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Impact of pretreatment low-abundance HIV-1 drug-resistant variants on virological failure among HIV-1/TB-co-infected individuals

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Objectives: To determine the impact of pretreatment low-abundance HIV-1 drug-resistant variants (LA-DRVs) on virological failure (VF) among HIV-1/TB-co-infected individuals treated with NNRTI first-line ART.

Methods: We conducted a case–control study of 170 adults with HIV-1/TB co-infection. Cases had at least one viral load (VL) ≥1000 RNA copies/mL after ≥6 months on NNRTI-based ART, and controls had sustained VLs <1000 copies/mL. We sequenced plasma viruses by Sanger and MiSeq next-generation sequencing (NGS). We assessed drug resistance mutations (DRMs) using the Stanford drug resistance database, and analysed NGS data for DRMs at ≥20%, 10%, 5% and 2% thresholds. We assessed the effect of pretreatment drug resistance (PDR) on VF.

Results: We analysed sequences from 45 cases and 125 controls. Overall prevalence of PDR detected at a ≥20% threshold was 4.7% (8/170) and was higher in cases than in controls (8.9% versus 3.2%), $P = 0.210$. Participants with PDR at ≥20% had almost 4-fold higher odds of VF (adjusted OR 3.7, 95% CI 0.8–18.3) compared with those without, $P = 0.104$. PDR prevalence increased to 18.2% (31/170) when LA-DRVs at ≥2% were included. Participants with pretreatment LA-DRVs only had 1.6-fold higher odds of VF (adjusted OR 1.6, 95% CI 0.6–4.3) compared with those without, $P = 0.398$.

Conclusions: Pretreatment DRMs and LA-DRVs increased the odds of developing VF on NNRTI-based ART, although without statistical significance. NGS increased detection of DRMs but provided no additional benefit in identifying participants at risk of VF at lower thresholds. More studies assessing mutation thresholds predictive of VF are required to inform use of NGS in treatment decisions.

Introduction

The success of scaling-up ART is greatly threatened by the emergence and transmission of HIV drug resistance (HIVDR). HIVDR that occurs in individuals who have not yet initiated ART, or who have prior ART use and are re-initiating first-line treatment, is known as pretreatment drug resistance (PDR).[1,2] PDR is associated with poor ART outcomes,[3,4] and there is strong evidence suggesting a substantial increase in levels of PDR in southern Africa, the region with the greatest HIV epidemic.[2,5,6] With evidence of increasing PDR, the WHO now recommends modifying the standard first-line ART regimen or introducing pretreatment HIVDR testing when NNRTI-PDR levels reach ≥10%.[1]

Changing the standard first-line ART regimen offers a more feasible alternative option to pretreatment HIVDR testing. The WHO recommends use of dolutegravir, an integrase strand transfer inhibitor (INSTI), that is cheaper and better tolerated compared with current NNRTIs.[7–9] Despite this recommendation, concerns over use of dolutegravir among specific subpopulations remain. This includes women of child-bearing age, due to potential for neural tube birth defects occurring when dolutegravir is used at the time of conception.[10–12] In addition, concerns also exist regarding co-administration of dolutegravir and TB treatment among HIV-1/TB-co-infected people, specifically from drug–drug interactions with rifampicin,[9] and in patients who do not tolerate dolutegravir.[13] This highlights potential for the continued use of NNRTIs, and the relevance of assessing the impact of NNRTI resistance mutations on ART.

Sanger sequencing has been the conventional method used for detecting HIV drug resistance mutations (DRMs). Sanger sequencing is relatively expensive, and does not reliably detect mutations that are not well represented, i.e. occurring at <20% of the viral population.[14,15] With advances in technology, next-generation sequencing (NGS) is becoming more popular and likely to replace Sanger sequencing in routine laboratory workflows.[14] NGS allows for multiplexing of samples, thereby reducing the cost of HIVDR testing. Furthermore, NGS has the ability to detect low-abundance drug-resistant variants (LA-DRVs), i.e. mutations occurring in <20% of the viral population.[14]

There is evidence suggesting that NNRTI-PDR detected by Sanger sequencing results in poor ART outcomes,[16–18] but there remains limited knowledge around the impact of LA-DRVs on treatment outcomes, and at different mutation frequencies. We sought to determine the impact of pretreatment LA-DRVs on virological failure (VF) among HIV-1/TB individuals treated with NNRTI-based first-line ART.

Patients and methods

Ethics

This research study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (reference number: BF340/17). Ethics approvals were also obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (reference numbers: E107/05 and BF051/09; Clinicaltrials.gov number NCT 01539005) for studies from which samples were obtained, and participants gave informed consent for sample storage and re-use.

Study design and study population

This was a nested case–control study aimed at determining the impact of pretreatment LA-DRVs on NNRTI-based ART outcomes. We defined VF as having at least one viral load (VL) ≥1000 HIV-1 RNA copies/mL in plasma after ≥6 months on NNRTI-based ART. De-identified remnant plasma samples from HIV-1/TB-co-infected adults (≥18 years) were obtained from the Starting Antiretroviral therapy at three Points in Tuberculosis (SAPiT) trial, and from a subsequent study known as the TB Recurrence upon Treatment with HAART (TRuTH).

The SAPiT trial was an open-label, randomized, controlled trial conducted by the Centre for AIDS Programme Research in Africa (CAPRISA) between June 2005 and July 2008, at the eThekwini Clinical Research Site (ECRS) in Durban, South Africa. The study investigated the effect of ART started during TB treatment (in two integrated-therapy groups) or after completion of TB treatment (in one sequential-therapy group) on mortality. The majority of participants received a once-daily regimen of efavirenz, lamivudine and didanosine (EFV/3TC/ddI) at ART initiation, but drug switches were allowed during the study. VLs were measured at the time of screening, at randomization and every 6 months thereafter. SAPiT study participants were enrolled into the TRuTH study, a prospective cohort study conducted between 2009 and 2013. The study aimed to assess TB recurrence among participants who were either cured or had successfully completed their TB treatment after 24 months follow-up in SAPiT. Details of the SAPiT and TRuTH studies have been published previously.[19–21]

In this analysis, we defined cases as participants with at least one VL ≥1000 copies/mL after ≥6 months of initiating an NNRTI-based ART. We defined controls as participants who had sustained virological suppression (VL <1000 copies/mL) after at least 6 months on ART (SAPiT ± TRuTH), and maintained viral suppression throughout the study follow-up period (5–6 years). Pre-ART samples from cases and controls were included in a 1:2 case:control ratio. A simple random sampling strategy was used to select unmatched controls. If plasma samples were not available for cases at their first high VL, we accessed a subsequent sample based on availability of remnant plasma. We excluded participants who had switched from an NNRTI-based regimen at the time of ART failure.

Laboratory methods

We retrieved plasma samples with VLs ≥1000 copies/mL from a −80°C biorepository and thawed them to room temperature prior to viral RNA extraction. For each sample, we centrifuged 500 μL of plasma at 23000 **g** for 1 h at 4°C to pellet the virus. We extracted viral RNA from 200 μL of pelleted plasma using a NucliSens EasyMAG HIV-1 (bioMérieux, Craponne, France) extraction system, and amplified the protease and reverse transcriptase genes using Southern African Treatment Resistance Network custom primers, as described previously.[22] We purified successfully amplified PCR products using a QIAquick PCR purification kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. To limit sample variability in the final sequencing product, we aliquotted purified PCR products of each sample for Sanger sequencing and NGS.

Sanger sequencing

In preparation for capillary electrophoresis, we performed sequencing reactions using a BigDye Terminator v3.1 kit (Applied Biosystems, Foster City, CA, USA), and sequencing reaction purifications using a BigDye XTerminator v3.1 purification kit (Applied Biosystems), according to the manufacturer's instructions. We performed capillary electrophoresis on an ABI 3730 genetic analyser and assessed the quality of sequences using Geneious software v8.1.9 (Biomatters Ltd, New Zealand).[23] We excluded sequences with incomplete reverse transcriptase (codons 1–254) genes, and detected DRMs using the Stanford University HIV drug resistance database (version 8.6).[24]

Next-generation sequencing

For NGS, we determined PCR product concentrations using a Qubit 3.0 fluorimeter (Life Technologies, Malaysia), diluted amplicons to 0.2 ng/μL and performed library preparation using the Nextera-XT DNA Library Preparation kit and Nextera Index kit (Illumina, San Diego, CA, USA), according to the manufacturer's instructions. In summary, library preparation involved kit-based enzymatic fragmentation of DNA, dual indexing of fragmented DNA and bead-based purification of amplicons using AMPure beads (Beckman Coulter, Brea, CA, USA). We performed quality control steps using the LabChip GX Touch (PerkinElmer, Hopkinton, MA, USA) to determine the amplicon size, and used the Qubit 3.0 fluorometer (Life Technologies, Malaysia) to determine library concentrations. We normalized each sample library to 4 nM concentration, pooled the normalized libraries and diluted them to a final concentration of 10 pM. We spiked the

10 pM library with 5% PhiX control, and sequenced it on an Illumina MiSeq platform using the MiSeq Nano Reagent Kit v2 (500 cycles).

For paired-end sequencing analysis, we used Genome Detective, a web-based tool for analysis of molecular sequence data (https://www.genomedetective.com).[25] In summary, the software used Trimmomatic for quality control in filtering sequences, for adaptor trimming and for checking for external contamination. It generated gene coverage plots and mapped them to a default HIV-1 subtype C reference sequence in Genome Detective. We excluded sequences with <100× depth of coverage or having incomplete reverse transcriptase (codons 1–254) genes. We aligned the sequences to an annotated HIV-1C reference sequence, and analysed for LA-DRVs in Geneious software v8.1.9 (Biomatters Ltd, New Zealand).[23]

Drug resistance analysis

We determined both Sanger sequencing and NGS DRMs based on the Stanford University HIV drug resistance database (version 8.6).[24] We estimated HIVDR prevalence at ≥20%, 10%, 5% and 2% thresholds, and defined drug resistance as having an NRTI resistance mutation or an NNRTI resistance mutation. We excluded individuals who did not have both an NGS and Sanger sequence either at the pre-ART timepoint or at VF. We excluded PI resistance mutations from our analysis, as none of the participants selected had initiated PI-based ART regimen.

Data analysis

We used STATA v13 (StataCorp, College Station, TX, USA) for statistical analysis. We used the Fisher's exact test and Wilcoxon rank-sum test (for categorical and continuous variables, respectively) to compare baseline demographics (i.e. sex and age) and clinical characteristics (CD4 count, VL, months on ART and SAPiT randomization arm) between cases and controls. We analysed the effect of PDR majority mutations on ART. To determine the impact of pretreatment LA-DRVs on VF, we excluded PDR majority mutations (i.e. mutations at ≥20% threshold) from the analysis. We further excluded thymidine analogue mutations (TAMs) D67NGE and K219NE, that do not confer any resistance to didanosine. We used univariable and multivariable logistic regression to assess the association between pretreatment LA-DRVs and VF. The model was adjusted for age, gender, CD4 count at ART initiation, VL at ART initiation, and randomization. We did not adjust for time on ART and TB medications, as controls were only sampled prior to treatment initiation, and TB medications were only taken for 6 months at SAPiT enrolment.

Results

Participant characteristics

We identified 99/642 cases with at least one VL ≥1000 copies/mL after receiving ART for at least 6 months, with approximately 50% (50/99) of the cases having two consecutive VLs ≥1000 copies/mL. From a total of 543 participants with sustained virological suppression, we selected 198 controls, giving a total of 297 participants (i.e. 99 cases and 198 controls). Of the 297 participants, 207 (69 cases and 138 controls) had appropriate samples available for testing. The median duration on ART for the 69 cases was 5 years (IQR 4.5–5.6) and the median duration on ART for the 138 controls was 6 years (IQR 5.0–6.3) (Figure S1, available as Supplementary data at *JAC* Online). We obtained complete NGS and Sanger sequence pairs for 170 participants (45 cases and 125 controls) (Figure 1, Table S1). Thirty-two of the 45 (71%) cases achieved viral suppression at subsequent points including those who switched ART.

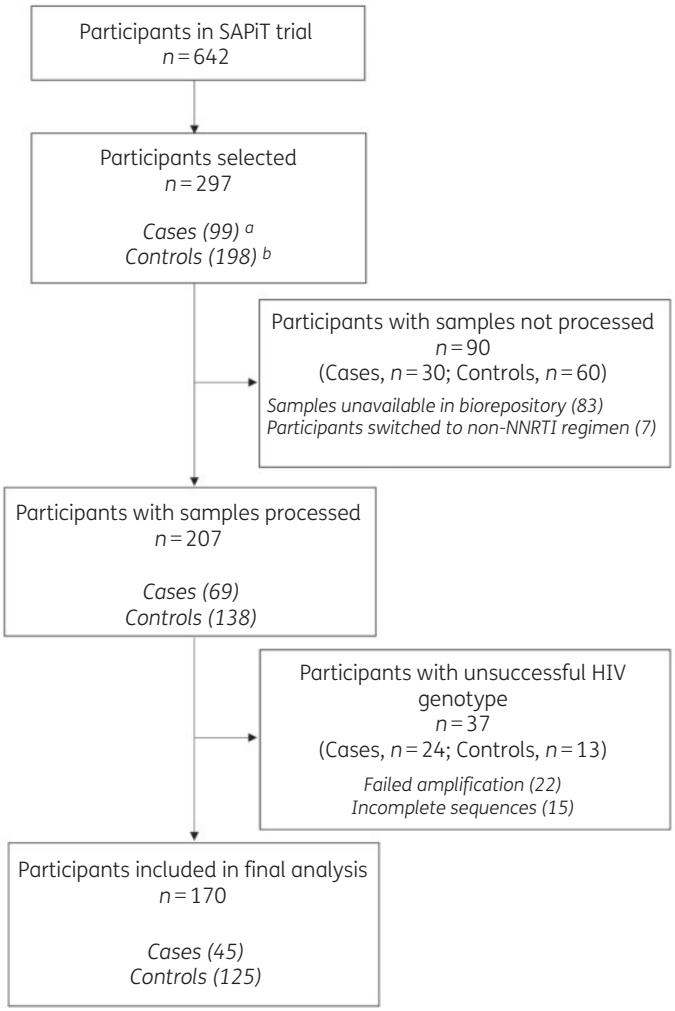

Figure 1. Summary flow chart of participants from selection to analysis. [a]Cases included all participants enrolled in the SAPiT trial that had viral loads ≥1000 copies/mL after ≥6 months on ART. [b]Controls were randomly selected from SAPiT trial participants to match cases at a 1:2 ratio.

All except two participants (168/170) received EFV/3TC/ddI at ART initiation. Of the two participants (i.e. controls), one received efavirenz with zidovudine and lamivudine, and the other received nevirapine with lamivudine and didanosine at ART initiation. Table 1 summarizes the demographic and clinical characteristics of the participants included in the final analysis. We did not observe any significant differences in demographic and clinical characteristics when comparing cases and controls.

HIV-1 drug resistance data before ART initiation

Overall, of 170 pre-ART sequences, all were subtype C. Eight (4.7%) had at least one DRM detected by Sanger sequencing and by NGS at a ≥20% threshold (four cases and four controls). NGS at a ≥20% threshold (i.e. majority drug resistance) was completely concordant with Sanger sequencing, so from this point onwards we only refer to NGS data. Seven of the eight sequences revealed single class resistance (two NRTI and five NNRTI), the other case

Table 1. Baseline characteristics of participants included in the final analysis

Characteristic	Total ($n=170$)	Cases ($n=45$)	Controls ($n=125$)	*P* value
Female, *n* (%)	99 (58.2)	29 (64.4)	70 (56.0)	0.326
Age in years, median (IQR)	34 (29–40)	35 (27–39)	34 (29–41)	0.420
Viral load (log_{10} copies/mL), median (IQR)[a]	5.3 (4.8–5.7)	5.2 (4.8–5.7)	5.3 (4.8–5.7)	0.496
CD4 count (cells/mm^3), median (IQR)	141 (58–224)	107 (42–219)	150 (83–225)	0.148
Treatment arms[b]				
early integrated, *n* (%)	50 (29.4)	12 (26.7)	38 (30.4)	
late integrated, *n* (%)	65 (38.2)	17 (37.8)	48 (38.4)	
sequential, *n* (%)	55 (32.4)	16 (35.6)	39 (31.2)	

Baseline refers to time of enrolment in the SAPiT trial.
[a]One case and two controls with missing viral loads prior to ART initiation.
[b]Early integrated arm, ART initiated within a month of starting TB treatment; late integrated arm, ART initiated within a month of completing intensive phase of TB treatment; sequential arm, ART initiated within a month of completing the continuation phase of TB treatment.

Table 2. Proportion of pre-ART NRTI and NNRTI resistance by NGS mutation thresholds

	Detection threshold			
Characteristics	2%	5%	10%	20%
Overall resistance (*n*=170)				
Any resistance, *n* (%)	31 (18.2)	13 (7.7)	11 (6.5)	8 (4.7)
Any NRTI resistance, *n* (%)	19 (11.2)	6 (3.5)	5 (2.9)	2 (1.2)
Any NNRTI resistance, *n* (%)	15 (8.8)	8 (4.7)	7 (4.1)	7 (4.1)
Cases (*n*=45)				
Any resistance, *n* (%)	11 (24.4)	7 (15.6)	7 (15.6)	4 (8.9)
Any NRTI resistance, *n* (%)	7 (15.6)	2 (4.4)	1 (2.2)	1 (2.2)
Any NNRTI resistance, *n* (%)	6 (13.3)	5 (11.1)	4 (8.9)	4 (8.9)
Controls (*n*=125)				
Any resistance, *n* (%)	20 (16.0)	6 (4.8)	4 (3.2)	4 (3.2)
Any NRTI resistance, *n* (%)	12 (9.6)	4 (3.2)	4 (3.2)	1 (0.8)
Any NNRTI resistance, *n* (%)	9 (7.2)	3 (2.4)	3 (2.4)	3 (2.4)

NGS, next-generation sequencing.

harboured both NRTI and NNRTI resistance. The most common mutations (K103N and V108I) were detected in 3/8 pre-ART sequences (Table S2). Table 2 summarizes the pre-ART drug class mutations observed by NGS at different mutation thresholds. Figure 2 shows the prevalence of majority DRMs and LA-DRVs at pre-ART, in cases and controls, respectively.

Impact of pretreatment majority DRMs and LA-DRVs on VF

The overall prevalence of pretreatment majority DRMs was higher in cases than in controls (8.9% versus 3.2%), $P=0.210$. Overall median time to VF among cases was 15.5 months (IQR 9.2–29.2). Cases with PDR at a ≥20% threshold had a shorter median time to VF (9.4 months, IQR 8.4–14.7) compared with cases without PDR mutations (16.3 months, IQR 9.2–30.4), $P=0.151$. Participants with pretreatment majority DRMs (i.e. at a ≥20% threshold) had almost 4-fold higher odds of VF [adjusted OR (aOR) 3.7, 95% CI 0.8–18.3] compared with those without ($P=0.104$). PDR prevalence increased to 18.2% (31/170) when LA-DRVs at ≥2% were included (24.4% among cases and 16.0% among controls), $P=0.260$. Of the 170 pre-ART sequences, 23 (13.5%, 7 cases and 16 controls) had LA-DRVs only (i.e. mutations at 2% to <20%), 2 with dual class resistance and 21 with single class resistance, i.e. NRTI (15) and NNRTI (6) resistance. Participants with pretreatment LA-DRVs had 1.6-fold higher odds of VF (aOR 1.6, 95% CI 0.6–4.3) compared with those without ($P=0.398$). The pre-ART LA-DRVs were as follows: 10.0% (17/170) for NRTIs (6 cases and 11 controls) and 4.7% (8/170) for NNRTIs (2 cases and 6 controls). The most common pre-ART DRM was K65R, occurring in 6 of 170 (3.5%) sequences (Figure 2a). The median frequency of K65R at pre-ART was 2.8% (IQR 2.2–3.1), and it occurred as the only mutation in five of the six sequences.

Twenty-eight of 31 (90%) participants harboured pretreatment NRTI- and/or NNRTI-LA-DRVs that are associated with resistance to the ART regimen the participants were taking. Of those, only

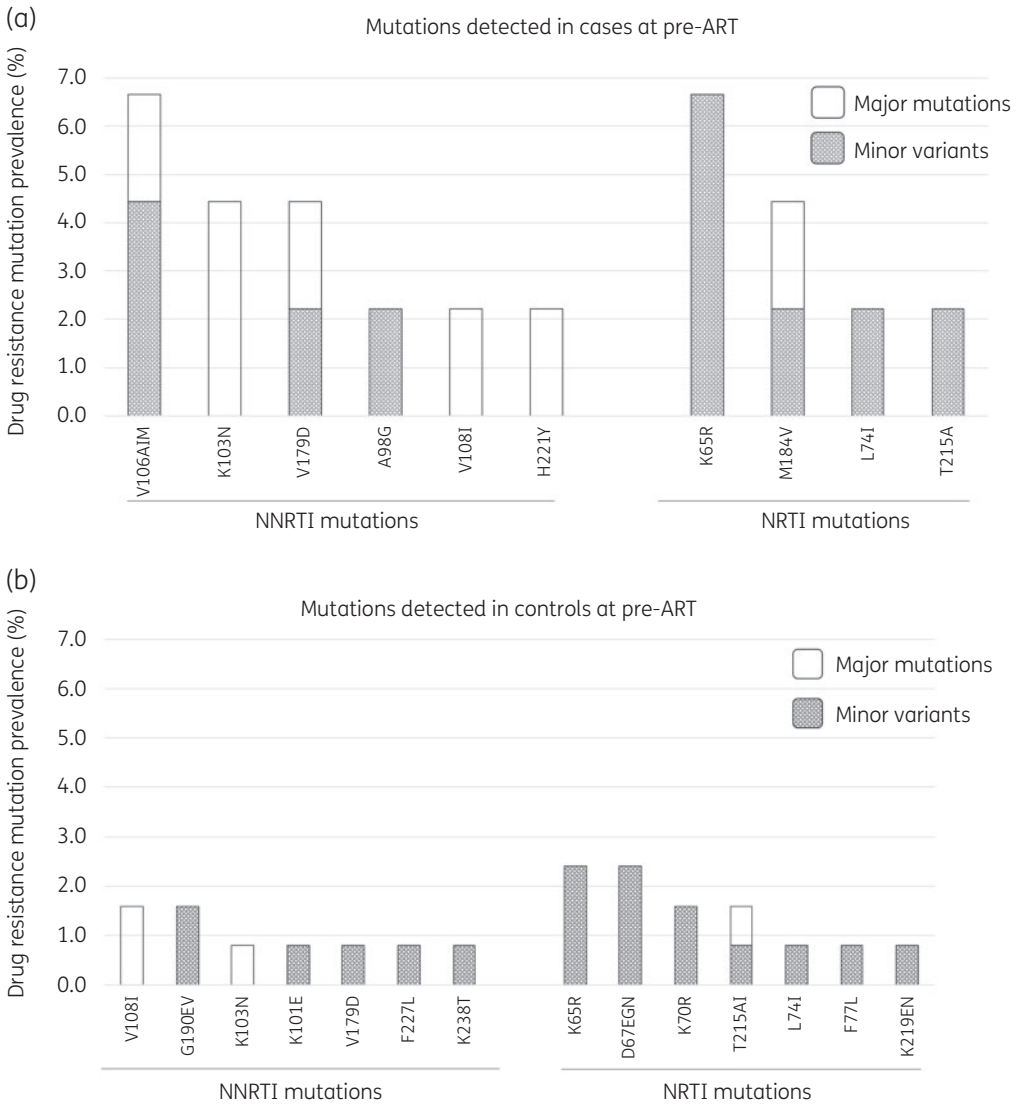

Figure 2. (a) Drug resistance mutations detected in cases with pre-ART resistance. (b) Drug resistance mutations detected in controls with pre-ART resistance. Major mutations represent mutations detected at frequencies of ≥20% threshold, and minor variants represents mutations detected at frequencies of between 2% and <20%.

three cases showed selection of LA-DRVs to majority DRMs at VF. The LA-DRVs selected for were K65R, L74I and V106AI mutations. The NNRTI mutation V106AI became a majority V106M mutation at VF in two cases, whilst the NRTI mutation L74I became a majority L74V mutation in one case, and the NRTI mutation K65R became a majority DRM in one case (Table S3). Despite K65R occurring as the most common DRM at pre-ART, the mutation was only selected for in one of the three cases.

Drug resistance data at virological failure

At VF, 80.0% (36/45) had majority DRMs (i.e. a ≥20% threshold), and the proportion increased to 84.4% (38/45) when LA-DRVs at 2% were included. Of the 36 with majority DRMs, 27 had dual class resistance and 9 had single class NNRTI resistance. The most common majority NNRTI mutation at VF was V106MI, occurring in 48.9% (22/45) of sequences, whilst M184VI was the most common NRTI mutation, occurring in 46.7% (21/45) of sequences. None of the baseline characteristics (i.e. sex, age, CD4 counts, VLs and study arm) was associated with VF (Table S4).

Discussion

In this analysis, we observed an increase in odds of VF among individuals with either pre-ART majority DRMs or LA-DRVs compared with those without, although without statistical significance. We observed that lowering the mutation detection threshold increased the detection of DRMs but had no additional benefit in identifying participants at risk of VF. We also observed that early versus delayed ART with rifampicin-based TB treatment did not enhance VF or the acquisition of DRMs (Table S4). However, our

definition of VF as having at least one VL ≥1000 copies/mL after ≥6 months on ART means that the proportions of DRMs reported are likely not to be reflective of those observed in people with prolonged viraemia on ART. This is considering that some participants might have suppressed their VL following intervention and therefore would not be ordinarily classified as VFs. Also, the lack of ART adherence data in this analysis means that we could not rule out poor ART adherence as a cause of VF amongst the cases.

A recent systematic review by the WHO HIVResNet working group showed that the majority of published studies (14 of 25) have reported no significant association between LA-DRVs and VF, among individuals exposed to first-line NNRTI-based regimens.[18] However, 11 of the 25 studies showed a higher risk of VF when individuals harbour NNRTI LA-DRVs prior to treatment initiation,[18] suggesting that LA-DRVs still play an important role in treatment response. The ANRS 12249 trial showed that having dual class resistance (NRTI and NNRTI) at 5% increased the time to viral suppression by almost 12 months (IQR 2.76–16.39) compared with not having PDR (median 3.48 months; IQR 2.79–5.78),[26] an outcome we could not determine in our study due to only three participants having dual class pretreatment LA-DRVs. However, despite the increased risk of VF with LA-DRVs at 5%, the ANRS 12249 trial further suggested that a combination of potent NRTI drugs with an NNRTI, coupled with good ART adherence, could result in short-term virological suppression even in the presence of pretreatment NNRTI DRMs.[26]

Controversy remains around the effect of pretreatment LA-DRVs on ART, as shown previously in the OCTANE trials. The OCTANE/A5208 trial 1,[27] done among women that had prior exposure to single dose nevirapine (sdNVP), suggested that pretreatment LA-DRVs have a significant impact on ART outcomes, whilst the follow-up OCTANE trial 2 showed no such impact among women without prior exposure to sdNVP.[28] The contradiction suggested that prior exposure to NNRTIs before ART initiation increases the mutant population size that can be selected for on NNRTI-based ART. This is supported by PDR data from a Kenyan cohort study showing that the number of pretreatment NNRTI mutations and their frequency (in a sample isolate) determine the risk of treatment failure, with a greater risk among individuals on nevirapine-based ART compared with efavirenz-based ART.[29] Given that any history of previous prevention of mother-to-child transmission (PMTCT) of HIV was not available, and noting the demographic distribution of participants included, it is likely that some women included in our analyses had undisclosed prior ART exposure for PMTCT.

The differences observed in the impact of pretreatment LA-DRVs on ART outcomes can be attributed to several factors. The WHO HIVResNet working group identified factors such as: the method of detecting HIVDR mutations; the analyses of mutations affecting one or more drug classes; the inclusion in some cases of majority DRMs; different mutation cut-off thresholds; variability in the stage of PDR detection; and differences in the number of individuals included in each study.[18] Therefore, lower frequency thresholds of mutations should be interpreted with caution. A multicountry nested case–control study showed a reduction in specificity from 98% (95% CI 95%–99%) at a ≥20% threshold, to 92% (95% CI 88%–95%) at a 1% threshold,[30] suggesting that the accuracy of resistance as a predictor of VF decreases with a reduction in the detection threshold. Such reductions in specificity could result in unnecessary modification of ART regimens, warranting the need for careful consideration of the benefits and shortcomings of detecting LA-DRVs for public health purposes.

The most common NNRTI mutations at VF occurred at positions where mutations are known to be highly selected for by efavirenz (i.e. positions 103, 106, 188 and 190),[24] suggesting adequate drug pressure for the selection of NNRTI DRMs. However, of greater relevance to the current ART programmes are NRTI resistance mutations. As more countries (including South Africa) roll out dolutegravir in combination with tenofovir and lamivudine (also known as TLD), ensuring the potency of the NRTI drugs is imperative to the success of the new ART regimen. The K65R mutation which causes high levels of resistance to tenofovir and intermediate resistance to lamivudine[24] is of greater concern in the TLD regimen. We detected the K65R mutation at a frequency of only 2%–5% pre-ART, evolving to a majority DRM in only one out of the three participants, and therefore we could not assess its impact on VF, with didanosine treatment. However, continued monitoring of NRTI resistance is warranted in order to avoid potential dolutegravir functional monotherapy due to pre-existing NRTI DRMs. Moreover, despite dolutegravir replacing NNRTIs in first-line ART, monitoring NNRTI resistance remains important in specific subpopulations, such as women of child-bearing age, HIV-1/TB-treated individuals and those who do not tolerate dolutegravir.

The findings from this study should be interpreted with consideration of the following limitations. Basing VF on only one VL result is not conventional. However, 84% (38/45) of the cases in this study already had majority DRMs, with over half of them having dual class NRTI and NNRTI resistance at VF, suggesting the need to consider early VL monitoring and switching of ART regimens before the accumulation of DRMs.[31] Secondly, about 43% (127/297) of the selected participants ended up being excluded from the analysis (Figure 1) due to the unavailability of stored samples and unsuccessful genotyping. However, there was no significant difference between the participants included and those excluded among the cases (Table S1). Lastly, most participants in this study initiated ART on didanosine, a drug that is not commonly used in current regimens.

In conclusion, in a population of individuals treated for HIV-1/TB initiated on NNRTI-based ART, we found that pretreatment LA-DRVs increased the odds of VF, although without statistical significance. Despite the ability of NGS to detect lower frequency mutations, LA-DRVs must be interpreted with caution to avoid misclassification of people as being at risk of VF and hence unnecessary modification of ART regimens. More studies investigating the impact of LA-DRVs on ART are required, including individuals with different clinical characteristics, and considering variables such as the time to VF due to outgrowth of LA-DRVs, the role of viral mutational loads and specific mutation cut-off thresholds that can inform use of NGS in guiding HIV treatment decisions at a public health level.

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This manuscript is dedicated to Babill Stray-Pedersen (1943–2019).

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Transparency declarations

None to declare.

Supplementary data

Figure S1 and Tables S1 to S4 are available as Supplementary data at *JAC* Online.

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