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Drug Resistance and Optimizing Dolutegravir Regimens for Adolescents and Young Adults Failing Antiretroviral Therapy.

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Abstract

Objectives: The integrase strand inhibitor dolutegravir (DTG) combined with tenofovir and lamivudine (TLD) is a single tablet regimen recommended for 1st, 2nd and 3rd line public health ART. We determined drug resistance mutations (DRMs) and evaluated the predictive efficacy of a TLD containing regimen for viremic adolescents and young adults in Harare, Zimbabwe.

Methods: We sequenced plasma viral RNA from HIV-1 infected adolescents and young adults on 1st and 2nd line ART with confirmed virologic failure (VL>1000 copies/ml) and calculated genotypic susceptibility scores (tGSS) to current 2nd, 3rd line and DTG regimens.

Results: 160 participants were genotyped; 112 (70%) on 1st line and 48 (30%) on 2nd line, median (IQR) age 18 (15-19) and duration of ART(IQR) was 6 (4-8) years. Major DRMs were present in 94% and 67% of 1st and 2nd line failures respectively ($p<0.001$). Dual class resistance to NRTIs and NNRTIs was detected in 96 (60%) of 1st line failures; PI DRMs were detected in a minority (10%) of 2nd line failures. A total genotypic susceptibility score (tGSS) ≤2 may risk PI or DTG monotherapy in 11% and 42% of 1st line failures switching to 2nd line PIs and TLD respectively.

Conclusion: Among adolescents and young adults, current PI based 2nd line therapies are poorly tolerated, more expensive and adherence is poor. In 1st line failure, implementation of TLD for many adolescents and young adults on long-term ART may require additional active drug(s). Drug resistance surveillance and susceptibility scores may inform strategies for the implementation of TLD.

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Role of each of the authors

ATM and CEN conceived the study. ATM, CEN, AM, JM, DK and VK supervised data collection and laboratory testing. JM, DK and VK performed data analysis. ATM, CEN, JM, DK and AM critically reviewed and finalized the paper. All authors contributed to subsequent drafts and reviewed and approved the final manuscript.

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Potential conflicts of interest. At the time of conception and the initial conduct of the study ATM had no reported conflicts of interest and was funded through an NIH K-grant. However, ATM is now an employee of Gilead Sciences. All other authors have no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

Keywords

HIV-1; HIV drug resistance; adherence; genotyping; adolescents and young adults; Zimbabwe

Introduction

HIV sero-prevalence in Zimbabwe remains high. Among adults (15–64 years), HIV prevalence is estimated to be 14.6% and approximately 4.7% among adolescents and young adults (15–24 years) [1,2]. To meet the WHO/UNAIDS goals of universal access to antiretroviral treatment (ART) to eliminate HIV by 2030, Zimbabwe has rapidly scaled up its treatment program and in 2016 adopted a policy of test and treat [3]. Although ART has delivered remarkable benefits, increased treatment has led to modest levels of virologic suppression among adolescents and young adults [2,4,5]. Recently a country-wide survey demonstrated that 87% of individuals are linked to care, but only 44% of adolescents and young adults (15–24 years) achieved virologic suppression of < 1,000 copies/ml [2].

Globally, adolescents and young adults pose significant challenges to treatment programs, particularly in low-and-middle income countries (LMICs) where weak health systems, reduced adherence and limited access to VL monitoring contribute to virologic failure [6,7]. These high failure rates lead to drug resistance, increasing morbidity, mortality and the potential for transmission of resistant viruses to sexual partners as well as newborns. Studies in Africa show high rates of acquired drug resistance among HIV infected individuals failing non-nucleotide reverse transcriptase inhibitors (NNRTI) based 1st line ART [8-10]. Muri et al (2017) found a high prevalence of ART associated drug resistance mutations (DRMs, 90%) among children and adolescents in rural Tanzania [11]. In Harare, drug resistance was documented in 68% of sequenced virus isolates in 2015 [5].

In contrast to the high rates of NNRTI associated DRMs in 1st line studies, virologic failure of protease inhibitor (PI) based ART regimens among adolescents and young adults is associated with poor adherence rather than resistance as the cause of failure [12-16].

In public health based approaches to ART in resource limited settings (RLS), guidelines are developed to simplify and streamline care with limited therapeutic options. In Africa, public health models of ART currently recommend tenofovir disoproxil fumarate/lamivudine(TDF +3TC) or zidovudine/lamivudine (AZT+3TC) as nucleos(t)ide reverse transcriptase inhibitors (NRTIs) in combination with either efavirenz (EFV) or nevirapine (NVP) as NNRTIs in 1st line therapy [17]. Upon virologic failure, empiric switching of confirmed 1st line failures to a ritonavir-boosted protease inhibitor (PI) with two new or recycled NRTIs is recommended [17]. In the absence of drug resistance monitoring, switches may occur unnecessarily or individuals may be switched to sub-optimal treatment.

An increasing prevalence of NNRTI associated mutations in naïve and experienced patients as transmitted and acquired resistance, respectively, has led to policy recommendations to adopt Integrase Strand Transfer Inhibitors (INSTIs) [18] in 1st line regimens [19-21]. Dolutegravir (DTG) is a potent INSTI with a high genetic barrier to resistance [22,23]. DTG in combination with tenofovir (TDF) and lamivudine (3TC) (TLD) is anticipated to replace

efavirenz (EFV) based regimens in LMICs [24]. Here we evaluated the potential effectiveness of a TLD regimen among adolescents and young adults failing current 1st and 2nd line regimens with multi-drug resistance (MDR).

Materials and Methods

Study design, population and settings

A cross sectional evaluation of adolescents and young adults aged 10–24 years with confirmed virologic failure, was conducted at the Parirenyatwa Hospital HIV ART treatment clinic (OI clinic) in Harare, Zimbabwe between June 2016 and June 2017. Those who were receiving ART and HIV care with VL>400 copies/ml in the previous month were identified and tested again within 1–2 weeks. Those with a confirmatory VL > 400 copies/ml were enrolled. Consenting participants had been receiving either 1st line (2 NRTIs + 1 NNRTI) or 2nd line ART (2NRTIs + a boosted PI) for at least 6 months. Genotype resistance testing carried out later was attempted only on those samples with VL> 1000 copies/ml.

Study procedures

Written informed consent was obtained from eligible participants aged 18–24 years, and assent as well as the informed consent from their legal guardians was obtained from children and adolescents aged 10–17 years. Socio-demographic (age and gender) and ART history data (treatment initiation date, treatment regimens, ART duration) were extracted from the medical records. Viral load (VL) and CD4 count were carried out in real time and results were returned to the clinicians managing the patients in contrast to genotyping results which were not available in real time.

Those suspected of treatment failure based on the clinical and/or immunological failure were managed according to the national ART standard treatment guidelines. Those with evidence of virologic failure (>400 copies/ml) received enhanced adherence counselling(EAC) for 3 months and repeat VL testing at the end of those 3 months. If the VL showed no improvement after the EAC, medication was switched to the next regimen.

Laboratory assessments

CD4 count was performed by Sysmex-Partec Cyflow^{R} Counter (Muenster, Germany) and VL was measured using the Cobas/Ampliprep v2.0 (Roche, USA). Both assays were performed in the Infectious Diseases Research Laboratory (IDRL), at the University of Zimbabwe. Viral HIV-1 RNA was isolated from plasma, and the Southern African Treatment Resistance Network (SATuRN) HIV drug resistance genotype was performed as previously described [25]. The protease and reverse transcriptase regions of the HIV pol gene were sequenced by Sanger sequencing at MCLab, Molecular Cloning Laboratories, USA. The sequences generated were assembled using Geneious software, version 8 [26]. HIVDRMs were analyzed using the Stanford HIV drug resistance database (HIVdb) [27]. The quality of sequences was assessed by phylogenetic tree reconstruction in Geneious v.8 and the REGA HIV-1 subtyping tool was used to determine HIV-1 subtype [28].

Genotypic analysis

Mutations at major NRTI, NNRTI and PI mutation sites/codons were identified using the International AIDS Society (IAS–USA) list of drug resistance mutations (2017) [29]. Genotypes were classified as wild-type, 1 class (NRTI or NNRTI only), 2 class (NRTI +NNRTI) or 3 class (NRTI+NNRTI+PI) resistance. Statistical analysis was performed using Stata version 14. Descriptive statistics were used to summarize the baseline demographic and clinical characteristics. Chi-square test or Fisher's exact test was used for comparison of proportions of DRMs across 1st line and 2nd line regimens. T-test and Mann Whitney U statistical tests were used to determine associations between explanatory variables (age, treatment characteristics, CD4 count and VL) and the presence of wild-type virus, one or two class resistance and multiple DRMs. Significance levels were set at $p = 0.05$. Total genotypic susceptibility score (tGSS) was calculated for the current ART regimens with switching to 2nd or 3rd line treatment as currently recommended in national treatment guidelines. These tGSS were compared to the implementation of tenofovir, lamivudine and dolutegravir (TLD) as a recommended ART regimen for 1st and 2nd line failures [18,21].

HIV drug resistance

Total genotypic susceptibility scores (tGSS) were calculated based on the number of 'active' drugs prescribed in their current regimen using the Rega Institute HIV Algorithm [30]. For each NRTI and NNRTI drug prescribed, a tGSS value of 1 was assigned if resistance was not identified, a value of 0.5 was assigned to intermediate resistance and 0 was assigned to drugs when mutations predicted high-level resistance. For ritonavir boosted protease inhibitor (PI) drugs, a GSS value of 1.5 was assigned if resistance interpretation identified no resistance, a value of 0.75 was assigned to intermediate resistance and 0 was assigned to drugs when mutations predicted high-level resistance. The arithmetic sum of the individual scores for the specific drugs prescribed provided the total tGSS of that treatment.

The estimated tGSS for their current failing regimen and the recommended 2nd or 3rd line ART was calculated based on the patient's treatment history. A second (future) tGSS was calculated for the anticipated new TLD, single tablet regimens (STR). For participants, currently on a TDF containing regimen, tGSS was calculated for a regimen of zidovudine (AZT), 3TC and boosted atazanavir (ATV/r) or boosted lopinavir (LPV/r). The tGSS for 3rd line ART regimens for participants on ATV/r or LPV/r with major PI DRMs was calculated for a regimen of darunavir/ritonavir, raltegravir or dolutegravir and optimized NRTIs as recommended by national ART guidelines [17]. Participants were grouped according to the number of active drugs prescribed: ≤2 and >2. A value of ≥3 indicates a fully active regimen. Although integrase sequencing was not included, all viruses in Zimbabwe were assumed to be wild type with respect to integrase, as INSTIs have not been introduced into the public treatment program [31-33]. The GSS to DTG was scored as 1.5.

Sequence accession number

Protease and partial Reverse transcriptase sequences in this study are available in GenBank under the following accession numbers: MK583768 – MK583927

Ethics

The study was reviewed and approved by the local institutional review board of the Joint Research and Ethics Committee of the University of Zimbabwe (JREC/185/15), by the Medical Research Council of Zimbabwe (MRCZ/A/1992) and the Research Council of Zimbabwe (RCZ/A/1992).

Results

Characteristics of study population

Of 789 VL tests performed between June 2016 and June 2017 among adolescents and young adults receiving ART and HIV care, 281 with a VL > 400 copies/ml (36% of virologic failure) were identified. Of the 281 participants screened for two detectable viral load tests >400 at least one month apart, 67 were excluded (47 did not meet the VL inclusion criteria and the remaining 20 declined to participate into the study). A total of 214 participants with a confirmatory VL (VL>400 copies/ml) were enrolled into the study. Genotype resistance testing was done on the 185 participants (86%, n=185/214) with VL>1000 copies/ml at the time of study enrolment. All sequences were confirmed as HIV-1 subtype C. Of the 185 participants, 160 (86%) were successfully genotyped; median (IQR) $\log_{10}$ VL of the 160 successfully genotyped was higher than the 25 that failed genotyping {$\log_{10}$ VL of 4.51(4.05–4.92) and $\log_{10}$ VL of 3.84(3.48–4.28) respectively, p=0.002}. One hundred and twelve participants (70%) were on 1st line ART with 67% (75/112) on TDF+3TC +EFV/NVP regimen and 33%(37/112) on AZT+3TC+EFV/NVP regimen. Forty-eight (30%) participants were on 2nd line ART with 58% (28/48) on TDF, 27% (13/48) on abacavir (ABC) and 15%(7/48) on AZT containing regimens. Of the 160 successfully genotyped samples, 83(52%) were from male participants. A large proportion of participants were likely long-term survivors of perinatal vertical transmission with a median (IQR) age at ART initiation of 10 (7–13) years. The median (IQR) age at study enrolment/treatment failure (TF) was 18 (15–19) years. The median (IQR) duration on ART was 6 (4–8) years, with no significant difference in duration on ART between subjects on 1st and 2nd line ART regimens (Table 1). The median (IQR) CD4 count was 197 (47–359) cells/mm^3 and median (IQR) $\log_{10}$ VL was 4.51 (4.05–4.93) copies/ml. Participants on 2nd line ART were older, with a median (IQR) age 18 (17–20) years and had a higher median (IQR) $\log_{10}$ VL of 4.76 (4.52–5.17) copies/ml compared to those on 1st line with a median (IQR) age 17(14–19) years and median (IQR) $\log_{10}$ VL of 4.39 (4.03–4.81) copies/ml (p<0.05) (Table1). Those failing 1st line therapy had slightly higher CD4 count than those failing 2nd line therapy median (IQR) CD4 count of 233 (64–376) cells/mm^3 vs median (IQR) CD4 count of 158 (31–307) cells/mm^3 respectively) (p=0.069) (Table 1).

Drug resistance based on genotype.

Drug resistance mutations (DRMs) were identified in 137/160 (86%) participants failing therapy; wild type sequences were identified in 23 (14%) participants. Resistance to NNRTIs only was identified in 41(26%) participants and defined as single class resistance. NRTI and NNRTI DRMs were detected in 96(60%), these were defined as having dual-class resistance (Table 2). Participants with wild-type or single class resistance were older median (IQR) age 18(16–20) years vs median (IQR) age 17(15–19) years respectively, p=0.03) and

had higher plasma median (IQR) $\log_{10}$ VL of 4.75(4.37–5.09) copies/ml vs median (IQR) $\log_{10}$ VL of 4.34(3.92–4.76) copies/ml (p=0.0001). No significant difference was observed in CD4 count, age at HIV diagnosis and duration on ART prior to study enrolment / treatment failure (TF) in those failing with single class vs dual class resistance, p>0.05 (Table 2).

DRMs in 1st and 2nd line virologic failures.

The frequency of detecting DRMs was higher in sequenced 1st line failures than in 2nd line failures (94% vs 67%, p<0.001) (Table3). Among NRTI drug resistance mutations, the K65R mutation was associated with TDF containing regimens compared with AZT regimens (OR=16.42, P=0.007). The proportion of tenofovir exposure was similar in 1st and 2nd line regimens (67% and 58% respectively (p>0.05). However, the K65R mutation was detected among 51% of 1st line TDF recipients and only 11% of 2nd line TDF recipients (P<0.001). Although all regimens included lamivudine, the M184V mutation was identified in 65% on 1st line and only 25% on 2nd line (p<0.001) (Table 3). Protease inhibitor associated mutations were detected in 10% (5/48) of 2nd line recipients, all of whom had drug resistance to all 3 classes (NRTI+NNRTI+PI) (Table 4).

Calculated tGSS to predict the activity of subsequent regimens.

Among the 112 participants (70%) failing on 1st line, 104 (93%) had tGSS≤2 on their current regimens and the remaining 8 (7%) had completely wild-type virus with a tGSS of >2 suggesting poor adherence to 1st line. The recommended 2nd line regimen as per the Zimbabwe national ART guideline would be 2NRTIs + ritonavir boosted PI. Among the 112 (70%) failing 1st line, 100 (89%) had a tGSS of >2 for recommended 2nd line ART regimens. A tGSS of ≤2 was observed in 12 (11%) of the participants. In these individuals, the boosted PI would be the only active agent resulting in PI monotherapy while on recommended 2nd line treatment (Table 5). If TLD is introduced as a 2nd line regimen, only 65 (58%) of those failing 1st line therapy have a predicted tGSS of >2 and the remaining 47 (42%) with a tGSS of ≤2 may be at risk for exposure to DTG monotherapy. Estimated susceptibility to TDF was 68%(108/160), to AZT was 87%(139/160) and 67%(107/160) were susceptible to abacavir (ABC) (Table 5). When we optimized the TLD WHO guidelines by substituting AZT for TDF to the TLD regimen, 96% (107/112) had a calculated tGSS of >2.

All 48 participants on 2nd line ART with virologic failure (including the 5 participants with multi-drug resistance) had tGSS>2 for the recommended 3rd line ART regimen in Zimbabwe i.e. Darunavir/ritonavir (DRV/r), raltegravir or dolutegravir and 2 optimized NRTIs [17]. Estimating implementation of dolutegravir + 2 optimized NRTIs, 92% (44/48) had a predicted tGSS of >2 and only 8% (4/48) may be at risk for exposure to DTG monotherapy with a tGSS of ≤2.

Predicted tGSS – susceptibility to newer (or 2nd line) NNRTI etravirine, rilpivirine and doravirine

Susceptibility to etravirine, rilpivirine and doravirine was calculated for all participants. All 160 participants were susceptible to doravirine. Eighty-three (52%) participants were

susceptible to etravirine (GSS to etravirine =1) and just over a third, 56(35%) participants were susceptible to rilpivirine (Table 5).

Discussion

Sequence analysis of the protease and reverse transcriptase genes of viremic adolescents and young adults on ART who are failing 1st and 2nd line ART treatment, confirms the widespread selection and persistence of NNRTI mutations. Genotypic resistance was identified in plasma viruses isolated from 93% and 67% of long-term 1st line and 2nd line ART recipients. Dual class resistance was identified in 71% and 33% of viremic individuals on 1st and 2nd line ART therapy respectively.

The high-levels of NRTI and NNRTI mutations among adolescents and young adults failing 1st line ART in our study are consistent with recent studies among children, adolescents and young adults in Africa [34-38]. These high rates of multi-class resistance among adolescents and young adults failing ART in RLS places them at risk of not having affordable treatment options in the future. This data emphasizes the need to develop novel therapies for hard-to treat populations such as perinatally infected children requiring life-long ART who have poor adherence as adolescents and young adults.

In contrast to NRTI and NNRTI resistance, PI resistance mutations were notably infrequent among 2nd line recipients. Similar studies have demonstrated that low frequency of PI resistance is likely indicative of poor adherence to the PI containing ART regimen[13,14,39,40]. In a recent study in Harare, young people were particularly prone to 2nd line failure without PI resistance, compared to adults [41]. Non-adherence to 2nd line regimens containing PI has been shown to be particularly important in young people [42-45]. In this current study, we found that although 2nd line regimens always included lamivudine, among participants without PI mutations only 16% had a M184V mutation, providing further evidence of limited exposure to their prescribed ART regimen due to non-adherence. Similarly, despite 58% of 2nd line participants receiving TDF containing regimens, K65R mutations were detected in only 11%, reflecting limited drug exposure and drug induced selection pressure. This data suggests that non-adherence to ART appears to be particularly problematic with 2nd line regimens. We postulate that this may be due to side effects associated with ritonavir boosted PIs and a high pill burden due to lack of single tablet regimens. Multi drug resistance (MDR) occurred in only 5 participants with major PI mutations who also had M184V (4) or K65R (1) and all 5 had persistent TAMS and NNRTI mutations (Table 5). Among adults in LMICs switching from 1st to 2nd line treatment, an inverse relationship between NRTI resistance on 1st line and viral suppression on 2nd line treatment is observed, suggesting that high levels of NRTI resistance and low tGSS (tGSS<2) on 1st line may be markers for adherence and a better predictor of outcome on PI based regimens [14,21,46,47].

The high prevalence of acquired and transmitted NNRTI and NRTI resistance mutations following the rapid scale-up of 1st line EFV regimens [18] has led to recent recommendations for switching to STR that include INSTIs. This data showing inadequate adherence to PI based regimens and high levels of acquired drug resistance to NRTI and

NNRTIs among adolescents and young adults, support implementation of INSTI therapies. The single tablet lower-cost TLD regimen as a 2nd line option may be more acceptable and effective than the current boosted PI regimens, particularly among adolescents and young adults. However, strategies to introduce dolutegravir (DTG) should be considered carefully to avoid exposure to potential DTG monotherapy because of extensive NRTI resistance. The data presented here shows the potential effectiveness of TLD among most (58%) of the participants currently failing 1st line regimens. However, we also show that with a predicted tGSS ≤2 for a TLD regimen, as many as 42% switching to a TLD regimen risk potential DTG monotherapy. An alternative INSTI regimen containing AZT could optimize virologic suppression in this population. However, the availability and cost of AZT, additional toxicities and the unwieldiness of adding a twice a day drug to a compact STR need to be taken into consideration. Further research on a TLD regimen in individuals with a predicted tGSS ≤2 for is required.

The limitations of our study were the small sample size, and the absence of measures of adherence such as PI or tenofovir drug levels in the hair [48] or red blood cells [49-51] which offer objective measures of drug exposure. However, these are balanced by a real-world observation of virologic failures after a median of > 6 years of treatment among adolescents in a resource limited setting. Moreover, these estimates of genotypic susceptibility based on detection of plasma RNA mutations by Sanger sequencing do not include low level variants (<20%) in plasma RNA or resistance mutations in archival proviral DNA [52-54]. Genotyping and the assessment of genotypic susceptibility scores (tGSS) is limited to only a few centers in LMICs.

The increasing prevalence of NNRTI resistance and the lower costs, added potency, reduced side-effects and increased tolerability of TLD as a single tablet regimen all speak to the introduction of this regimen on a broad scale. Based on our observations of multidrug resistance among adolescents and young adults failing 1st line, tenofovir/lamivudine/efavirenz (TLE), we sound a note of caution in the implementation of INSTIs as 2nd line treatment for those failing extensive 1st line NNRTI based treatment. When we optimized a TLD regimens by hypothetically substituting AZT for TDF in the TLD regimen, interestingly 97% (155/160) were susceptible with a tGSS of >2. The reinforcement of enhanced adherence counseling, the addition of potent new agent(s), or as an example, recycling AZT among 1st line virologic failures, may present strategies to reduce the risk of selecting INSTI resistance and maintain virologic suppression. Novel NRTIs or NNRTIs may be required with INSTIs, while the possibility of an AZT and INSTI containing regimen should be considered to optimize virologic suppression.

Conclusion

A significantly higher proportion of adolescents failing NNRTI based 1st line regimens had clinically significant DRMs compared to those failing PI based 2nd line regimens. The introduction of the STR, TLD, with a high genetic barrier to resistance in LMICs, may optimize long term ART treatment success. However, this should be accompanied by frequent virus load monitoring to avoid DTG monotherapy and further limiting options for this vulnerable and hard to treat population.

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Table 1.

Demographic and baseline clinical characteristics of participants by ART regimens.

Characteristics	**All (n=160)**	**1st line ART (n=112)**	**2nd line ART (n=48)**	**P value**
Gender				
Male	83(52%)	60(54%)	23(48%)	0.512
Age at study enrolment/VF median (IQR) years	18(15-19)	17(14-19)	18(17-20)	**0.035** *
Age at HIV diagnosis median(IQR) years	10(7-13)	10(7-14)	11(7-12)	0.866
Age at ART initiation median(IQR) years	11(9-14)	11(9-14)	12(8-14)	0.584
Plasma VL, median(IQR) log_{10} copies/ml	4.51(4.05-4.93)	4.39(4.03-4.81)	4.76(4.52-5.17)	**0.002** *
CD4 at study enrolment/VF, median(IQR) cells/mm^3	197(48-359)	233(64-376)	158(31-307)	0.069
Duration on ART prior to VF, median years(IQR)	6(4-8)	6(4-8)	7(5-9)	0.143
Median duration years(IQR) on 1st line before 2nd line	NA	NA	4.5(2.0-6.3)	NA
Duration on PI based ART prior to enrolment, mean years(±SD) *	NA	NA	3.6(±1.2)	NA

VF virologic failure; IQR interquartile range; ART antiretroviral therapy; VL viral load; PI protease inhibitor; SD standard deviation; NA non-applicable.

* 48 participants were on PIs, these consisted of Atazanavir/ritonavir (ATV/r) in 33 (69%) and Lopinavir/ritonavir (LPV/r) in 15 (31%).

Table 2:

Socio-demographic and clinical characteristics of the 160 participants with available viral sequences.

Characteristics	**All patients (n=160)**	**WT or 1 DRM to 1 class DRM(NNRTI) (n=64)**	**DRMs to 2 classes (NRTI+NNRTI) (n=96)**	**P value**
Age at study enrolment, median(IQR), years	18(15-19)	18(16-20)	17(15-19)	**0.03***
Gender, n (%)				
Male	83(52%)	30(47%)	53(55%)	0.30
Age at HIV diagnosis, median(IQR), years	10(7-13)	11(7-14)	10(7-13)	0.32
Age at ART initiation, median(IQR), years	11(9-14)	12(8-14)	11(9-13)	0.16
Plasma VL, median(IQR) log10 copies/ml at study enrolment/VF	4.51(4.05-4.93)	4.75(4.37-5.09)	4.34(3.92-4.76)	**0.0001***
CD4 at study enrolment/ VF, median cells/mm^3(IQR)	197(48-359)	195(73-367)	197(46-346)	0.44
Duration on ART prior to study enrolment / VF median(IQR), years	6(4-8)	6(4-8)	7(5-9)	0.143

WT wild type; DRM drug resistance mutation; NRTI nucleoside/nucleotide reverse transcriptase inhibitor; NNRTI non- NRTI; IQR interquartile range; VL viral load; SD standard deviation.

Table 3:

Comparison of the frequency of DRMs between 1st and 2nd line recipients

Mutations	Total n (%)	1st line 112(70%)	2nd line 48(30%)	P value
Total DRMs	137(86)	105(94)	32(67)	**P<0.001***
NRTIs	96(60)	80(71)	16(33)	**P<0.001***
M184V	85(53)	73(65)	12(25)	**P<0.001***
K65R	41(25)	38(34)	3(6)	**P<0.001***
K70R	9(6)	6(5)	3(6)	P=0.812
TAMs	38(24)	29(26)	9(19)	P=0.345
NNRTIs	137(85)	105(93)	32(67)	**P<0.001***
K103N	58(36)	51(45)	7(15)	**P<0.001***
Y181C	45(28)	31(27)	14(29)	P=0.82
G190A	57(35)	44(39)	13(27)	P=0.150
V106AM	45(28)	41(36)	4(8)	**P<0.001***
Mutations to NRTIs+NNRTIs	96(60)	80(71)	16(33)	**P<0.001***
PI mutations	5(3)	0	5(10)	#
M46I	4(3)	0	4(8)	#
N88S	2(1)	0	2(4)	#
I84IV	1(1)	0	1(2)	#

DRMs drug resistance mutations; NRTIs nucleoside/nucleotide reverse transcriptase inhibitors; TAMs thymidine analogue mutations; NNRTIs non-NRTIs, PI protease inhibitor; 1st line regimen was comprised of 2NRTIs+ NNRTI; 2nd line regimen was comprised of 2NRTIs + PI; # not computed.

Table 4:

Multi-drug resistance (MDR) among the 5 participants with PI DRMs on 2nd line regimens.

#Number	Age (years)	Gender	Duration On 2nd line ART prior to enrolment (years)	PI DRMs	NRTI DRMs	NNRTI DRMs
1	18	Male	4	M46I, I54V, N88S	M184V, T215Y	Y181C, G190A, H222Y
2	12	Male	4	M46I, I84IV, N88NS	M184V, K70R, T215F, K219Q, D67N, T69D	V108I, Y181C
3	17	Female	4	V82M	M184V, K70R, D67G, T69D, K219Q	A98G, K101E, Y181C, G190A
4	15	Male	1	M46I	M184V, K65R, A62V	A98G, Y181C, G190A
5	21	Female	3	M46I, L90M	M184V, M41L, K70N, L74I, V75T, L210W, T215Y	K101E, E138A, G190A

PI protease inhibitor; NRTIs nucleoside/nucleotide reverse transcriptase inhibitors; DRMs drug resistance mutations; NNRTIs non NRTIs.

Table 5:

tGSS to infer adherence to current regimens and to predict response to subsequent regimens.

Characteristics of all viremic participants (n=160)	**1st line regimen**	**2nd line regimen**
	n=112(70%)	**n=48(30%)**
Current regimens tGSS		
tGSS≤2	104(93%)	6(12.5%)
tGSS>2	8(7%)	42(87.5%)
Predicted subsequent regimens as per the Zimbabwe national ART Guidelines		
tGSS≤2	12 (11%)	0(0%)
tGSS>2	100(89%)	48(100%)
Estimated TLD susceptibility as a subsequent regimen per WHO guidelines		
tGSS≤2	47(42%)	4(8%)
tGSS>2	65(58%)	44(92%)
Estimated TLD by substituting AZT for TDF as subsequent regimens		
tGSS≤2	5(4%)	0(0%)
tGSS>2	107(96%)	48(100%)
Susceptibility to doravirine		
GSS=1	112(100%)	48(100%)
Susceptibility to etravirine		
GSS=1	52(46%)	31(65%)
Susceptibility to rilpivirine		
GSS=1	32(29%)	24(50%)

tGSS total genotypic susceptibility score, ART antiretroviral therapy; TLD tenofovir/lamivudine/dolutegravir; WHO World Health Organization; AZT zidovudine; TDF tenofovir; GSS genotypic susceptibility score; The preferred 2nd line regimens for adolescents (>=10 years) and adults as per the Zimbabwe national ART guidelines is AZT+3TC+ATV/r or LPV/r (if TDF was used in 1st line ART) and TDF+3TC+ATV/r or LPV/r (if AZT was used in 1st line ART). The preferred 3rd line treatment is darunavir/ritonavir (DRV/r) + raltegravir/dolutegravir +2 optimized NRTIs.