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Title: Drug Resistance among Adolescents and Young Adults with Virologic Failure of 1st Line ART and Response to 2nd Line Treatment.

Running title: HIV drug resistance and 2nd line treatment

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Abstract

Introduction: Barriers to sustainable virologic suppression (VS) of HIV infected adolescents and young adults include drug resistance mutations (DRMs) and limited treatment options which may impact the outcome of 2nd line antiretroviral therapy (ART).

Methods: We sequenced plasma viral RNA from 74 adolescents and young adults (16-24 years) failing 1st line ART at Newlands Clinic, Zimbabwe between October 2015 and December 2016. We evaluated 1st line nucleoside reverse transcriptase inhibitor (NRTI) susceptibility scores to 1st and 2nd line regimens. Boosted PI based ART was provided and viral load (VL) monitored for ≥ 48 weeks. Fisher's exact test was used to evaluate factors associated with VS on 2nd line regimens, defined as VL < 1000 copies/ml (VS_{1000}) or < 50 copies/ml (VS_{50}).

Results: The 74 participants on 1st line ART had a median (IQR) age of 18 (16-21) years and 42 (57%) were female. The mean (±SD) duration on ART was 5.5 (±3.06) years and the median (IQR) $\log_{10}$ VL was 4.26 (3.78 - 4.83) copies/ml. After switching to a 2nd line PI regimen, 88% suppressed to < 1000 copies/ml and 76% to < 50 copies/ml at ≥ 48 weeks. A new NRTI was associated with increased VS_{50} (p=0.031).

Conclusions: These 74 adolescents and young adults failing 1st line ART demonstrated high levels (97%) of DRMs despite enhanced adherence counseling. Switching to new NRTIs in 2nd line improved VS. With the widespread adoption of generic dolutegravir, lamivudine and tenofovir combinations in Africa, genotyping to determine NRTI susceptibility may be warranted.

Introduction

Though there has been an increase in access to antiretroviral therapy (ART) in sub-Saharan Africa (SSA) and other low and middle-income countries (LMICs), HIV and AIDS remains a major public health burden. Zimbabwe has an HIV prevalence of 14.6% among the adult population (15-64 years) and approximately 4.7% among adolescents and young adults (15-24 years) [1]. The roll out of ART has resulted in a significant reduction in HIV/AIDS-related morbidity and mortality [2,3] and has transformed the disease from being life-threatening into a chronic and manageable condition among individuals with good adherence. To meet the WHO/UNAIDS goals of universal access to treatment to eliminate HIV by 2030, Zimbabwe adopted a policy of test and treat in 2016 [4].

Public health ART, as recommended by the WHO is possible because of access to low cost generic fixed dose combinations (FDC) [5–7]. Antiretroviral therapy, clinical support and adherence counseling to maintain virologic suppression (VS; the third 90) is particularly challenging for adolescents and young adults living with HIV [8–11]. In the last few years in Africa, single dose daily combination therapy in both 1st and 2nd line treatment has become more tolerable, acceptable and effective than multi-pill regimens [12–14].

A substantial change in public health ART in Africa is the roll-out of FDC of tenofovir, lamivudine and dolutegravir (TLD) recommended by WHO for 1st and 2nd line treatment [15]. Among adolescents and young adults, TLD may be a more economical, convenient and better-tolerated regimen. However, the effectiveness of TLD as a FDC may be reduced by accumulated nucleotide reverse transcriptase inhibitor (NRTI) resistance mutations.

Here, we evaluated adolescents and young adults who had received an enhanced adherence counseling (EAC) for virologic failure (VF) after treatment with recommended, available FDCs. Patterns of HIV drug resistance mutations (HIV DRMs) and 1st line NRTI drug susceptibility which may impact virologic response to 2nd line protease inhibitor (PI) based ART were estimated. As these adolescents and young adults were switched to 2nd line treatment and followed for $\geq$ 48 weeks, we evaluated the relationship between DRMs, genotypic susceptibility and the impact of DRMs on viral load suppression (VLS).

Materials and Methods

Study design

This was a retrospective analysis of 74 adolescents and young adults failing recommended 1st line treatment from a cohort of 726 HIV infected adolescents and young adults receiving care and treatment at Newlands Clinic Harare, Zimbabwe between October 2015 and December 2016. Genotyping was performed after VF (defined as viral load, VL>1000 copies/ml) was recorded, following weekly enhanced adherence counseling group intervention (EACGI) for 12 weeks. Second-line treatment was initiated and six-monthly VL measures were continued after the switch. (Figure 1).

Study setting

Newlands Clinic is a private voluntary organization founded in Harare, Zimbabwe in 2004 with the aim of providing comprehensive ART services to people living with HIV from poor communities in and around Harare. Nurses with counseling qualifications provide routine adherence and psychosocial counseling at each clinic visit [16]. For HIV infected adolescents and young adults with VF on ART, an EACGI is provided through a psychological and mental health approach. The EACGI accommodates 8-15 young people as 12 weekly one and half hour-long sessions to facilitate adherence and provide peer support though weekly group meetings. The clinic provides 1st and 2nd line ART as recommended by the Ministry of Health and Child Care ART program. First-line ART was provided as FDCs of tenofovir disoproxil fumarate/lamivudine (TDF+3TC), abacavir/lamivudine (ABC+3TC) or zidovudine/lamivudine (AZT+3TC). These were combined with either efavirenz (EFV) or nevirapine (NVP). Second-line treatment was with a PI, either ritonavir boosted atazanavir (ATV/r) or ritonavir boosted lopinavir (LPV/r) with TDF +3TC or ABC+3TC or AZT+3TC.

Laboratory methods

The plasma VL were obtained in real time for clinical management with the Cobas Ampliprep/TaqMan48 HIV-1 quantification system (Roche Diagnostics, USA). For HIV-1 drug resistance genotyping, ribonucleic acid (RNA) was extracted using the QIAMP Viral RNA kit (Qiagen, Germany), as per manufacturers' instructions. The extracted RNA samples were reverse transcribed and amplified using the Southern African Treatment Resistance Network (SATuRN) protocol on the PTC-200 Peltier thermocycler (MJ research, Temecula,

CA USA) at the University of Zimbabwe, College of Health Sciences. The SATuRN/Life Technologies genotyping method is a fully integrated protocol for surveillance and monitoring of drug resistance. The SATuRN protocol was designed to be an affordable protocol implementing mostly open source and open access bioinformatics resources for the interpretation of HIV drug resistance [17]. The amplicons generated were sequenced by Sanger sequencing at Molecular Cloning Laboratories, San Francisco, California, USA. The quality of the consensus sequences generated was verified by phylogenetic tree reconstruction in Geneious software, version 8 (http://www.geneious.com) [18]. HIV drug resistance mutations were determined using the Stanford HIV drug resistance database (HIVdb) (http://hivdb.stanford.edu) [19] and the HIV-1 subtypes with the REGA HIV subtyping tool [20].

Virologic analysis and drug resistance mutations

Virologic suppression was categorized as VL<50 copies/ml (VS_{50}) and VL between 50-1000 copies/ml (Low-level viremia, LLV) at $\geq$ 48 weeks. For clinical management of the participants, the WHO (Public Health ART) guidelines define VS, as VL< 1000 copies/ml (VS_{1000}) and VF as VL >1000 copies/ml on two consecutive VL measurements within a 3-month interval with adherence support between measurements [21].

Genotypes were classified as wild-type, non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations only, or 2 class resistant with mutations to both NNRTI and NRTI drugs. Susceptibility to 2nd generation NNRTIs etravirine (ETR) and rilpivirine (RPV) was estimated based on detection of L100I, K101EP, Y181CIV, Y188L, G190EQ and M230L as major resistance mutations [22,23]. Major NRTI, NNRTI and PI mutation sites/codons were determined using the Stanford HIVdb. Total genotypic susceptibility scores were calculated for 1st line regimens (GSS1). For each NRTI and NNRTI drug prescribed, a GSS value of 1 was assigned if resistance was not identified, a value of 0.5 was assigned to intermediate resistance and 0 was assigned when mutations predicted high-level resistance.

Total genotypic susceptibility scores were also calculated for 2nd line regimens (GSS2). For this, 1st line NRTI genotypic results were used and a value of 1.5 was assigned to boosted PIs (bPIs). These GSS2 were based on ritonavir bPIs with 2 NRTIs; ABC or TDF or AZT and

3TC. Total genotypic susceptibility scores were calculated based on the number of 'active' drugs prescribed using the Rega Institute HIV algorithm on the Stanford HIVdb [24]. The arithmetic sum of the individual scores for the specific drugs prescribed provided the total GSS.

Statistical analysis

Descriptive statistics were used to summarize the baseline demographic and clinical characteristics as well as DRMs among the participants. Fisher's exact test, Kruskal-Wallis H test or Anova test and Student's t test were used to evaluate factors (age, ART duration, NRTI mutations on 1st line failure, GSS and mean VL at 24 and 48 weeks on 2nd line ART) associated with VS on 2nd line ART, categorized as VL<50 copies/ml (VS_{50}) and VL between 50-1000 copies/ml (LLV). Significance levels were set at P= 0.05. All statistical analysis was performed using Stata version 14 (StataCorp LP, College Station, Texas, USA; 800-STATA-PC).

Ethics approval and consent to participate

Ethical approval was obtained from the Biomedical Research Training Institute-Institutional Review Board (AP142/2017) and the Medical Research Council of Zimbabwe (MRCZ/E/191). Permission to conduct the research was approved by the Newlands Clinic's research board.

Results

Participant Characteristics

Among 726 adolescents and young adults (16 - 24 years) who were receiving 1st line ART at the Newlands Clinic between October 2015 and December 2016, 74 (10%) had a confirmed VL > 1000 copies/ml after 12 weeks of EACGI. The 74 participants, median (IQR) age of 18 (16 – 21) years were receiving treatment with single daily dose regimens; TDF+3TC+EFV/NVP 54 (73%) and ABC+3TC+EFV/NVP 3 (4%), or twice daily regimens of AZT+3TC+EFV/NVP 17(23%). Their demographic and clinical characteristics are shown in (Table 1).

Of the 74 participants failing NNRTI based 1st line ART, 62 (84%%) were switched to a bPI based 2nd line ART, one (1%) remained on 1st line ART and suppressed at ≥ 48 weeks of

follow up. Among the 62 participants switched to a bPI based 2nd line ART, 60 (82%) were followed up to ≥ 48 weeks while 2 (3%) participants had their last VL measured at 24 weeks. The remaining 11 (15%) participants left clinical care and were lost to follow up (Figure 1).

Drug resistance at 1st line failure.

HIV DRMs were detected in 72/74 (97%) participants after EAC and confirmed VF. The frequency of NNRTI DRMs is shown in Figure 2a. NRTI DRMs were found in 84% with the most common being M184V (72%), K65R (41%) and thymidine analogue mutations (32%) (Figure 2b). Additional NRTI mutations, L74V and Y115F were identified among all 3 participants on ABC at 1st line ART failure (Figure 2b). The combination of K65R and M184V, associated with ABC, TDF and 3TC resistance was identified in 28/74 (38%) participants. NRTI and NNRTI DRMs (2 class resistance) were detected in 62/74 (84%). Susceptibility to new 2nd generation NNRTIs, ETR and RPV, was reported as 31(42%) to ETV and 19(35%) to RPV.

NRTI drug switching from 1st to 2nd line ART regimen.

After 1st line treatment failure, sixty two of the 74 (84%) participants switched to a 2nd line treatment which included continuation of 3TC and either a change to a new or recycled NRTI and ATV/r (54/62) or LPV/r (8/62). Genotyping results were not always available in real time to health care providers. In 23/60 (38%) cases, participants remained on the same NRTI combination. This was based on a reluctance to use AZT+3TC (a recommended NRTI combination after failure of a TDF based regimen) [25]. The 37 who switched to a new NRTI backbone demonstrated VS_{50} in 86% (32/37) compared to 61% (14/23) who continued on the same NRTI backbone (p=0.031) (Fischer's exact test). The 37 participants who switched to a new NRTI backbone were most often switching from TDF to ABC (See supplementary Figure). Upon switching to a bPI regimen, significant differences were not observed in VS_{50} at ≥ 48 weeks; 58% (11/19) of the participants who remained on TDF compared to the 76% (19/25) who changed to a new NRTI backbone respectively (p=0.327) (Chi-Square test) (Supplementary Figure).

The detection of M184V mutation at 1st line treatment failure was significantly associated with VS_{50} (87%) on 2nd line treatment compared to LLV (57%) and VF (57%) (p=0.038) (Kruskal-Wallis H test). The detection of K65R was more frequent in both VS_{50} (44%) and LLV (71%) categories compared to VF (14%) (p=0.106) (Kruskal-Wallis H test).

Comparing genotypic susceptibility among these three categories on their 1st line regimen, a significant greater susceptibility was seen among VF (median GSS1 of 1) compared to LLV (median GSS1 of 0.5) and those who achieved VS_{50} (median GSS1 of 0.5) (p= 0.042) (Kruskal-Wallis H test). No significant difference was seen on 2nd line regimens among VF (median GSS2 of 2.5), LLV (median GSS2 of 2) and those who achieved VS_{50} (median GSS2 of 2.25) (p=0.154) (Kruskal-Wallis H test) (See Table 2). Among the 46 who achieved VS_{50} on 2nd line ART, all 8 (100%) participants on LPV/r achieved VS_{50} compared to the 38 (70%) who achieved VS_{50} on ATV/r (p=0.10) (Fisher's exact test)

Discussion

Adolescents and young adults globally pose challenges to HIV treatment programs, particularly in LMICs where weak health systems, lack of support for adherence and retention and limited access to VL monitoring contribute to VF [26,27]. While ART has been effective in decreasing AIDS-related deaths among adults, benefits among adolescents and younger adults have been limited [21]. In SSA, poor adherence and loss to follow up among adolescents and young adults have been reported compared to older individuals living with HIV [28–30]. In 2015 in Zimbabwe, VF rates were nearly 40% among 15-19 year olds [1,31]. Policy and guidelines support service delivery to improve adherence, viral suppression and clinical outcomes among adolescents [32]. Similarly, the WHO guidelines encourage EAC before switching to a new regimen [21].

In a clinic in Harare, we documented the patterns of DRMs and evaluated 2nd line treatment outcomes among HIV-1 infected adolescents and young adults who had failed a 1st line ART regimen despite EAC for 12 weeks. Genotyping after failure demonstrated that 97% had at least one clinically significant HIV DRM and resistance to two drug classes (NNRTI and NRTI) was identified among 62/74 (84%). Although

DRMs were common after 1st line ART failure , switching to 2nd line bPI based ART demonstrated suppression to < 1000 copies/ml in 53/60 (88%), consistent with studies among HIV infected children and adults where VS in response to 2nd line regimens range from 70% to 95% after 48 weeks of follow-up [33–39].

We observed that overall, 1st line ART failures who changed to a new NRTI were more likely to achieve VS on 2nd line regimens compared to those who continued on the same NRTI (p=0.031) (Fisher's exact test). Genotyping before the switch to 2nd line regimens demonstrated M184V (72%) and K65R (41%) at 1st line ART failure. The WHO recommendations for NRTI switching suggest that 1st line TDF recipients should be switched to AZT or ABC [21]. Based on the presence of a K65R mutation, AZT is the preferred NRTI [40–43]. Here, continuation of TDF from 1st to 2nd line regimens resulted in 58% suppression, while switching from TDF to AZT or ABC demonstrated modestly higher VS_{50} in 2nd line regimens, 80% and 73% respectively.

Our study identified a small, but interesting subgroup with persistent LLV and with high levels of NRTI drug resistance. Low-level viremia (from 50-1000 copies/ml) may be a precursor of VF, raising concerns about the long-term durability of 2nd line treatment. Hence, optimization of NRTI and intensification of treatment in patients with persistent LLV has been considered [44–46] to achieve VS (VL<50 copies/ml). In a recent review, Ryscavage et al (2014) concluded that resistance genotyping should be considered in patients with persistent LLV when feasible, and treatment should be modified if resistance is detected [47].

Alternatively, ATV/r in 2nd line regimens may also be linked to LLV and risk of VF. Recent systematic reviews of data comparing drugs used for 2nd line ART reported low- to very-low-quality evidence for using ATV/r or DRV/r (once-daily) over LPV/r (twice-daily) as the preferred bPI option (https://www.who.int/hiv/topics/treatment/)

[48]. Interestingly, all 8/8 (100%) participants switched to LPV/r based treatment achieved VS_{50} compared to 38/46 (70%) of those switched to ATV/r. Although, this finding was not statistically significant (p=0.10).

Genotypic assessment of drug susceptibility and the prediction of treatment outcome has been well studied in resource rich settings [49–51]. However, in 2nd line studies in LMICs, increased genotypic susceptibility to NRTI drugs and fewer mutations has been associated with VF of 2nd line as documented in adults [52–56]. This provides indirect evidence that those failing 2nd line regimens may be inconsistently adherent. The participants, studied here, who failed 2nd line regimens with high VL had a distinct lack of NRTI mutations. Additional barriers to VLS in adolescents include drug toxicity and intolerance, psychological problems (depression, anxiety, trauma) and social barriers including stigma have been linked to poor treatment adherence in adolescents and young adults [57–60].

Important limitations of the study are that genotyping results after 1st line ART failure were not always available in real time to health care providers for the optimization of ART before switching to 2nd line regimens. In addition, limited adherence and ATV/r as 2nd line ART may have reduced the rate of VS_{50}. Hence, the contribution of 1st line NRTI resistance to 2nd line treatment are only estimates. Other limitations of our study include the small sample size and the follow up of 2nd line treatment for only ≥ 48 weeks. The results suggest that genotyping results after 1st line ART failure are of limited value and that non-adherence most likely accounts for high level VF. The presence of both K65R and M184V among those treated with TDF or ABC and 3TC does provide evidence for genotypic resistance and virtual PI monotherapy in many 2nd line recipients. Long-term follow-up is needed to confirm whether virologic suppression to < 50 copies is sustained.

In African Public Health treatment programs, 2nd line PI based daily FDC treatment is provided to a growing population of highly NRTI and NNRTI experienced patients [15,25]. Here, VS to levels <1000 and < 50 copies/ml was found in 88% and 76% respectively at ≥ 48 weeks. High rates of VS similar to those reported in other studies provide reassurance of effective VS on a PI based 2nd line ART [35–37]. However, when the NRTI backbone is not optimized after failure of 1st line ART, concern is raised about the durability of 2nd line treatment, particularly with ATV/r as the sole active agent in a population where many decades of continued treatment are anticipated. The costs, long-term adherence to bPIs, together with the increasing prevalence of NNRTI DRMs support the recent recommendations to adopt Integrase Strand Transfer Inhibitors such as dolutegravir (DTG)

[15]. The single tablet lower-cost TLD with a high genetic barrier to resistance may be more acceptable and effective than the current bPI and NNRTI regimens [15,61–63] . This may extend suppressive ART treatment, particularly among adolescents and young adults failing current 1st and 2nd line regimens with multi-drug resistance. However, 2nd and 3rd line ART including DTG require active adherence support and frequent VL monitoring to prevent selection of resistance to DTG, further limiting options for this vulnerable and hard to treat population.

Conclusion

Clinically significant HIV DRMs were frequently detected (97%) among adolescents and young adults failing 1st line ART. When switched to 2nd line ART, VS was higher in participants who received a new NRTI backbone as expected. Genotyping adds little to the choice of 2nd line NRTI regimens, except that the absence of 1st line NRTI resistance appears to be a predictor of poor adherence and subsequent ART failure. The roll-out of TLD may improve ART treatment success in LMICs particularly among this hard to treat population.

Sequence Data

HIV-1 drug resistance sequence data are available on Genbank (accession numbers MK893083 - MK893156).

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Authors' disclosure

All authors have no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

Authors' contributions

DK, CC, TS, TM, JM and VK conceived the study. TS, TM, BV and VK supervised data collection and laboratory testing. JM, DK, VK and TMa performed data analysis. JM, TS, TM,

CC, BV and DK critically reviewed and finalized the paper. All authors contributed to subsequent drafts and reviewed and approved the final manuscript.

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Table 1: Participant characteristics at the time of the study

Characteristics	Participants (N=74)
Age in years, median(IQR)	18 (16 – 21)
Gender, n (%)	
Female	42 (57)
Male	32 (43)
Viral load (log_{10} copies/ml), median(IQR) at 1st line failure	4.26 (3.78 - 4.83)
CD4 count (cells/mm^3), median (IQR)	336 (163 - 444)
Duration on ART, mean years (± SD)	5.5 (± 3.0)
1st line ART regimens, n (%)	
TDF + 3TC + EFV/NVP	54 (73%)
AZT + 3TC + EFV/NVP	17(23%)
ABC +3TC + EFV/NVP	3 (4%)
2nd line ART regimens at switch, n (%)	
TDF +3TC+ATV/r	23 (37%)
AZT+3TC+ATV/r	9 (15%)
AZT+3TC+LPV/r	2 (3%)
ABC+3TC+ATV/r	22 (35%)

ABC+3TC+LPV/r	6 (10%)

IQR Inter quartile range; SD Standard deviation; TDF Tenofovir disoproxil fumarate; 3TC Lamivudine; EFV Efavirenz; NVP Nevirapine; AZT Zidovudine; ABC Abacavir; ATV/r Atazanavir/ritonavir; LPV/r Lopinavir/ritonavir.

Table 2: Characteristics associated with virologic outcome after switch to 2nd line ART.

Characteristics	**VS (VL<50cp/ml) n=46**	**LLV (50-1000cp/ml) n=7**	**VF (VL≥1000cp/ml) n=7**	**P value**
Age in years, median (IQR)	18(16-22)	17(16-20)	16(15-21)	0.686
ART duration in years, median (IQR)	6(3 -8)	4(2-6)	7(5-10)	0.241
M184V, n (%) at 1st line failure	40(87%)	4(57%)	4(57%)	**0.038***
K65R, n (%) at 1st line failure	20(43%)	5(71%)	1(14%)	0.106
Median(IQR) GSS1	0.5 (0.5-1)	0.5(0.5-0.5)	1(1-2)	**0.042***
Median(IQR) GSS2	2.25(1.50-2.50)	2(2.00-3.00)	2.5(2.50-3.50)	0.154
Median(IQR) viral load at 24 and 48 weeks on 2nd line ART	1.46 (1.35-1.73)	2.71(2.24-3.12)	4.78(3.28-5.30)	**P<0.001***

VS Virologic suppression (VS<50 cp/ml), LLV Low level viremia (50-1000 cp/ml), VF Virologic failure (VL≥1000 cp/ml), M184V mutation causing high level resistance to lamivudine (3TC) and emtricitabine (FTC), K65R Mutation causing high level resistance to tenofovir (TDF) and decrease susceptibility to abacavir (ABC), Median viral load at 24 and 48 weeks while on 2nd line PI based regimen.

Figure legends

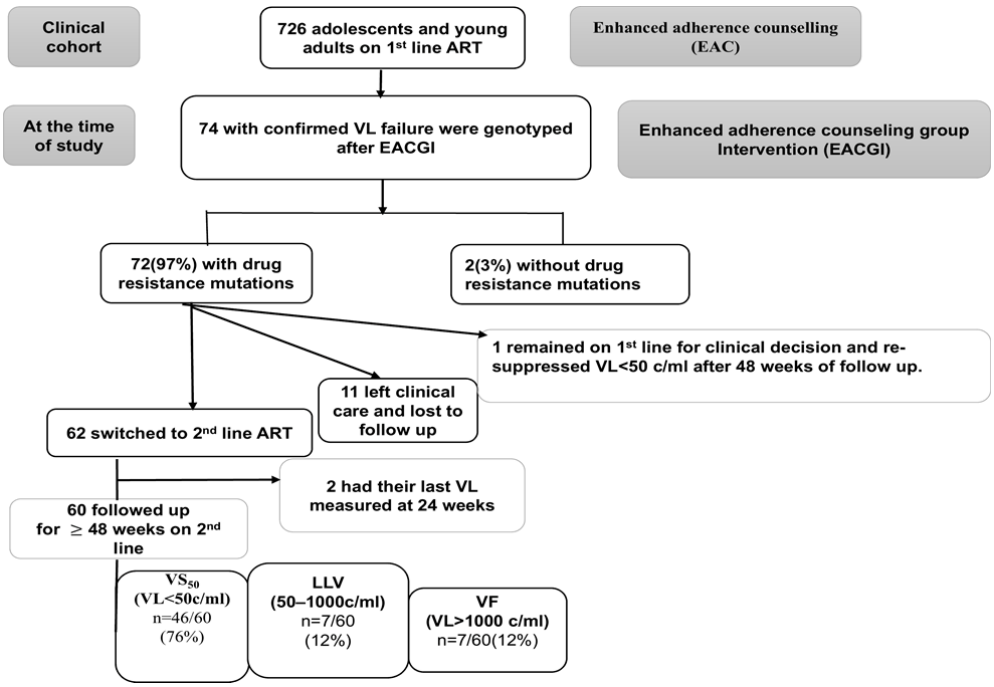


Figure 1: Summary of the participants at 1st line ART and outcomes after switch to 2nd line ART (ART Antiretroviral therapy, EAC Enhanced adherence counseling, VL Viral load, EACGI Enhanced adherence counselling group intervention, bPI Boosted protease inhibitor, VS Virologic suppression, VS_{50} virologic suppression VL<50 copies/ml, LLV Low level viraemia, VF Virologic failure VL>1000 copies/ml).

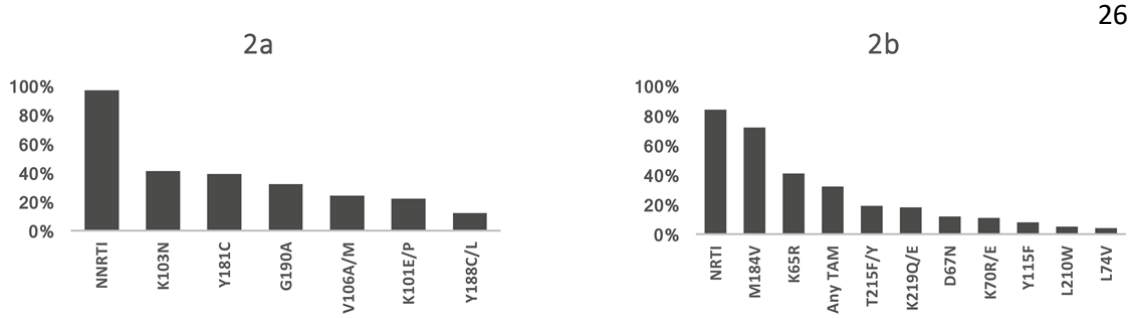
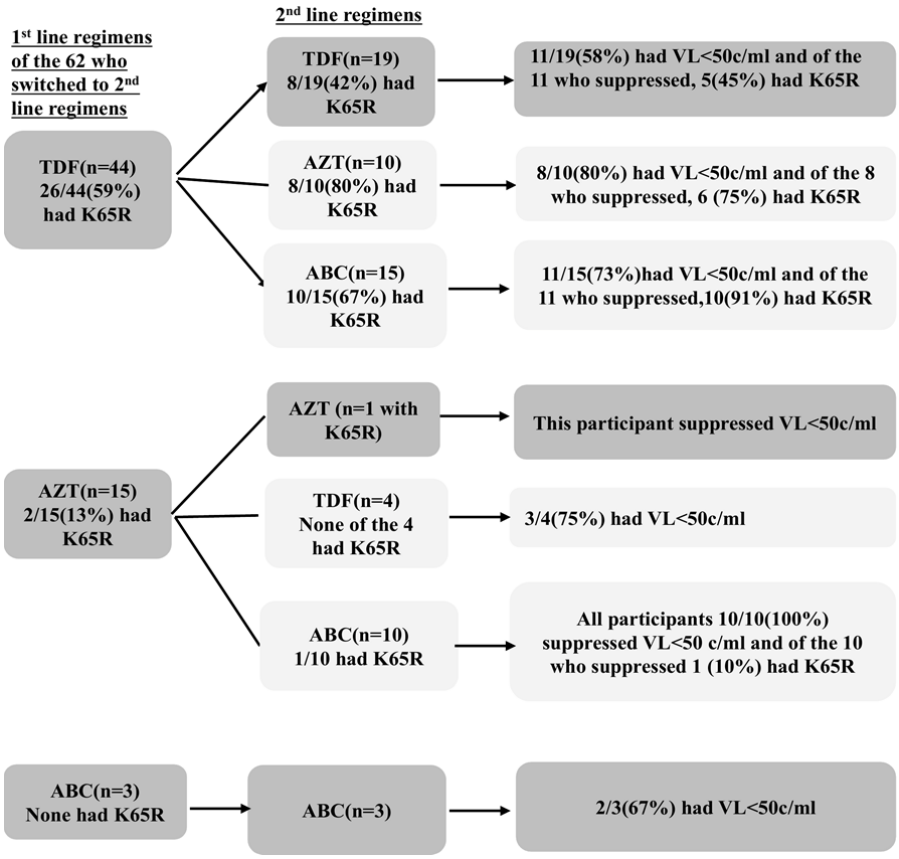


Figure 2a and 2b: Drug resistance mutations among the 74 participants failing 1st line ART (2a: Frequency of observed non-nucleotide reverse transcriptase inhibitors (NNRTI) drug resistance mutations; 2b: Frequency of observed nucleotide reverse transcriptase inhibitors (NRTI) drug resistance mutations).



Supplemental Figure: NRTI backbone switched from 1st line to 2nd line ART among the 62 participants (NRTI Nucleotide reverse transcriptase inhibitors; TDF Tenofovir disoproxil fumarate; AZT Zidovudine; ABC Abacavir; VL Viral load. Some participants remained on the same NRTI backbone after switching to PI based 2nd line regimen and others switched to a new NRTI backbone).