated with an increased risk of presenting with VF.[65] Taken together, studies that reported the association of viral blip or unconfirmed LLV with VF support our findings. It is challenging to compare studies of LLV due to the use of different VF thresholds,[2–4] LLV duration[6,15,66–69] and number of VL measurements.[6,36,38,40] However, our findings support the lowering of VF thresholds to VL > 50 copies/mL, as per the current EACS guidelines,[4] and highlight the potential role of LLV as a warning of higher risk of VF. Study findings revealed that it is very important to closely monitor individuals with LLV by repeating VL testing upon detection of VL > 50 copies/mL with intensified adherence assessment and support.

Table 3. Prevalence of HIV DRMs stratified by LLV group at enrolment

Resistance measure	Total $N=157$	Low LLV $N=124$	High LLV $N=33$	*P* value
Any mutation, *n* (%)	57 (36) 95% CI: 28–43	43 (35) 95% CI: 26–44	14 (42) 95% CI: 25–61	0.46
Resistance category, *n* (%)				
NRTI-associated	17 (11)	13 (10)	4 (12)	0.74
NNRTI-associated	50 (32)	40 (32)	10 (30)	0.83
PI-associated	7 (4)	6 (5)	1 (3)	0.63
INSTI-associated	6 (4)	4 (3)	2 (6)	0.41

Numbers in the 'Any mutation' row are the number of participants with at least one mutation associated with any of the four drug classes (NNRTI, NRTI, PI and INSTI), Numbers in the 'Resistance category' rows are the number of participants with at least one mutation in any specific drug resistance class. Comparison of proportion test was used to test the difference in the prevalence of DRMs amongst the two VL groups. Proportions with 95% CI were estimated using the binomial exact method. Note that the sum of the number of persons with individual classes of mutations will exceed the number of persons with any mutation, as some people had more than one class of DRM.

Table 4. Prevalence of major HIV DRMs stratified by LLV group at enrolment

Resistance measure	Total ($N=332$)	LLV (51–999 copies/mL) $N=157$	VL ≥ 1000 copies/mL $N=175$	*P* value
Any mutation, *n* (%)	84 (25) 95% CI: 21–30	31 (20) 95% CI: 14–27	53 (30) 95% CI: 24–38	0.04
Resistance category, *n* (%)				
NRTI-associated	54 (16)	17 (11)	37 (21)	0.01
NNRTI-associated	47 (14)	15 (10)	32 (18)	0.04
PI-associated	13 (4)	7 (4)	6 (3)	0.6
INSTI-associated	7 (2)	6 (4)	1 (1)	0.08

Numbers in the 'Any mutation' row are the number of participants with at least one mutation associated with any of the four drug classes (NNRTI, NRTI, PI and INSTI), Numbers in the 'Resistance category' rows are the number of participants with at least one mutation in any specific drug resistance class. Comparison of proportion test was used to test the difference in the prevalence of DRMs amongst the two VL groups. Proportions with 95% CI were estimated using the binomial exact method. Note that the sum of the number of persons with individual classes of mutations will exceed the number of persons with any mutation, as some people had more than one class of DRM.

Our study had several limitations. We could not establish whether detected DRMs were present at prior LLV events or were newly accumulated DRMs during LLV periods, as we assessed LLV and viral sequence at only one timepoint. In addition, the vast majority of our sequences were from proviral DNA, and the mutations identified in the proviral DNA might not be identical to those in the plasma HIV-1 RNA. However, studies have shown relative concordance in DRMs between the two[34,70–72] and US clinical guidelines support the use of proviral DNA genotyping for VL = 200–1000 copies/mL but interpretation with caution.[3] We adjusted for hypermutations to avoid the overestimation of mutations. Due to the lack of more frequent VL measurements during the follow-up, we could not identify individuals who may have shifted from one VL group to another, and we only had follow-up VL for a subset of participants on ART who had viral sequences at baseline (as only some PWH with sequences in BCPP were enrolled in the longitudinal survey cohort). At the time of enrolment there were few participants ($n=64$) who were on ART for <6 months (1.4%). However, the majority of these had suppressed VL (66%; 42/64) and 34% had LLV

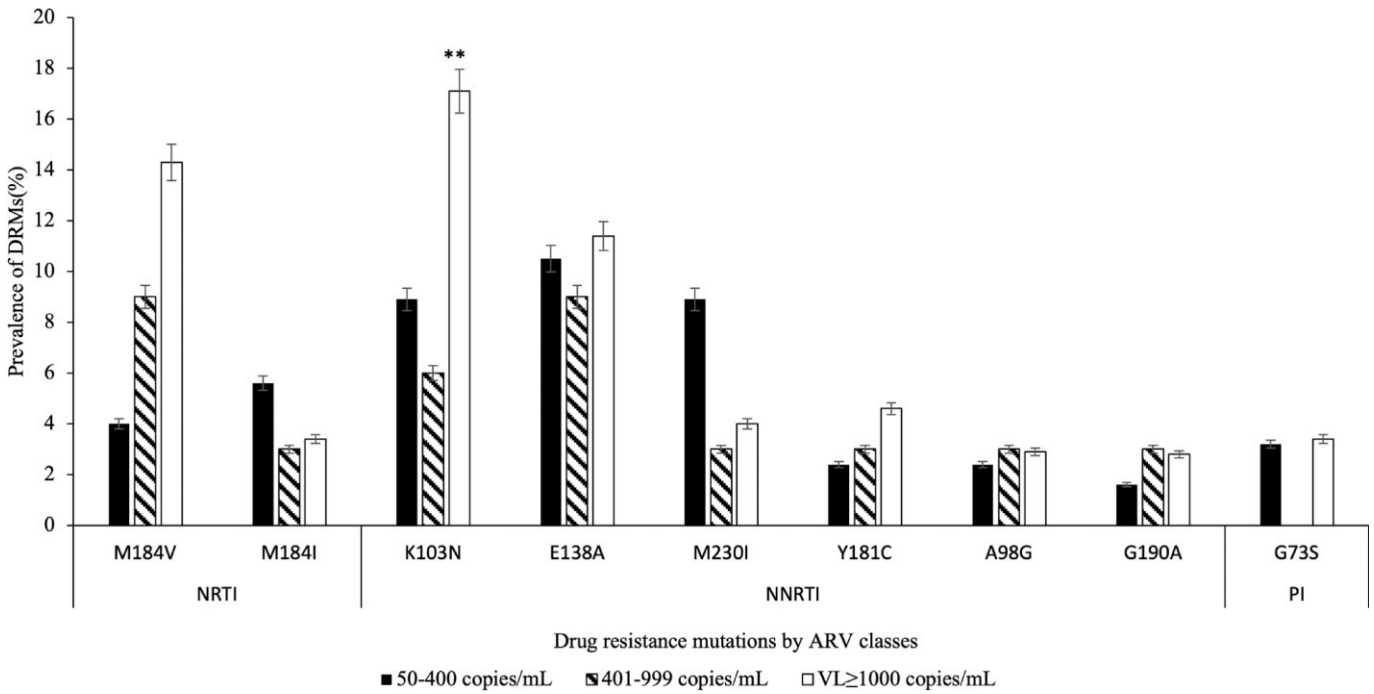

Figure 3. DRMs in adults on ART ($N=332$) with detectable plasma HIV-1 RNA > 50 copies/mL, stratified by VL groups and drug classes (including only DRMs with an overall prevalence of >2%). ARV, antiretroviral. Note that prevalence of all INSTI-associated mutations was less than 2%. **Statistically higher compared with the whole LLV group ($P=0.01$).

Table 5. Factors associated with VF among BCPP participants who had follow-up VL result

Risk factor at study entry	VF at follow-up ($N=24$)	Non-VF at follow-up ($N=1685$) (95% CI)	Univariate OR	*P* value	Multivariate OR (95% CI)	*P* value
VL group, *n* (%)						
Suppressed ($N=1666$)	12 (0.7)	1654 (99.3)	1 (ref)	<0.001	1 (ref)	<0.001
LLV ($N=43$)	12 (27.9)	31 (72.1)	53.4 (19.0–150.0)		36.1 (9.5–136.9)	
LLV groups, *n* (%)[a]						
Low LLV ($N=34$)	8 (24)	26 (76)	1 (ref)		[b]	
High LLV ($N=9$)	4 (44)	5 (56)	2.6 (1.0–7.3)	0.07		
Major DRM at LLV, *n* (%)						
Absent ($N=27$)	5 (19)	22 (81)	1 (ref)		[b]	
Present ($N=16$)	7 (44)	9 (56)	3.4 (1.0–17.1)	0.13		
Sex *n* (%)						
Female ($N=1296$)	14 (1)	1282 (99)	1 (ref)		1 (ref)	0.11
Male ($N=413$)	10 (2.4)	403 (97.6)	2.3 (0.89–5.8)	0.09	2.4 (0.8–7.2)	
ART regimen						
First line ($N=1507$)	15 (1)	1492 (99)	1 (ref)		1 (ref)	
Second line ($N=111$)	7 (6)	104 (94)	6.7 (2.6–17.3)	<0.001	2.6 (0.77–9.0)	0.12
Age at enrolment (years), median (IQR)	35.6 (30.5–41.8)	42.1 (36.5–50.2)	0.94 (0.91–0.97)	<0.001	0.93 (0.89–0.98)	<0.01
Duration on ART at VF, median (IQR)	8.5 (7.6–12.8)	8.2 (4.9–12.1)	1.1 (0.91–1.3)	0.36	[b]	

All column variables represent those who experienced VF at follow-up while rows represent those who were virally suppressed or had LLV at study entry. REF, reference.

[a]Low LLV was used as a reference.

[b]Excluded from multivariate analysis because of collinearity or low numbers.

(27%; 17/64 and 8%; 5/64 had low LLV and high LLV, respectively). We excluded all these participants from the analysis. Our study was performed before the widespread rollout of dolutegravir-based ART in Botswana; only 7.0% of our participants were taking dolutegravir and studies in populations taking dolutegravir-based regimens are warranted. However, our study had the advantage of the large sample size and the sampling design of the BCPP cohort providing good representativeness of the Botswana population. The other strength of our study is that we were able to detect DRMs from both plasma and proviral DNA in settings of LLV, where diagnosis of DRMs using plasma is limited by VL thresholds of >1000 copies/mL with most commercially available genotyping kits.[20]

In conclusion, in a well-characterized large population-based group of adults with HIV on ART in Botswana, persons with VF had higher prevalence of major DRMs than persons with LLV (although similar prevalence of any DRM). A single episode of LLV was strongly associated with subsequent VF among these individuals who were predominantly taking NNRTI-based ART. Our findings show that any detectable VL between 50 and 1000 copies/mL leads to poorer treatment outcomes, therefore we highlight that it is very important that persons with LLV are provided with enhanced adherence support and more frequent VL monitoring.

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Transparency declarations

None to declare.

Author contributions

S.G., O.T.B. and S.M. conceived the study and prepared the first draft. R.S. and E.R. reviewed the manuscript and provided comments. O.T.B., S.G., S.M. and M.S.M. finalized the manuscript based on feedback from other authors. S.G., S.M., O.T.B., V.N., M.E., T.G., J.M.M. and S.L. collected or prepared the data. S.M., S.G., V.N., M.Z.S., D.M., B.M., T.D., O.T.B. and P.M. conducted and supervised the laboratory experiments. O.T.B., S.M., S.G. and V.N. analysed the sequences. O.T.B., N.O.M., I.G., W.T.C., K.M. and S.M. did the statistical analysis and data presentation. S.M., S.G., T.G., M.P.H., J.M.M., M.O.M., T.M., M.E. and S.L. helped provide overall guidance to the conduct of the study. M.P.H., J.M.M., M.M., V.N., S.M., S.G., M.E. and S.L. were involved in the origination and development of the concept of the study.

Data availability

All relevant data are presented within the paper and the Supplementary data, figures and tables. HIV-1 sequences and associated clinical data are available on reasonable request through the PANGEA consortium (www.pangea-hiv.org). BCPP data are available at https://data.cdc.gov/Global-Health/Botswana-Combination-Prevention-Project-BCPP-Publi/qcw5-4m9q.

Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online.

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