ORIGINAL ARTICLE

Drug Resistance in HIV Following First-line ART Failure: Insights from a Cross-sectional Study in India

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Abstract

Introduction: Our study assesses human immunodeficiency virus (HIV) drug resistance (HIVDR) in patients failing first-line (1L) antiretroviral therapy (ART) with dual nucleoside analog reverse transcriptase inhibitor (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) regimens in India.

Methods: In this cross-sectional study, consecutive HIV-1–infected patients aged 13 years or older, failing 1L ART after at least 12 months exposure, underwent HIV genotyping and drug resistance testing (DRT) using the ViroSeq™ HIV-1 Genotyping System and the Stanford HIV-1 Database, with HIVDR classification based on a penalty score of ≥30.

Results: Among 115 eligible participants, 110 underwent DRT, revealing efavirenz (EFV) or nevirapine (NVP) resistance rates of 85.3% (n = 93/109) and 87.2% (n = 95/109), respectively, and substantial cross-resistance to rilpivirine (RPV) (37.6%, n = 41/109), etravirine (ETV) (30.3%, n = 33/109), and doravirine (DOR) (60.5%, n = 66/109).

The cohort was categorized into 3 groups based on their previous ARV drug exposure: group A (36.4%, n = 40) with prior TA exposure (AZT or d4T) but no TFV exposure; group B (19.1%, n = 21) with prior nonconcomitant exposure to both TAs and TFV; and group C (44.5%, n = 49), exposed to TFV only. Despite group B's 1L ART regimen failure with TFV, the prevalence of AZT resistance was similar (difference in proportions, ΔP: 14.6%, p = 0.277) between group A [57.5% (n = 23/40)] and group B [42.9% (n = 9/21)].

TFV resistance was comparable (ΔP: 0.8%,p = 0.947) between group A (32.5%, n = 13/40) and group B (33.3%, n = 7/21), despite group A's lack of TFV exposure, and was also similar to the TFV-only–exposed group (group C: 38.8%, n = 19/49).

Regarding distinct DRM patterns, the prevalence of K65R DRM was higher (ΔP: 22.4%, p = 0.060) among TFV-only–exposed patients (group C: 36.7%, n = 18/49) compared with PLH exposed to both TAs and TFV (group B: 14.3%, n = 3/21), whereas multiple TAMs occurred at similar rates (ΔP: 12.1%, p = 0.367) among TA-exposed patients [group A: 55.0% (n = 22/40) vs group B: 42.9% (n = 9/21)].

Conclusion: The research provides insights into the complexities of HIVDR, emphasizing the interplay of resistance patterns and the role of drug exposure history, especially in the context of resistance to TFV and second-generation NNRTIs.

Clinical significance: Ensuring adequate drug exposure history in patients can prevent poor outcomes in PLH being treated with ART due to resistance. Resistance profiling is especially relevant following first-line ART failure.

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Introduction

A 0.22% prevalence of human immunodeficiency virus (HIV) in India emphasizes the imperative for effective antiretroviral therapy (ART) strategies.[1,2] Dolutegravir (DTG) as a first-line (1L) treatment has enhanced efficacy and reduced resistance,[3] but a considerable number of persons living with HIV (PLH) still use non-nucleoside reverse transcriptase inhibitor (NNRTI) regimens.

NNRTIs, lauded for their affordability and effectiveness, historically formed the cornerstone of HIV elimination efforts.[4,5] Concerns surrounding NNRTI resistance, especially with suboptimal adherence, have underscored the need for alternative approaches.[6] Understanding NNRTI resistance remains relevant, even as their role shifts in primary regimens, as they continue to be part of alternative 1L strategies recommended by the World Health Organization (WHO).[7]

Nucleoside analog reverse transcriptase inhibitors (NRTIs), particularly tenofovir (TFV), remain crucial backbone agents even with DTG-based 1L strategies. Moreover, their enduring relevance, unlike NNRTIs, extends into the realm of second-line (2L) ART, highlighting the complexities of NRTI resistance beyond 1L therapy.[7,8] The potential impact of TFV resistance on future treatment outcomes necessitates thorough evaluation.

The scope of NNRTIs has gained renewed vigor with second-generation NNRTIs, offering the potential for tailored therapies for both ART-naïve PLH and those facing treatment failure across multiple drug classes.[9–12] However, their susceptibility to resistance, including cross-resistance from first-generation NNRTI drug-resistance mutations (DRMs),[10,13,14] is a less explored aspect that is crucial, especially in cohorts heavily exposed to NNRTIs.

Our study comprehensively evaluates HIV drug resistance (HIVDR) in a sizable cohort of PLH who failed on a 1L dual NRTI + NNRTI regimen. Our particular focus is on resistance to TFV and second-generation NNRTIs within this context.

Methods

Study Design, Subjects, and Sample Size

This institutional-based cross-sectional study included consecutive HIV-1-infected patients aged ≥13 years, on 1L ART for ≥12 months between July 2019 and May 2021. Inclusion criteria were: (1) PLH on a 1L ART regimen with a dual NRTI backbone [lamivudine (3TC) or emtricitabine (FTC) along with tenofovir (TFV) or zidovudine (AZT)] and a single NNRTI core agent [efavirenz (EFV) or nevirapine (NVP)], (2) failing 1L ART [defined as 2 consecutive viral load (VL) measurements ≥1,000 cp/mL, with 6–8 weeks of enhanced adherence in between], and (3) a most recent VL meeting the threshold for HIV sequencing (VL ≥2,000 cp/mL).

Exclusions comprised: (1) PLH on 2L ART, (2) prior exposure to abacavir (ABC),

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integrase strand transfer inhibitors (INSTI), or boosted protease inhibitors (b/PI), (3) individuals denying consent, and (4) those with sequencing failures. The study patients' HIV-1 management adhered to WHO recommendations from 2016, involving dual NRTI + NNRTI as 1L ART, with EFV as the preferred NNRTI.[15,16] Notably, second-generation NNRTIs were not available in India during the study period. Consequently, none of the patients were exposed to doravirine (DOR), rilpivirine (RPV), or etravirine (ETV). The study received ethical approval from the Institutional Ethics Committee.

According to WHO guidelines, to assess clinic-level HIVDR 12 months after ART initiation at a sentinel site, screening 115 patients, allowing for a 20% attrition rate due to missing data and genotyping failures, effectively results in a sample size of 96 participants consecutively failing 1L ART.[17,18]

Genotype Sequencing and Sequence Analysis

Samples with VL ≥2,000 cp/mL underwent HIV genotyping and drug resistance testing (DRT) using the ViroSeq™ HIV-1 Genotyping System [reverse transcriptase (RT) gene (codons 1–240), protease gene (codons 1–99), and integrase-encoding regions]. PI, NRTI, and NNRTI DRMs were identified using ViroSeq software™ in conjunction with the updated (version 9.1, update 2022-06-02) Stanford HIV-1 Database (hivdb.stanford.edu).[19]

HIVDR interpretation is based on penalty scores: susceptible (Sus), potential low-level resistance (<15; Pot-LLR), low-level resistance (15–29; LLR), intermediate resistance (30–59; IR), and high-level resistance (≥ 60; HLR).[19] In cases of nucleotide mixtures, these were considered mutant based on their impact on encoded amino acids.[20] Multidrug resistance referred to resistance to ≥2 drugs from the 1L ART regimen.[21,22] To classify patients failing 1L ART as having HIVDR in this study, a penalty score of ≥30 (IR or HLR) was used.

Data Collection and Statistical Analysis

Demographic and clinical data were collected through interviews and treatment record reviews using semistructured case report forms. Blood samples were collected for plasma VL, CD4+ cell count, and DRT. Screening for opportunistic infections (OIs) and relevant investigations were conducted. All patients provided informed consent and were informed of the results.

As general considerations, categorical variables are presented as percentages with 95% confidence intervals (CI) (1-sample binomial test, Clopper–Pearson exact method). Continuous variables are described with either mean ± standard deviation (SD) or median and interquartile range (IQR), as applicable. Between-group comparisons were made using the Chi-squared test or Fisher exact test, depending on expected counts. Statistical significance was set at $p < 0.05$ (2-tailed), with corrections for multiple comparisons where necessary. Statistical analyses were performed using Excel for Microsoft Office 365 (Microsoft Corporation, Redmond, WA, USA), Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, version 23.0; IBM Corp., Armonk, NY, USA), and Prism GraphPad 8.1 (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

Results

Demographic and Baseline Characteristics, and First-line Antiretroviral Therapy Regimens

Out of 115 eligible participants, 110 underwent HIV genotyping and DRT (Fig. 1). The median age of the study participants was 41 (IQR 13; range 13–78) years, with 24.5% (n = 27) females. The median 1L ART duration was 81 (IQR 79; range 12–199) months (Table 1).

DRMs were anticipated to align with the patients' exposure to specific antiretroviral (ARV) agents, leading to 3 groups based on their ARV drug exposure: group A, prior TAs (AZT or d4T) but no TFV exposure (36.4%, n = 40); group B, prior nonconcomitant exposure to TAs and TFV [TA exposure for 40 (median) months followed by TFV exposure for 63 (median) months] (19.1%, n = 21); and group C, TFV-only exposure (44.5%, n = 49). Demographics and baseline characteristics are shown in Table 1.

The predominant HIV-1 genotype was C (genotype C: 99.1%, n = 109; genotype A: n = 1) (Table 1). Overall, 13.8% (n = 15/109) exhibited complete susceptibility across drug classes (NNRTI, NRTI, and PI), with varying percentages across exposure groups: group A, 15.0% (n = 6/40); group B, 9.5% (n = 2/21); and group C, 14.6% (n = 7/48).

Resistance to Non-nucleoside Reverse Transcriptase Inhibitors

Predicted susceptibility to NNRTIs after 1L ART failure: One (out of 110) sample was excluded due to missing data. NNRTI resistance (IR or HLR to at least 1 NNRTI) was detected in 86.2% [(n = 94/109), 95% CI: 78.3–92.1] of PLH failing 1L ART. Overall, 22.9% [(n = 25/109), 95% CI: 15.4–31.9] of PLH displayed resistance to all NNRTIs.

Resistance to EFV and NVP: EFV and NVP resistance were observed in 85.3% [(n = 93/109), 95% CI: 76.4–90.7] and 87.2% [(n = 95/109), 95% CI: 78.5–92.2] of PLH, respectively (Fig. 2).

Resistance to second-generation NNRTIs: Cross-resistance (IR or HLR) to RPV occurred in 37.6% (n = 41/109, 95% CI: 28.5–47.4), to ETV in 30.3% (n = 33/109, 95% CI: 21.8–39.8), and to DOR in 60.5% (n = 66/109, 95% CI: 50.2–69.2) (Fig. 2).

ETV resistance was twice as frequent [difference in proportions (ΔP): 20.7% (95% CI: 0.2–41.3), p = 0.038] in NVP-exposed compared with EFV-exposed patients. A similar trend [ΔP: 29.8% (95% CI: 8.7–50.8), p = 0.005] was observed for RPV resistance in NVP-exposed compared with EFV-exposed patients. In contrast, DOR resistance was markedly more frequent [ΔP: 43.6% (95% CI: 24.5–62.6), $p < 0.001$] in EFV-exposed [74.6% (n = 47/63), 95% CI: 63.9–85.4] than in NVP-exposed [31.0% (n = 9/29), 95% CI: 15.3–50.8] patients (Table S1).

Patterns of NNRTI DRMs: The most common NNRTI DRM was K103N/S [49.5% (n = 54/109), 95% CI: 38.9–58.4], followed by V106M [29.4% (n = 32/109), 95% CI: 20.2–37.9], G190A [16.5% (n = 18/109), 95% CI: 10.1–24.8], and Y181C [13.8% (n = 15/109), 95% CI: 7.9–21.7] (Fig. 3). K103N/S occurred in isolation (absence of major NNRTI DRMs) in only 10.1% (n = 11/109, 95% CI: 5.1–17.3) of PLH.

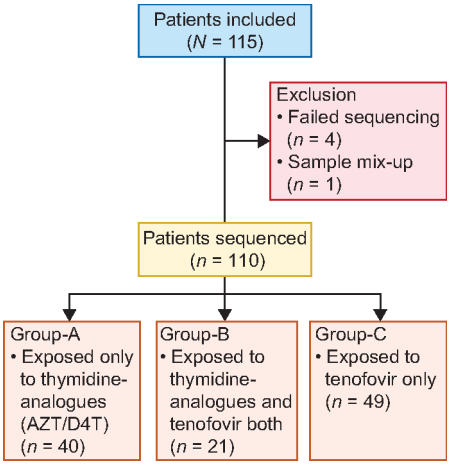

Fig. 1: Patient flow. This figure illustrates the flow of patients and their inclusion in the study. Out of the initial 115 people living with HIV (PLH) who met the inclusion criteria, 4 samples failed sequencing, and 1 sample was excluded due to a mix-up. The remaining 110 PLH were categorized into 3 groups: group A, group B, and group C. Within our cohort, 48.2% (n = 53) started 1L-ART before 2014, when AZT was predominantly used in 1L-ART, of which 90.6% (n = 48/53) had been exposed to AZT, and 30.2% (n = 16/53) had received both AZT/D4T (thymidine analogs, TAs) and TFV. The remaining 57 PLH initiated 1L-ART after 2014, with the majority (89.5%, n = 51/57) having received TFV and 8.8% (n = 5/57) with exposure to both TAs and TFV

Table 1: Baseline characteristics

Characteristics	*Total (n = 110)*	*Group A (n = 40)*	*Group B (n = 21)*	*Group C (n = 49)*
Sex				
Male (%, *n*)	75.5% (83)	70% (28)	81% (17)	77.6% (38)
Female (%, *n*)	24.5% (27)	30% (12)	19% (4)	22.4% (11)
Age				
Age (median, IQR, range) years	41 (IQR: 13, range: 13–78)	45 (IQR: 12, range: 13–62)	40 (IQR: 14, range: 21–55)	40 (IQR: 10, range: 26–78)
Year of initiation of ART				
During or after 2014* (%, *n*)	51.8% (57)	15% (6)	23.8% (5)	93.9% (46)
Before 2014 (%, *n*)	48.2% (53)	85% (34)	76.2% (16)	6.1% (3)
Duration of first-line ART				
First-line ART (median, IQR, range) months	–	–	40 (IQR: 69, range: 8–112) (*n* = 19)	–
Alternative first-line ART (median, IQR, range) months	–	–	63 (IQR: 69, range: 4–137) (*n* = 19)	–
Overall (median, IQR, range) months	81 (IQR: 79, range: 12–199)	124 (IQR: 62, range: 19–199)	103 (IQR: 38, range: 59–198)	35 (IQR: 43, range: 12–120)
Cytosine analog exposure				
3TC only (%, *n*)	97.3% (107)	100% (40)	90.5% (19)	98% (48)
FTC only (%, *n*)	0.9% (1)	0% (0)	4.8% (1)	0% (0)
Both 3TC and FTC (%, *n*)	1.8% (2)	0% (0)	4.8% (1)	2% (1)
First-line NNRTI agent				
EFV (%, *n*)	57.3% (63)	22.5% (9)	33.3% (7)	95.9% (47)
NVP (%, *n*)	26.4% (29)	67.5% (27)	4.8% (1)	2% (1)
Both EFV and NVP (%, *n*)	16.4% (18)	10% (4)	61.9% (13)	2% (1)
Adherence to regimen prior to diagnosis of first-line ART failure				
≥95% (%, *n*)	32.3% (30)	34.4% (11)	16.7% (3)	37.2% (16)
85–94% (%, *n*)	25.8% (24)	25% (8)	33.3% (6)	23.3% (10)
<85% (%, *n*)	41.9% (39)	40.6% (13)	50% (9)	39.5% (17)
Not assessed/ doubtful (*n*)	*n* = 17	*n* = 8	*n* = 3	*n* = 6
Immunovirological status				
Baseline PVL (median, IQR, range) Log10 Copies/mL	4.8 (IQR: 1.3, range: 3.3–7.9)	4.4 (IQR: 1.4, range: 3.3–6.6)	4.7 (IQR: 1.3, range: 3.7–6.8)	4.9 (IQR: 1.4, range: 3.3–7.9)
PVL 2000–1,00,000 copies/mL (%, *n*)	53.6% (59)	55% (22)	57.1% (12)	51% (25)
PVL 1,00,000–3,00,000 copies/mL (%, *n*)	21.8% (24)	22.5% (9)	28.6% (6)	18.4% (9)
PVL >3,00,000 copies/mL (%, *n*)	24.5% (27)	22.5% (9)	14.3% (3)	30.6% (15)
CD4 (median, IQR, range) cells/mm^3	169 (IQR: 212, range: 7–892)	189 (IQR: 259, range: 7–892)	164 (IQR: 202, range: 11–400)	150 (IQR: 179, range: 10–779)
CD4 200–499 200 cells/mm^3 (%, *n*)	33.6% (37)	35.0% (14)	38.09% (8)	30.6% (15)
Advanced HIV (CD4 <200 cells/mm^3) (%, *n*)	53.6% (59)	47.5% (19)	57.1% (12)	57.1% (28)
Coinfections				
VDRL reactive (*n*)	–	–	–	–
HBsAg reactive (*n*)	*n* = 4	*n* = 1	*n* = 0	*n* = 3
Hepatitis-C (n)	*n* = 1	*n* = 1	*n* = 0	*n* = 0
HIV-1 genotype				
C (%, *n*)	99.1% (109)	100% (40)	100% (21)	98% (48)
A (%, *n*)	0.9% (1)	0% (0)	0% (0)	2% (1)

*2014 marks the year of implementation of TFV in national program; 3TC, lamivudine; ART, antiretroviral therapy; EFV, efavirenz; FTC, emtricitabine; HBsAg, hepatitis-B surface antigen; IQR, interquartile-range; NVP, nevirapine; PVL, plasma viral-load

Resistance to Nucleoside Reverse Transcriptase Inhibitors

Predicted susceptibility to NRTIs after 1L ART failure: Among the 110 participants, 80.0% [(*n* = 88/110), 95% CI: 71.3–87.0] showed resistance (IR or HLR) to 3TC and FTC due to M184V/I DRMs. TFV and AZT resistance occurred in 35.5% [(*n* = 39/110), 95% CI: 26.6–45.1] and 31.8% [(*n* = 35/110), 95% CI: 23.3–41.4] of PLH, respectively, with no significant difference (p = 0.568). Overall, 16.4% (*n* = 18/110, 95% CI: 9.9–24.6) had dual resistance to TFV and AZT, whereas 49.1% [(*n* = 54/110), 95% CI: 39.4–58.8] remained susceptible to both. TFV susceptibility with

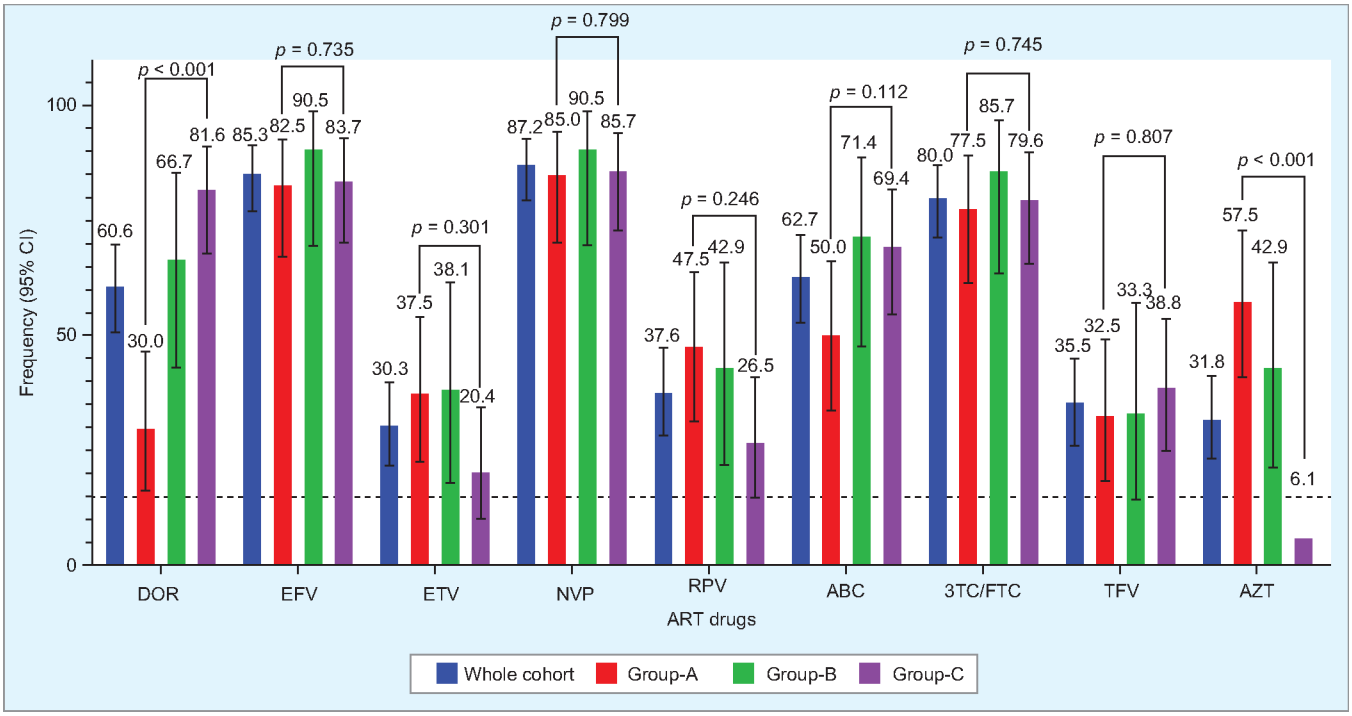

Fig. 2: Resistance to NRTI and NNRTI. The figure illustrates the frequency of drug resistance (IR or HLR) to specific antiretroviral agents (ARVs) in the entire cohort as well as in subgroups A, B, and C, denoted by blue, red, green, and purple bars, respectively. Error bars represent the 95% confidence interval (CI), and corresponding *p*-values indicating differences in proportions among groups A to C are provided; 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; AZT, zidovudine; DOR, doravirine; EFV, efavirenz; ETV, etravirine; FTC, emtricitabine; NVP, nevirapine; RPV, rilpivirine

AZT resistance was observed in 48.6% (n = 17/35) of PLH, and AZT susceptibility with TFV resistance in 53.8% (n = 21/39).

Resistance to AZT: AZT resistance was comparable [ΔP: 14.6% (95% CI: −11.5–40.8), p = 0.277] among groups with prior TA exposure [group A: 57.5% (n = 23/40, 95% CI: 40.8–72.9) vs group B: 42.9% (n = 9/21, 95% CI: 21.8–65.9)], despite failure of a TFV-containing 1L ART regimen in group B. Three PLH in group C had AZT resistance (Fig. 2).

Resistance to TFV: TFV resistance was comparable [ΔP: 0.8% (95% CI: −24.0–25.7), p = 0.947] among groups with prior TA exposure [group A: 32.5% (n = 13/40, 95% CI: 18.6–49.1) vs group B: 33.3% (n = 7/21, 95% CI: 14.6–56.9)], despite group A's lack of TFV exposure. TFV resistance among the TFV-only–exposed group [group C: 38.8% (n = 19/49, 95% CI: 25.2–53.7)] was similar to group A [ΔP: 6.2% (95% CI: −13.6–26.2), p = 0.539] and to group B [ΔP: 5.4% (95% CI: −18.9–29.8), p = 0.666] (Fig. 2).

Patterns of NRTI DRMs: The TFV DRM K65R occurred in 19.1% (n = 21/110, 95% CI: 12.2–27.7) of patients, and multiple (≥ 2) thymidine analog mutations (TAMs) occurred in 30.9% (n = 34/110, 95% CI: 22.4–40.4) [TAM-1: 6.4% (n = 7/110), TAM-2: 10.0% (n = 11/110), mixed TAM-1 and TAM-2 pattern: 14.5% (n = 16/110)].

Among TFV-exposed patients, K65R occurred more frequently in the TFV-only–exposed group [group C: 36.7% (n = 18/49, 95% CI: 23.4–51.7)] compared with PLH exposed to both TAs and TFV [group B: 14.3% (n = 3/21, 95% CI: 3.1–36.3)], showing a trend toward statistical significance [ΔP: 22.4% (95% CI: 2.3–42.6), p = 0.060] (Fig. 2).

Multiple TAMs occurred in 55.0% (n = 22/40, 95% CI: 38.5–70.7) of group A and 42.9% (n = 9/21, 95% CI: 21.8–65.9) of group B. This difference was not statistically significant [ΔP: 12.1% (95% CI: −14.0–38.3), p = 0.367]. Multidrug NRTI DRMs, including T69INS and the Q151 complex, were not observed (Fig. 2).

Distinctive patterns of K65R were observed. K65R occurred in isolation or with M184V/I ± other TFV DRMs (K70E or Y115F) in 57.1% (n = 12/21, 95% CI: 34.0–78.1) of PLH harboring K65R. Among the remaining 9 sequences, K65R coexisted with a single TAM (not compromising AZT) in 8 sequences [group C: n = 7/8 (K65R + M41L: n = 5/8; K65R + K219E: n = 2/8); group B: n = 1/8 (K65R + D67N)]. One group B sequence harbored M41L + K65R + M184V + T215YS, compromising both AZT and TFV.

Three patients in group C had multiple TAMs despite no documented TA exposure. Post hoc recategorization into group B yielded similar results (Table S2 and Table S3). Brief results are presented below.

Group A (TA-only exposure) had a higher occurrence of multiple TAMs [55.0% (n = 22/40, 95% CI: 38.5–70.7)] compared with 50.0% (n = 12/24, 95% CI: 29.1–70.9) in group B (TA exposure followed by virological failure on a TFV backbone), but the difference was not statistically significant [ΔP: 5.0% (95% CI: −20.3–30.3), p = 0.698]. Accordingly, AZT resistance was 57.5% (n = 23/40, 95% CI: 40.9–72.9) in group A and 50.0% (n = 12/24, 95% CI: 29.1–70.9) in group B, with no significant difference [ΔP: 7.5% (95% CI: −17.7–32.7), p = 0.560] (Table S2).

K65R occurred more frequently in group C (TFV-only exposure) [39.1% (n = 18/46, 95% CI: 25.1–54.6)] compared with group B [12.5% (n = 3/24, 95% CI: 2.7–32.4)], with a statistically significant difference [ΔP: 26.6% (95% CI: 7.3–45.9), p = 0.021]. However, TFV resistance, although higher in group C [41.3% (n = 19/46, 95% CI: 26.9–56.8)] than in group B [29.2% (n = 7/24, 95% CI: 12.6–51.1)], did not reach statistical significance [ΔP: 12.1% (95% CI: −10.9–35.2), p = 0.318]. As expected, K65R was not observed in group A, yet TFV resistance in group A [32.5% (n = 13/40, 95% CI: 18.6–49.1)] was comparable to group B [ΔP: 3.3% (95% CI: −19.9–26.6), p = 0.781] and group C [ΔP: 8.8% (95% CI: −11.5–29.1), p = 0.399] (Table S2).

Resistance to Protease Inhibitor

PI resistance was observed in only 6 PLH, with no DRMs detected for commonly used PIs, including atazanavir, darunavir, and lopinavir.

Discussion

Our study reaffirms the widespread occurrence of NRTI resistance, particularly against TFV, in

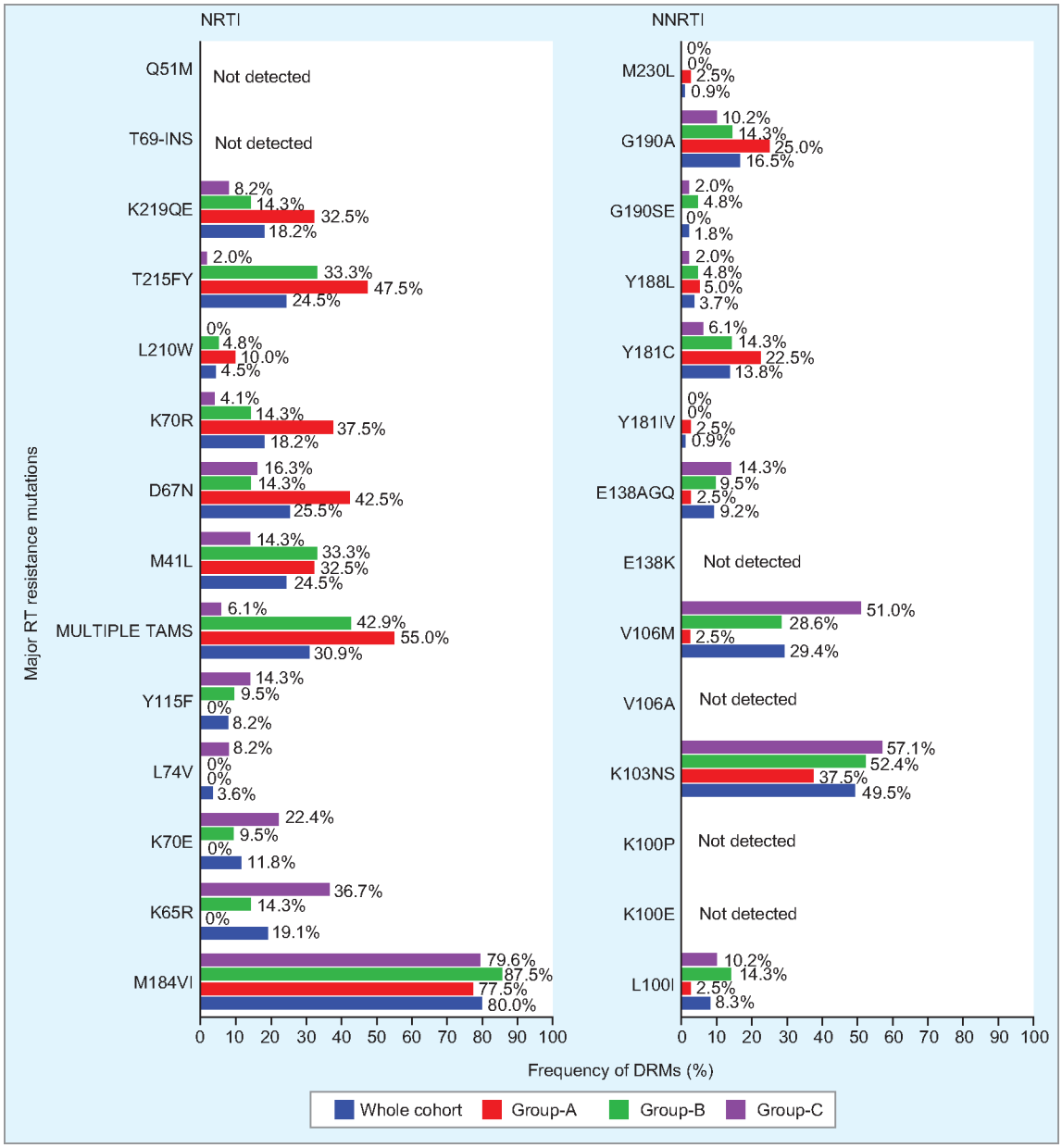

Fig. 3: Major RT resistance mutations. This figure displays the frequency of major NRTI and NNRTI drug-resistance mutations (DRMs) in the entire cohort, as well as in subgroups A, B, and C, represented by blue, red, green, and purple bars, respectively; DRMs, drug-resistance mutations; RT, reverse transcriptase

PLH failing 1L ART with dual NRTI + NNRTI, consistent with recent findings highlighting 58–86% TFV resistance, largely associated with the K65R DRM.[23,24] Notably, the international TENORES study, a comprehensive assessment of HIVDR following the scale-up of WHO-recommended TFV-based ART, revealed TFV resistance rates ranging from 20 to 50%, with higher prevalence in low- and middle-income countries (LMICs) across sub-Saharan Africa.[25] In the Indian context, Dinesha et al.'s recent study echoes this trend, revealing TFV resistance linked to the K65R DRM in 28.1% of patients failing TFV-based regimens, with 10.6% of the K65R-negative subgroup exhibiting TFV resistance.[26] Our findings indicate an overall TFV resistance rate of 37.14% among PLH failing a TFV-containing regimen, underscoring the need to address emerging challenges related to TFV resistance, particularly in LMICs where alternative treatment options may be limited.

In our cohort, K65R DRM, a key TFV DRM, was present in nearly 20% of cases. While the escalating prevalence of K65R DRM appears promising for AZT-based 2L ART,[7] the existence of unconventional resistance profiles within a subset of patients complicates matters. This intriguing observation in our study centers on patients who were sequentially exposed to TAs (AZT or d4T), followed by TFV in their 1L ART regimens (group B). While TFV-selected DRMs were expected to be prevalent in this subcohort, we observed a lower prevalence of the K65R DRM (20.4%) and a higher occurrence of multiple TAMs (38.09%). Previous research has documented the occurrence of multiple TAMs in up to 40% of patients failing TFV-based 1L ART, with 13.3–27.6% of such patients not exhibiting susceptibility to AZT.[6,13,23,27,28] Notably, a large secondary analysis of data from the TenoRes study by Gregson et al. indicated that TAMs specifically selected by AZT or d4T were present in approximately 16% of patients failing TFV-based first-line ART.[29] These resistance patterns suggest potential challenges for both TFV and AZT in 2L ART, regardless of the presence of TFV in the failing regimen.

The often understudied phenomenon of cross-resistance to TFV in individuals with no prior TFV exposure and resistance

to AZT in patients failing TFV-based 1L ART holds important implications for future ART regimens for such patients. Given WHO's public health approach of changing the NRTI backbone agent during the transition from first-line to second-line ART[7], addressing and comprehending resistance to both AZT and TFV within this context becomes paramount.

Another intriguing observation in our study is the coexistence of K65R and TAMs in PLH failing TFV-based 1L ART, despite their known antagonism, highlighting diverse coexistence patterns of K65R and TAMs and emphasizing distinct TAM profiles in the presence of K65R.[29,30] Specifically, our findings showed that in 55% of K65R DRM cases, K65R was detected alone or with M184V/I ± other TFV DRMs, such as K70E or Y115F. In 40% of cases, K65R coexisted with a non-AZT-compromising TAM. Notably, 1 sequence displayed M41L + K65R + M184V + T215YS.

In summary, our findings have 3 important implications for TFV resistance. First, virological failure on TFV-containing 1L ART is influenced by both the failing regimen and prior TA exposure, leading to TAM accumulation and impacting AZT efficacy. Second, our precise treatment records enabled categorization of PLH failing 1L ART into 3 different exposure groups, wherein the occurrence of multiple TAMs in PLH sequentially exposed to TAs and TFV reflects mechanisms of TAM emergence in TFV-based 1L ART failure, as outlined by Gregson et al.,[29] including pretreatment resistance, programmatic substitution (occult treatment failure during programmatic substitution to TFV), and undisclosed ART exposure. Third, our observation of nearly 40% AZT resistance in those failing TFV-based 1L ART but with previous exposure to TAs highlights the importance of accurate treatment histories and genotypic drug resistance testing and challenges WHO's 2L ART recommendations[7] for TFV-based 1L ART failures. This is especially relevant for patients with complex treatment backgrounds who initiated ART before TFV inclusion in the national program and experienced interruptions, where substituting TFV with AZT in second-line ART might compromise treatment efficacy.

In the context of NNRTI resistance, it is unsurprising that a high percentage (86.2%) of patients failing 1L ART with dual NRTI + NNRTI exhibited NNRTI resistance, primarily involving EFV or NVP.[6,13,24,26,28,31,32] The prominence of these NNRTIs has historically been central to combination ART, but their susceptibility to resistance due to factors such as low genetic barriers and longer half-lives, especially with suboptimal adherence,[33,34] highlights the need for a critical appraisal of these drugs. As DTG gains prominence as the preferred 1L ART, our findings remain relevant, shedding light on the progression and patterns of DRMs, potentially impacting transmission. A significant aspect of our study focuses on the emergence of resistance to second-generation NNRTIs, an unexplored facet in India.

The primary NNRTI DRM identified in our study was K103N/S (48.6%), followed by V106M (28.4%), G190A (16.5%), and Y181C (13.8%), consistent with findings from broader studies in Brazil,[35] South Africa,[13,27] and China.[36] Notably, Y181C is more prevalent in patients exposed to NVP, while V106M is associated with EFV use.[13] In the Indian context, a systematic review and meta-analysis by Karade et al. documented the relative prevalence of K103N, Y181C, and G190A mutations in Indian PLH.[37] Our findings are in agreement with these studies, confirming the prevalence of specific mutations in Indian PLH. In contrast, a 2017 Indian study by Dutta et al. found Y188L as the most common DRM (18.18%), followed by K103N (6.81%).[38]

The prevalence of the K103N/S DRM in our study is noteworthy, even though it does not directly affect second-generation NNRTIs (ETV and RPV).[27,39] Despite this, our study found substantial resistance rates for both ETV (30.3%) and RPV (37.6%). This trend could be linked to the relatively limited occurrence of K103N/S in isolation, consistent with findings from previous studies.[13,28] The presence of the Y181C mutation, while less frequent than K103N/S, is concerning due to its association with resistance against both ETV and RPV.[40] These findings suggest that >33% of our patients exhibited resistance to second-generation NNRTIs to which they had not been previously exposed.

Our study sets the stage for the introduction of second-generation NNRTIs into India's public health programs. However, concerns arise about their future effectiveness, particularly with approximately 33% of patients on first-line NNRTI-based ART potentially experiencing issues with the RPV + CAB ART regimen, necessitating guided DRT. Similarly, the presence of ETV resistance in a significant proportion of viremic patients on 1L ART suggests that the use of ETV in subsequent ART regimens and tailored therapy, including third-line salvage regimens, should also be guided by DRT.[13,24] Strengthening HIV genotyping and DRT is critical as these second-generation NNRTIs are integrated into India's public health framework.

Regarding DOR, a new-generation NNRTI of interest due to its effectiveness in patients with K103N and G190A DRMs and currently under investigation in combination with islatravir, a nucleoside reverse transcriptase translocation inhibitor,[41,42] our study found a considerable prevalence of DOR resistance (59.6%) in patients failing first-line ART, with V106M emerging as the second most prevalent NNRTI DRM (28.4%). Notably, the prevalence of DOR resistance in our Indian cohort differs from African (84.8%)[10] and European (nearly 20%)[43,44] cohorts, possibly due to variation in the occurrence of the V106M DRM. Regular surveillance is crucial to assess the feasibility of DOR implementation, given the substantial prevalence of DOR resistance in our cohort.

The less frequently examined drug ABC showed substantial resistance at 62.7% in our cohort of patients failing 1L ART, consistent across all subcohorts, suggesting limited efficacy in those unable to tolerate AZT in second-line ART.[13] Fortunately, no PI mutations were identified, representing a favorable outcome.

The findings of our study should be considered within the context of certain limitations. DRT was conducted after prolonged viremia without considering episode durations, limiting insights into the temporal progression of resistance. Additionally, the potential influence of CD4 cell count or viral load on DRMs was not assessed. The lack of pretreatment drug resistance information may have led to an overestimation of resistance levels due to unrecognized transmitted DRMs. Furthermore, the cross-sectional design of our study is inherently prone to bias.

Nonetheless, our study comprehensively analyzes HIV drug resistance after 1L ART failure in India in a sizeable population. Subgrouping by drug exposure offers a distinctive perspective on HIVDR, particularly regarding TFV and AZT, which is crucial for informing ART switch strategies. Our assessment of NNRTI resistance, including second-generation NNRTIs, emphasizes the potential strategic use of DTG to address resistance concerns and ensure sustained efficacy of these agents in future ART regimens.

Conclusion

Our comprehensive study on HIV drug resistance after 1L ART failure in India emphasizes the importance of TFV and AZT resistance, especially in the context of subsequent ART regimens. Our findings also highlight the resistance profiles of second-generation NNRTIs and suggest that DTG integration could address resistance concerns, ensuring ongoing NNRTI efficacy in future ART regimens.

Clinical Significance

Ensuring adequate drug exposure history in patients can prevent poor outcomes in PLH being treated with ART due to resistance.

Resistance profiling is especially relevant following first-line ART failure. NNRTIs remain viable ART options in Indian PLH despite the presence of DRMs.

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References

1. Flexner CW, Kashuba A. Editorial: new drugs for HIV: quo vadis? Curr Opin HIV AIDS 2022;17:1–3.
2. Vitoria M, Rangaraj A, Ford N, et al. Current and future priorities for the development of optimal HIV drugs. Curr Opin HIV AIDS 2019;14:143–149.
3. Kanters S, Vitoria M, Zoratti M, et al. Comparative efficacy, tolerability and safety of dolutegravir and efavirenz 400mg among antiretroviral therapies for first-line HIV treatment: a systematic literature review and network meta-analysis. EClinicalMedicine 2020;28.
4. Nanfuka M, Forrest JI, Zhang W, et al. Durability of non-nucleotide reverse transcriptase inhibitor-based first-line ART regimens after 7 years of treatment in rural Uganda: a prospective cohort study. Med (United States) 2021;100(19):E25763.
5. Amin J, Becker S, Belloso W, et al. Efficacy of 400 mg efavirenz versus standard 600 mg dose in HIV-infected, antiretroviral-naive adults (ENCORE1): a randomised, double-blind, placebo-controlled, non-inferiority trial. Lancet 2014;383(9927):1474–1482.
6. Parbie PK, Abana CZY, Kushitor D, et al. High-level resistance to non-nucleos(t)ide reverse transcriptase inhibitor based first-line antiretroviral therapy in Ghana; a 2017 study. Front Microbiol 2022;13:973771.
7. World Health Organization. Update of recommendations on first- and second-line antiretroviral regimens [Internet]. WHO; 2019. Available from: http://www.who.int/hiv/pub/arv/arv-update-2019-policy/en/
8. Paton NI, Musaazi J, Kityo C, et al. Dolutegravir or darunavir in combination with zidovudine or tenofovir to treat HIV. N Engl J Med 2021;385(4):330–341.
9. Rhee SY, Schapiro JM, Saladini F, et al. Potential role of doravirine for the treatment of HIV-1-infected persons with transmitted drug resistance. AIDS Res Ther 2023;20(1):1–7.
10. Steegen K, Moorhouse M, Wensing AMJ, et al. Is there a role for doravirine in African HIV treatment programmes? A large observational resistance study in South Africa. J Int AIDS Soc 2021;24(5):e25706.
11. Avihingsanon A, Hughes MD, Salata R, et al. Third-line antiretroviral therapy, including raltegravir (RAL), darunavir (DRV/r) and/or etravirine (ETR), is well tolerated and achieves durable virologic suppression over 144 weeks in resource-limited settings: ACTG A5288 strategy trial. J Int AIDS Soc 2022;25(6).
12. Moorhouse MA, Cohen K. The role of rilpivirine in Southern Africa. South Afr J HIV Med 2019;20(1).
13. Steegen K, Bronze M, Papathanasopoulos MA, et al. HIV-1 antiretroviral drug resistance patterns in patients failing NNRTI-based treatment: results from a national survey in South Africa. J Antimicrob Chemother 2017;72(1):210–219.
14. Steegen K, Chandiwana N, Sokhela S, et al. Impact of rilpivirine cross-resistance on long-acting cabotegravir-rilpivirine in low and middle-income countries. AIDS 2023;37(6):1009–1011.
15. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. Geneva: World Health Organization; 2016.
16. National AIDS Control Organization; Ministry of Health and Family Welfare; Government of India. National technical guidelines on antiretroviral treatment [Internet]. 2018. Available from: https://naco.gov.in/sites/default/files/NACO%20-%20National%20Technical%20Guidelines%20on%20ART_October%202018.pdf
17. Jordan MR, Bennett DE, Wainberg MA, et al. Update on World Health Organization HIV drug resistance prevention and assessment strategy: 2004–2011 [Internet]. Clin Infect Dis. 2012;54:S245 [cited 2024 Jul 20]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3338313/
18. Jordan MR, Bennett DE, Bertagnolio S, et al. World Health Organization surveys to monitor HIV drug resistance prevention and associated factors in sentinel antiretroviral treatment sites. Antiviral Ther 2008;13:15–23.
19. Stanford Database (HIVdb). HIV drug resistance database, version 9.1 update 2022-06-02 [Internet]. 2022 [cited 2022 Oct 7]. Available from: https://hivdb.stanford.edu/
20. World Health Organization. HIV drug resistance: WHO/HIVResNet HIV drug resistance laboratory operational framework, 2nd edition. Geneva: World Health Organization; 2020.
21. Hosseinipour MC, Gupta RK, Van Zyl G, et al. Emergence of HIV drug resistance during first- and second-line antiretroviral therapy in resource-limited settings. J Infect Dis 2013;207(Suppl 2).
22. Gupta-Wright A, Fielding K, van Oosterhout JJ, et al. Virological failure, HIV-1 drug resistance, and early mortality in adults admitted to hospital in Malawi: an observational cohort study. Lancet HIV 2020;7(9):e620–e628.
23. Parker Hudson F, Mulenga L, Westfall AO, et al. Evolution of HIV-1 drug resistance after virological failure of first-line antiretroviral therapy in Lusaka, Zambia. Antivir Ther 2019;24(4):291–300.
24. Chimukangara B, Lessells RJ, Sartorius B, et al. HIV-1 drug resistance in adults and adolescents on protease inhibitor-based antiretroviral therapy in KwaZulu-Natal Province, South Africa. J Glob Antimicrob Resist 2022;29:468–475.
25. Gregson J, Tang M, Ndembi N, et al. Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicentre retrospective cohort study. Lancet Infect Dis 2016;16(5):565–575.
26. Dinesha TR, Gomathi S, Boobalan J, et al. Genotypic HIV-1 drug resistance among patients failing tenofovir-based first-line HAART in South India. AIDS Res Hum Retroviruses 2016;32(12):1234–1236.
27. Chimukangara B, Lessells RJ, Singh L, et al. Acquired HIV drug resistance and virologic monitoring in a HIV hyper-endemic setting in KwaZulu-Natal Province, South Africa. AIDS Res Ther 2021;18(1):74.
28. Miti S, Handema R, Mulenga L, et al. Prevalence and characteristics of HIV drug resistance among antiretroviral treatment (ART) experienced adolescents and young adults living with HIV in Ndola, Zambia. PLoS One 2020;15.
29. Gregson J, Kaleebu P, Marconi VC, et al. Occult HIV-1 drug resistance to thymidine analogues following failure of first-line tenofovir combined with a cytosine analogue and nevirapine or efavirenz in sub-Saharan Africa: a retrospective multi-centre cohort study. Lancet Infect Dis 2017;17(3):296–304.
30. Chaplin B, Imade G, Onwuamah C, et al. Distinct pattern of thymidine analogue mutations with K65R in patients failing tenofovir-based antiretroviral therapy. AIDS Res Hum Retroviruses 2018;34(2):228–233.
31. Clipman SJ, Solomon SS, Srikrishnan AK, et al. Antiretroviral drug resistance in HIV sequences from people who inject drugs and men who have sex with men across 21 cities in India. Open Forum Infect Dis 2022;9(10).
32. Ekstrand ML, Shet A, Chandy S, et al. Suboptimal adherence associated with virological failure and resistance mutations to first-line highly active antiretroviral therapy (HAART) in Bangalore, India. Int Health 2011;3(1):27–34.
33. Carr A, Mackie NE, Paredes R, et al. HIV drug resistance in the era of contemporary antiretroviral therapy: a clinical perspective. Antiviral Ther 2023;28.
34. Mackie N. Resistance to non-nucleoside reverse transcriptase inhibitors [Internet]. In: Antiretroviral Resistance in Clinical Practice. 2006 [cited 2023 Oct 21]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK2249/
35. Chaves YO, Pereira FR, De Souza Pinheiro R, et al. High detection rate of HIV drug resistance mutations among patients who fail combined antiretroviral therapy in Manaus, Brazil. Biomed Res Int 2021;2021.
36. Cao P, Su B, Wu J, et al. Treatment outcomes and HIV drug resistance of patients switching to second-line regimens after long-term first-line antiretroviral therapy: an observational cohort study. Med (United States) 2018;97(28).
37. Karade S, Chaturbhuj DN, Sen S, et al. HIV drug resistance following a decade of the free antiretroviral therapy programme in India: a review. Int J Infect Dis 2018;66:33–41.
38. Dutta N, Nandi S, Guha SK, et al. Emergence of HIV drug-resistant mutations in East Indian population after failure of first-line antiretroviral therapy. HIV AIDS Rev 2017;16(4):258–264.
39. Verbon A, Rijnders BJA. Successful switch to rilpivirine/tenofovir/emtricitabine in HIV-1-infected patients with an isolated K103N mutation acquired during prior nonnucleoside reverse transcriptase inhibitor therapy. HIV Med 2014;15(10):611–614.
40. Diphoko T, Gaseitsiwe S, Kasvosve I, et al. Prevalence of rilpivirine and etravirine resistance mutations in HIV-1 subtype C-infected patients failing nevirapine or efavirenz-based combination antiretroviral therapy in Botswana. AIDS Res Hum Retroviruses 2018;34(8):667.
41. Molina JM, Yazdanpanah Y, Afani Saud A, et al. Islatravir in combination with doravirine for treatment-naive adults with HIV-1 infection receiving initial treatment with islatravir, doravirine, and lamivudine: a phase 2b, randomised, double-blind, dose-ranging trial. Lancet HIV 2021;8(6):e324–e333.
42. Rock AE, Lerner J, Badowski ME. Doravirine and its potential in the treatment of HIV: an evidence-based review of the emerging data. HIV AIDS (Auckl) 2020;12:201–210.
43. Soulie C, Santoro MM, Storto A, et al. Prevalence of doravirine-associated resistance mutations in HIV-1-infected antiretroviral-experienced patients from two large databases in France and Italy. J Antimicrob Chemother 2020;75(4):1026–1030.
44. Sterrantino G, Borghi V, Callegaro AP, et al. Prevalence of predicted resistance to doravirine in HIV-1-positive patients after exposure to non-nucleoside reverse transcriptase inhibitors. Int J Antimicrob Agents 2019;53(4):515–519.

Table S1: Resistance to second generation NNRTIs

	EFV in first-line ART (n = 63)	*NVP in first-line ART (n = 29)*	*Diff. in proportions (95% CI)*	*p*
ETV% [(*n*) 95% CI]	20.6% (*n* = 13/63) 95% CI: 11.5–32.7	41.4% (*n* = 12/29) 95% CI: 23.5–61.06	20.7% (95% CI: 0.2–41.3)	0.038
RPV [(*n*) 95% CI]	25.4% (*n* = 16/63) 95% CI: 15.3–37.9	55.2% (*n* = 16/29) 95% CI: 35.7–73.6	29.8% (95% CI: 8.7–50.8)	0.005
DOR [(*n*) 95% CI]	74.6% (*n* = 47/63) 95% CI: 63.9–85.4	31.03% (*n* = 9/29, 95% CI: 15.3–50.8)	43.6% (95% CI: 24.5–62.6)	<0.001

n = 18 PLH who were exposed to both EFV and NVP in their 1L ART were excluded

Table S2: Drug susceptibility profile and DRMs after recategorizing three patients in group B rather than in group C

Characteristics	*Total (n = 110)*	*Group A (n = 40)*	*Group B (n = 24)*	*Group C (n = 46)*
Drug-susceptibility profile				
DOR	60.5 (*n* = 66, 95% CI: 50.2–69.2)	30 (*n* = 12, 95% CI: 16.6–46.5)	70.8 (*n* = 17, 95% CI: 48.9–87.4)	80.4 (*n* = 37, 95% CI: 66.1–90.6)
EFV	85.3 (*n* = 93, 95% CI: 76.4–90.7)	82.5 (*n* = 33, 95% CI: 67.2–92.7)	91.7 (*n* = 22, 95% CI: 73–99)	82.6 (*n* = 38, 95% CI: 71.7–93.5)
ETV	30.3 (*n* = 33, 95% CI: 21.6–39.5)	37.5 (*n* = 15, 95% CI: 22.7–54.2)	37.5 (*n* = 9, 95% CI: 18.8–59.4)	19.6 (*n* = 9, 95% CI: 9.4–33.9)
NVP	87.2 (*n* = 95, 95% CI: 78.5–92.2)	85 (*n* = 34, 95% CI: 70.2–94.3)	91.7 (*n* = 22, 95% CI: 73–99)	84.7 (*n* = 39, 95% CI: 74.5–95.1)
RPV	37.6 (*n* = 41, 95% CI: 28.2–47)	47.5 (*n* = 19, 95% CI: 31.5–63.9)	45.8 (*n* = 11, 95% CI: 25.6–67.2)	23.9 (*n* = 11, 95% CI: 12.6–38.8)
ABC	62.7 (*n* = 69, 95% CI: 53–71.8)	50 (*n* = 20, 95% CI: 33.8–66.2)	75 (*n* = 18, 95% CI: 53.3–90.2)	67.4 (*n* = 31, 95% CI: 52–80.5)
3TC/FTC	80 (*n* = 88, 95% CI: 71.3–87)	77.5 (*n* = 31, 95% CI: 61.5–89.2)	87.5 (*n* = 21, 95% CI: 67.6–97.3)	78.3 (*n* = 36, 95% CI: 63.6–89.1)
Tenofovir	35.5 (*n* = 39, 95% CI: 26.6–45.1)	32.5 (*n* = 13, 95% CI: 18.6–49.1)	29.2 (*n* = 7, 95% CI: 12.6–51.1)	41.3 (*n* = 19, 95% CI: 27–56.8)
AZT	31.8 (*n* = 35, 95% CI: 23.3–41.4)	57.5 (*n* = 23, 95% CI: 40.9–73)	50 (*n* = 12, 95% CI: 29.1–70.9)	0 (*n* = 0, 95% CI: 0–7.7)
NRTI-DRMs				
M184VI	80 (*n* = 88, 95% CI: 71.3–87)	77.5 (*n* = 31, 95% CI: 61.5–89.2)	87.5 (*n* = 21, 95% CI: 67.6–97.3)	78.3 (*n* = 36, 95% CI: 63.6–89.1)
K65R	19.1 (*n* = 21, 95% CI: 12.2–27.7)	0 (*n* = 0, 95% CI: 0–8.8)	12.5 (*n* = 3, 95% CI: 2.7–32.4)	39.1 (*n* = 18, 95% CI: 25.1–54.6)
K70E	11.8 (*n* = 13, 95% CI: 6.4–19.4)	0 (*n* = 0, 95% CI: 0–8.8)	12.5 (*n* = 3, 95% CI: 2.7–32.4)	21.7 (*n* = 10, 95% CI: 10.9–36.4)
L74V	3.6 (*n* = 4, 95% CI: 1–9)	0 (*n* = 0, 95% CI: 0–8.8)	4.2 (*n* = 1, 95% CI: 0.1–21.1)	6.5 (*n* = 3, 95% CI: 1.4–17.9)
Y115F	8.2 (*n* = 9, 95% CI: 3.8–15)	0 (*n* = 0, 95% CI: 0–8.8)	8.3 (*n* = 2, 95% CI: 1–27)	15.2 (*n* = 7, 95% CI: 6.3–28.9)
≥ 2 TAMS	30.9 (*n* = 34, 95% CI: 22.4–40.4)	55 (*n* = 22, 95% CI: 38.5–70.7)	50 (*n* = 12, 95% CI: 29.1–70.9)	0 (*n* = 0, 95% CI: 0–7.7)
M41L	24.5 (*n* = 27, 95% CI: 16.8–33.7)	32.5 (*n* = 13, 95% CI: 18.6–49.1)	41.7 (*n* = 10, 95% CI: 22.1–63.4)	8.7 (*n* = 4, 95% CI: 2.4–20.8)
D67N	25.5 (*n* = 28, 95% CI: 17.6–34.6)	42.5 (*n* = 17, 95% CI: 27–59.1)	20.8 (*n* = 5, 95% CI: 7.1–42.2)	13 (*n* = 6, 95% CI: 4.9–26.3)
K70R	18.2 (*n* = 20, 95% CI: 11.5–26.7)	37.5 (*n* = 15, 95% CI: 22.7–54.2)	16.7 (*n* = 4, 95% CI: 4.7–37.4)	2.2 (*n* = 1, 95% CI: 0.1–11.5)
L210W	4.5 (*n* = 5, 95% CI: 1.5–10.3)	10 (*n* = 4, 95% CI: 2.8–23.7)	4.2 (*n* = 1, 95% CI: 0.1–21.1)	0 (*n* = 0, 95% CI: 0–7.7)
T215FY	24.5 (*n* = 27, 95% CI: 16.8–33.7)	47.5 (*n* = 19, 95% CI: 31.5–63.9)	33.3 (*n* = 8, 95% CI: 15.6–55.3)	0 (*n* = 0, 95% CI: 0–7.7)
K219QE	18.2 (*n* = 20, 95% CI: 11.5–26.7)	32.5 (*n* = 13, 95% CI: 18.6–49.1)	16.7 (*n* = 4, 95% CI: 4.7–37.4)	6.5 (*n* = 3, 95% CI: 1.4–17.9)
T 69 INS	0 (*n* = 0, 95% CI: 0–3.3)	0 (*n* = 0, 95% CI: 0–8.8)	0 (*n* = 0, 95% CI: 0–14.2)	0 (*n* = 0, 95% CI: 0–7.7)
Q51M	0 (*n* = 0, 95% CI: 0–3.3)	0 (*n* = 0, 95% CI: 0–8.8)	0 (*n* = 0, 95% CI: 0–14.2)	0 (*n* = 0, 95% CI: 0–7.7)
NRTI-DRMs (*n* = 109)				
L100I	8.3 (*n* = 9, 95% CI: 3.8–15.1)	2.5 (*n* = 1, 95% CI: 0.1–13.2)	16.7 (*n* = 4, 95% CI: 4.7–37.4)	8.9 (*n* = 4, 95% CI: 2.5–21.2)
K100E	0 (*n* = 0, 95% CI: 0–3.3)	0 (*n* = 0, 95% CI: 0–8.8)	0 (*n* = 0, 95% CI: 0–14.2)	0 (*n* = 0, 95% CI: 0–7.9)
K100P	0 (*n* = 0, 95% CI: 0–3.3)	0 (*n* = 0, 95% CI: 0–8.8)	0 (*n* = 0, 95% CI: 0–14.2)	0 (*n* = 0, 95% CI: 0–7.9)
K103NS	49.5 (*n* = 54, 95% CI: 38.9–58.4)	37.5 (*n* = 15, 95% CI: 22.7–54.2)	50 (*n* = 12, 95% CI: 29.1–70.9)	58.7 (*n* = 27, 95% CI: 44.5–72.9)
V106A	0 (*n* = 0, 95% CI: 0–3.3)	0 (*n* = 0, 95% CI: 0–8.8)	0 (*n* = 0, 95% CI: 0–14.2)	0 (*n* = 0, 95% CI: 0–7.9)
V106M	29.4 (*n* = 32, 95% CI: 20.2–37.9)	2.5 (*n* = 1, 95% CI: 0.1–13.2)	33.3 (*n* = 8, 95% CI: 15.6–55.3)	50.0 (*n* = 23, 95% CI: 35.6–64.4)
E138K	0 (*n* = 0, 95% CI: 0–3.3)	0 (*n* = 0, 95% CI: 0–8.8)	0 (*n* = 0, 95% CI: 0–14.2)	0 (*n* = 0, 95% CI: 0–7.9)
E138AGQ	9.2 (*n* = 10, 95% CI: 4.5–16.2)	2.5 (*n* = 1, 95% CI: 0.1–13.2)	16.7 (*n* = 4, 95% CI: 4.7–37.4)	11.1 (*n* = 5, 95% CI: 3.7–24.1)
Y181IV	0.9 (*n* = 1, 95% CI: 0–5)	2.5 (*n* = 1, 95% CI: 0.1–13.2)	0 (*n* = 0, 95% CI: 0–14.2)	0 (*n* = 0, 95% CI: 0–7.9)
Y181C	13.8 (*n* = 15, 95% CI: 7.9–21.7)	22.5 (*n* = 9, 95% CI: 10.8–38.5)	12.5 (*n* = 3, 95% CI: 2.7–32.4)	6.7 (*n* = 3, 95% CI: 1.4–18.3)
Y188L	3.7 (*n* = 4, 95% CI: 1–9.1)	5 (*n* = 2, 95% CI: 0.6–16.9)	4.2 (*n* = 1, 95% CI: 0.1–21.1)	2.2 (*n* = 1, 95% CI: 0.1–11.8)
G190SE	1.8 (*n* = 2, 95% CI: 0.2–6.5)	0 (*n* = 0, 95% CI: 0–8.8)	4.2 (*n* = 1, 95% CI: 0.1–21.1)	2.2 (*n* = 1, 95% CI: 0.1–11.8)
G190A	16.5 (*n* = 18, 95% CI: 10.1–24.8)	25 (*n* = 10, 95% CI: 12.7–41.2)	20.8 (*n* = 5, 95% CI: 7.1–42.2)	6.7 (*n* = 3, 95% CI: 1.4–18.3)
M230L	0.9 (*n* = 1, 95% CI: 0–5)	2.5 (*n* = 1, 95% CI: 0.1–13.2)	0 (*n* = 0, 95% CI: 0–14.2)	0 (*n* = 0, 95% CI: 0–7.9)

Patients in group A, who were exposed in their first-line ART to thymidine analogs only, had a discernibly higher occurrence of multiple TAMS, 55.0% (*n* = 22/40; 95% CI: 38.5–70.7), compared to patients in group B, who were exposed to thymidine analogs before failing on a TFV backbone, 50.0% (*n* = 12/24; 95% CI: 29.1–70.9), the difference not being remarkably significant [difference in proportions: 5.0% (95% CI: −20.3 to 30.3); *p* = 0.698]. This corresponded with AZT resistance being detected with a frequency of 57.5% (*n* = 23/40; 95% CI: 40.9–72.9) in group A and 50.0% (*n* = 12/24; 95% CI: 29.1–70.9) in group B, the difference not being statistically significant [difference in proportions: 7.5% (95% CI: −17.7 to 32.7); *p* = 0.560].

The incidence of K65R among patients in group C, who were exposed to TFV only, was 39.1% (*n* = 18/46; 95% CI: 25.09–54.6), and among those in group B, who were exposed to thymidine analogs before failing on a TFV backbone, was 12.5% (*n* = 3/24; 95% CI: 2.7–32.4), the difference being evidently significant [difference in proportions: 26.6% (95% CI: 7.3–45.9); *p* = 0.021]. Yet TFV resistance, though distinctly higher in group C, 41.3% (*n* = 19/46; 95% CI: 26.9–56.8), compared to group B, 29.2% (*n* = 7/24; 95% CI: 12.6–51.09), was not large enough to attain statistical significance [difference in proportions: 12.1% (95% CI: −10.9 to 35.2); *p* = 0.318]. As expected, K65R was not observed in patients in group A, who had no TFV exposure, yet the incidence of TFV resistance in group A, 32.5% (*n* = 13/40; 95% CI: 18.6–49.1), was comparable to that in group B (difference in proportions: 3.3% [95% CI: −19.9 to 26.6]; *p* = 0.781) and group C [difference in proportions: 8.8% (95% CI: −11.5 to 29.1); *p* = 0.399].

Table S3: Drug susceptibility profile and DRMs after recategorizing three patients in group B rather than in group C and sensitivity analysis of key results

Scenario	*Comparison*	*Group A (%)*	*Group B (%)*	*ΔP (%)*	*p-value*	*Statistical significance*
Scenario 1	Multiple TAMs	55.0% (22/40)	42.9% (9/21)	12.1	0.367	Not significant
	AZT-resistance rates	57.5% (23/40)	50.0% (12/24)	7.5	0.560	Not significant
Scenario 2	Multiple TAMs	55.0% (22/40)	50.0% (12/24)	5.0	0.698	Not significant
	AZT-resistance rates	57.5% (23/40)	50.0% (12/24)	7.5	0.560	Not significant

Interpretation

Scenario 1 represents original handling of data, and scenario 2 represents *post hoc* recategorization of 3 patients in group B rather than in group C based on drug resistance testing results.

In both scenarios:

Multiple TAMs: There is no statistically significant difference between group A and group B.

AZT resistance rates: There is no statistically significant difference between group A and group B.

These findings suggest that the presence of multiple TAMs and AZT resistance rates do not significantly differ between group A (TAs-only exposure) and group B (TAs exposure and subsequent virological failure on a TFV backbone) across both scenarios.