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ORIGINAL ARTICLE

Prevalence of HIV-1 transmitted drug resistance and viral suppression among recently diagnosed adults in São Paulo, Brazil

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Abstract

HIV-1 transmitted drug resistance (TDR) mutations may reduce the efficacy of antiretroviral therapy (ART), but pre-treatment testing to determine the virus genotype can improve the efficacy of ART. Unfortunately, issues related to cost and logistics of pre-treatment testing limit its use in resource-limited settings. We studied 596 ART-naive individuals who were newly diagnosed from 2014 to 2016 in São Paulo, Brazil, to evaluate TDR and virological outcome after 48 weeks of genotype-guided therapy. One or more TDR (based on the WHO surveillance list) was observed in 10.9% (CI 95%, 8.6–13.6) of the sequences, the most common of which was the K103 N mutation, which confers resistance to first-generation drugs of the non-nucleoside reverse transcriptase inhibitor (NNRTI) antiretroviral drug class. Dual-class (1%, 6/596) and triple-class (0.34%, 2/596) resistance were uncommon. After 48 weeks of treatment with ART, infection was suppressed to below 200 copies/mL in most patients (95%), with full suppression (RNA target not detected) in 65%. The following characteristics at patient enrollment were independently associated with a lack of full suppression: CD4 T cell counts below 500 cells/μL, viremia above 100,000 copies/mL, older age, and TDR to NNRTI. The rates of resistance were intermediate, but genotype-guided therapy resulted in high rates of viral suppression. The observed resistance profile should not be an obstacle to the use of the dolutegravir-based regimen now recommended in Brazil, but genotype testing may be warranted before initiating first-generation NNRTI-based regimens.

Introduction

The benefits of combination antiretroviral therapy (ART) to treat human immunodeficiency virus (HIV)-1 infection are well established, but virus resistance is an important factor in treatment failure [1]. Viral suppression is associated with immunological recovery, favorable clinical outcome [2], and improved life expectancy, which in some recent studies approaches that of the general population [3]. However, incomplete viral suppression favors the emergence of drug resistance, increases the probability of virologic failure, and may compromise subsequent treatment [4]. Some circulating viruses may be resistant and, if transmitted, may impact treatment response. Therefore, the use of a pre-treatment genotype test is recommended in many countries [1]. However, issues related to cost and logistics limit the use of the test in resource-limited settings. Plasma HIV-1 RNA quantitation is the preferred method for measuring the viral load (VL) to monitor the response to ART [5]. Viral suppression should be achieved within three to six months on ART. The limit of detection (LoD) of different commercial and in-house assays varies from 20 to 400 copies per mL (c/mL), and the results are reported either by numeric value or as "below the LoD." The Abbott rtPCR assay (USA) that

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is currently used in the Brazilian public system has an LoD of 40 c/mL. The results that are below the LoD and with no viral RNA detected are reported as "target not detected" (TND). The level of viremia that defines satisfactory viral suppression varies with the definition purpose. The Brazilian Ministry of Health and the World Health Organization (WHO) adopt a VL of 1,000 c/mL to define viral suppression in the cascade of care framework [6]. Many clinical trials use < LoD (below limit of detection) to define virological success [7, 8]. Many clinical guidelines define virological failure as a VL above 200 c/mL [9–11]. Although the benefit of very stringent suppression levels has not been clearly proven [12], TND, full viral suppression, has been correlated with a lower risk of viral rebound when compared to very low viremia levels, e.g., < 20 c/mL [13] and < 40 c/mL or 40-49 c/mL [14]. Additionally, observational studies in Canada [15] and Africa [16] have shown an association between lowest viremia levels and favorable clinical outcome. These findings support the concept that the lower the viremia, the better. The Brazilian Ministry of Health has provided free access to ART since the nineties and started recommending universal treatment in 2013. An efavirenz-based regimen was recommended as the first-line choice of treatment from 2002 to 2017 when efavirenz was substituted for dolutegravir. Low or intermediate TDR rates have been documented in São Paulo and other areas of Brazil in most of the studies using Sanger population sequencing from plasma HIV RNA. [17–21]. To investigate the evolution of TDR prevalence in the area and the impact of TDR on ART response, we analyzed HIV sequences from 596 ART-naïve patients from São Paulo diagnosed from 2014 to 2016. The state of São Paulo has the largest population of people living with HIV/AIDS in Brazil, with over 200,000 patients currently on ART. In this study, the virological response after 48 weeks on ART was documented and the predictors of failure evaluated for individuals followed at the public HIV outpatient clinics in São Paulo.

Patients and methods

As part of an open access initiative to provide pre-treatment genotype testing in the São Paulo public health system, consecutive blood samples from ART-naïve HIV-positive patients who were newly diagnosed at public outpatient clinics between January 2014 and April 2016 were sent to the Adolfo Lutz Retrovirus Laboratory. The National ART Dispensation database SICLOM was reviewed to eliminate cases of unreported ART at study inclusion and to identify the regimen start date, ART regimen used, and subsequent ART changes up to the virological outcome assessment date. The virological outcome was assessed in the National HIV Laboratory database, SISCEL. The first VL result available after 48 weeks of ART initiation was recorded, and suppression rates were calculated considering four different metrics: 1,000 c/mL; 200 c/mL; < 40 c/mL (below LoD); and RNA target not detected (TND).

Sequences were obtained from plasma viral RNA that was extracted either manually (QIAmp Viral RNA Mini Kit, QIAGEN, Hilden Germany) or automatically (M2000 extractor, Abbott, USA) and retrotranscribed and amplified using an in-house PCR assay [19]. Briefly, amplification of a portion of the HIV polymerase region (codon 1-235 related to HXB2, accession number MG211834-MG212426) was carried out using retrotranscribed RNA from a one-step reaction (RT-PCR), followed by nested PCR using SuperScript III and High-Fidelity Taq polymerase (Life Technologies, USA). A unique fragment of approximately 1,112 bp was sequenced after Big Dye incorporation using eight primers to cover part of the *pol* region (codon 1 to 235). Sequences were edited manually using Sequencher 4.7 software (Gene Codes, USA), ReCall (beta v3.01), or both.

HIV genotyping was performed according to Stanford HIV Database Genotyping Resistance Interpretation (GRIHIVdb), and the results were reported to clinical sites to support patient management strategies. For purposes of the study, sequences were reanalyzed by using the Calibrated Population Resistance (CPR) tool (CPR Version 6.0, Stanford Database, SDRM 2009) and referencing a standard mutation list of surveillance drug-resistance mutations adopted by WHO as transmitted resistance (TDR).

HIV subtypes were determined using the NCBI Genotyping and REGA HIV Subtyping tools and confirmed by phylogenetic analysis using BEAST v1.7.4 under the GTR + I + G model. Recombinant genomes were evaluated by jpHMM (jumping profile Hidden Markov Model, http://jphmm.gobics.de/) and SCUEAL Datamonkey, http://www.datamonkey.org/dataupload_scueal.php.

Statistical analysis

The data were analyzed using Epi Info 6 (CDC, USA), STATA (v8 Stata Corp, USA), and Graph Pad Prism (v5, USA), applying a level of statistical significance of $p < 0.05$ (two-tailed test). Results of continuous variables were expressed as the median and interquartile range (IQR: 25-75). Mid-p 95% was used to calculate the confidence interval. Dichotomous variables were compared using the Pearson chi or Fisher exact test, and continuous variables were compared using the Mann-Whitney or Kruskal-Wallis test. For logistic regression, variables were dichotomized as viremic/not viremic at the different cutoffs (TND, < LoD, < 200 c/mL, and < 1,000 c/mL). To assess associations with viral suppression, we included the following variables: gender; age (≤ 25 years,the lower quartile, vs. older); CD4 T cell counts (below vs. above 500 cells/

μL); viremia (below vs. above 100,000 c/mL); B/non-B HIV subtype; presence or absence of (i) non-nucleoside reverse transcriptase inhibitor (NNRTI), (ii) nucleoside reverse transcriptase inhibitor (NRTI), or (iii) protease-inhibitor (PI) mutations; use of an NNRTI-based first-line ART vs. other regimens at enrollment; and ART regimen change vs. no change, before virological outcome assessment. Variables with $p = 0.2$ or lower were retained for adjusted analyses.

The study was approved by the institutional ethical committee (CEPIAL - CAAE 022980012.6.0000.0059) and was supported in part by grants FAPESP 2013/19441-7 and FAPESP PPSUS2016/14813-1.

Results

Patient characteristics

Between January 2014 and April 2016, pre-ART genotypic resistance testing was conducted using unique samples from 622 HIV-1-infected individuals. After checking the treatment database, 26 patients were excluded from the study because there was documentation that they had received ART previously; the remaining 596 ART-naïve individuals were included in the analyses. Most of the cases were recent diagnoses at the time of genotypic resistance testing. The median time from diagnosis to testing was 33 days (IQR: 22–68), with only 38 cases (6.3%) and 14 cases (2.4%) with durations longer than one and two years, respectively. The characteristics of the study population are summarized in Table 1.

Table 1 Baseline demographic and laboratory characteristics of the study population (n = 596)

Variable	N (%) or median (IQR)
Age (years)	29 (25-35)
Male gender, *n* (%)	525 (88.1)
CD4 T cell count (cells/mm^3)	497 (323-654)
Viral load ($\log_{10}$)	4.49 (3.94-5.08)
HIV-1 subtype, n (%)	
B	434 (72.8)
C	85 (14.3)
D	1 (0.2)
F	48 (8.1)
G/AG	2 (0.3)
BF recombinants	18 (3.0)
Other recombinants	8 (1.3)

Values are expressed as median and IQR (25th-75th percentiles) or as number of cases (percentage in parentheses). HIV subtypes were determined based only on a partial pol sequence; other recombinants included 2 BD, 1 CF and 5 BC

Transmitted drug resistance mutations

The viruses in 65 out of the 596 cases harbored at least one TDR (10.9%, 95% CI: 8.6-13.6) in the HIV-1 polymerase. Mutations associated with NNRTI resistance, present in 41 cases (6.9%), were the most common, followed by mutations associated with NRTI resistance (22; 3.7%) and PI resistance (13; 2.2%). Dual-class mutations were observed in six (1%) cases, two NRTI + NNRTI, two NRTI + PI and two NNRTI + PI, and triple-class mutations were observed in two (0.34%) sequences. The NRTI drugs used in the currently recommended combination, tenofovir (TDF) and lamivudine (3TC), were predicted to be fully active in 97.5% and 98.8% of the cases, respectively. Integrase resistance was not evaluated. Table 2 shows TDR prevalence estimates from previous studies that used similar methodology [18, 19], showing a small but nonsignificant increase ($p = 0.4$) in the proportion of sequences with TDR.

Using the clinical Stanford mutation list (GRI-HIVdb), which includes additional mutations not in the CPR/WHO list, at least one mutation associated with NNRTI resistance was detected in 113 (19%) sequences, while mutations associated with NRTI resistance and major mutations associated with PI resistance were detected in 30 (5.3%) and 13 (2.2%) of the sequences, respectively. Overall, males had a similar proportion of sequences with TDR (10.9%) than females (11.3%, $p = 0.92$). Figure 1 shows the observed mutations from the 596 sequences.

HIV-1 subtypes

Subtype B was the predominant subtype (72.8%), followed by subtypes C (14.3%), and F (8.1%). Two cases of subtype AG, one subtype D, and 26 cases of different recombinant

Table 2 Transmitted drug resistance mutations in São Paulo State, Brazil

	2010-2013	2011-2013	2014-2016
CPR all	7.6% (17/225)	9.2% (20/217)	10.9% (65/596)
CPR NRTI	1.3% (3/225)	3.6% (8/217)	3.7% (22/596)
CPR NNRTI	4.8% (11/225)	4.6% (10/217)	6.9% (41/596)
CPR PI	1.3% (3/225)	1.8% (4/217)	2.2% (13/596)
CPR 2/3 class	0.8% (2/225)	0.9% (2/217)	1% (6/596)
IAS all	14.2% (32/225)	17.0% (37/217)	22% (131/596)

Transmitted drug-resistance-mutation (TDR) rates were estimated for each antiretroviral therapy (ARV) class from this study (2014-16) and previous published evaluations conducted by our group using similar sampling and methodology (2010-2013 from Ferreira et al; and 2011-2013 from Guimarães et al; 2015). TDR is shown by ARV class, with cases with resistance to two or three ARV classes and the total number of drug resistance mutations (DRMs) according to the Calibrated Population Resistance (CPR) list and according to the International AIDS Society–USA (IAS–USA) resistance mutation list

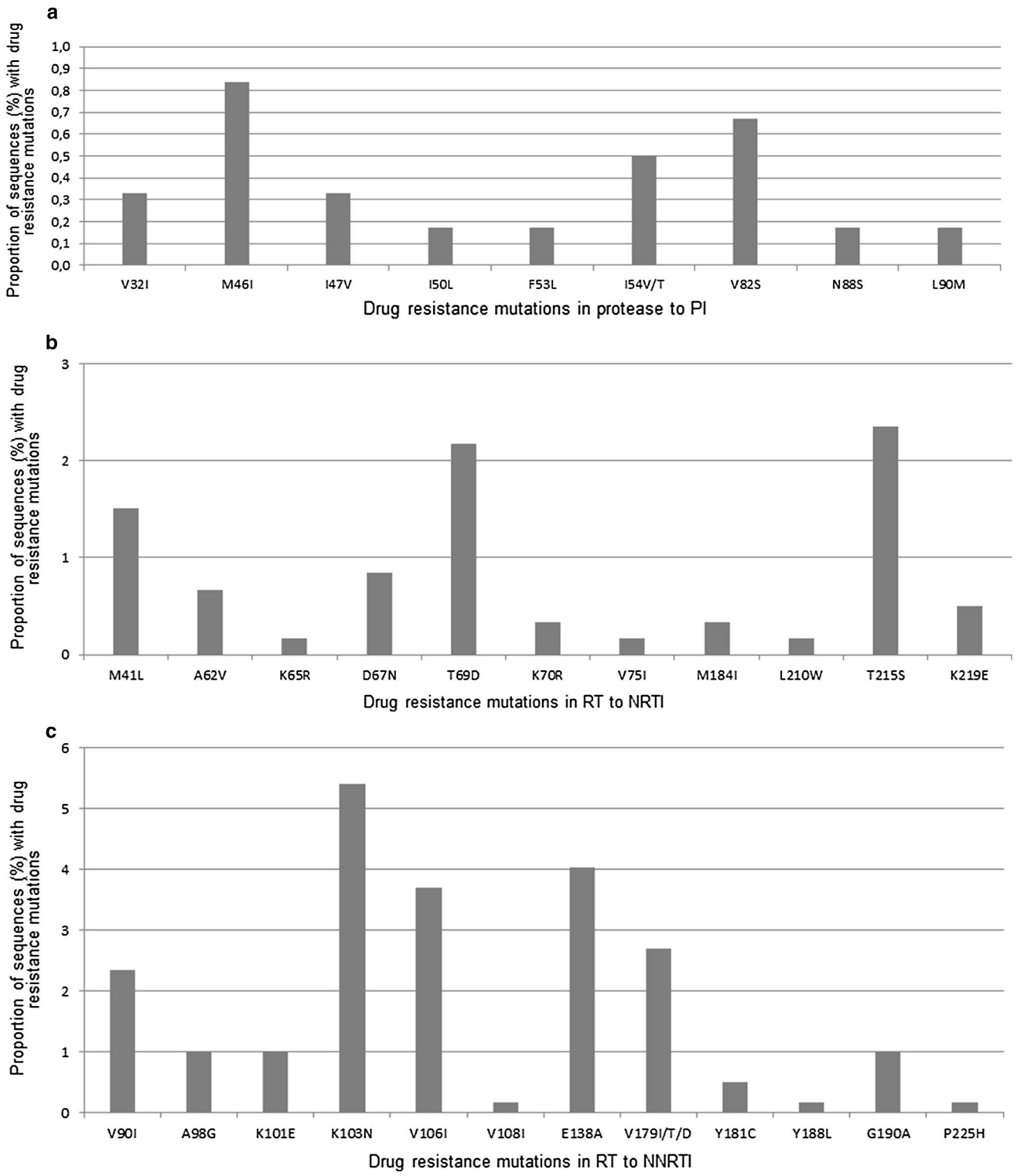

Fig. 1 Proportion of sequences with mutations in the protease and reverse transcriptase (RT) at each resistance-associated codon according to antiretroviral drug class, including a) protease inhibitors, b) nucleoside RT inhibitors (NRTI), and c) non-nucleoside RT inhibitors (NNRTI). Some mutations in RT are shown that are not on the CPR/WHO list, including A62 V and V75I, associated with NRTI resistance, and several minor (accessory) mutations associated with resistance to the NNRTI class (V90I, A98G, V106I, V179I/T/D), as well as one major but polymorphic NNRTI mutation: E138A (according to the Stanford HIV Resistance Database)

patterns (4.3%) with mostly BF mosaics were also identified (Table 1). Women tended to have a higher proportion of HIV-1 F or F mosaics then men (22.5% vs. 10.5%, $p=0.003$). We observed some differences in the rate of TDR within subtypes. At least one mutation was observed in 10.7% of all subtype B (46/428), 4.7% of subtype C, 20.8% of subtype F, and 18.2% of BF mosaic sequences. Using subtype B as the reference, subtype F had comparatively more mutations ($p=0.003$). The proportion of mutations associated with NNRTI resistance was similar (7.4% vs. 5.5%, $p=0.66$) with the observed difference due to mutations associated with NRTI (10.4 vs. 3%, $p=0.011$) and PI (6.3% vs. 1.9%, $p=0.055$) resistance.

Because of the higher proportion of subtype F in women and the possibility of previous undocumented ART exposure from mother-to-child transmission prophylaxis, we analyzed men separately. Although smaller, the difference pattern persisted for NRTI mutations (10.8% vs. 2.6%, $p=0.08$). Similar significant differences were also observed when F genomes were compared to all the other subtypes and recombinant *pol* genomes, with more mutations associated with NRTI resistance (11.3% vs. 2.7%, $p<0.001$) and PIs (5.6% vs. 1.7% $p=0.03$) but not associated with NNRTI resistance (7.0% vs. 6.9%, $p=0.95$).

Subtype C viruses tended to have fewer TDR mutations, but the differences were generally not significant, except when NRTI mutation rate were compared to those of subtype F (0% vs. 14.8%, $p<0.007$).

First-line ART regimens

Data on first-line ART was available for 566 (95%) of the 596 patients. ART was initiated at a median of 99 days (IQR: 45-227) after HIV diagnosis and 49 days (IQR: 14-147) after genotyping sampling, with 54 initiating therapy before the availability of test results. During the study, the most common regimen, tenofovir disoproxil fumarate, lamivudine and efavirenz (TDF/3TC/EFV), was used by 72% of patients following the Brazilian guidelines. Alternative regimens included TDF plus 3TC and atazanavir/ritonavir (TDF/3TC/ATZ/r) in 14% of the patients, zidovudine/3TC and lopinavir/ritonavir (AZT/3TC/LPV/r) in 5%, TDF/3TC/LPV/r in 3%, and other combinations in 6%. Patients used one (70%) to five (0.2%) regimens during the observation period. Although we were not able to document the reason for therapy change, only 3.2% of patients using an NNRTI-based first-line regimen had an NNRTI TDR mutation identified, compared to 11.2% of those initiating a PI-based regimen ($p=0.001$). Moreover, most patients (80%) on an NNRTI-based regimen that harbored an NNRTI TDR changed regimens. However, patients on NNRTI-based regimens were less likely to change than those on other ART combinations (17.6% vs. 33.6%, $p<0.001$). First-line LPV/r-based regimens were the most likely to be changed, with 50% patients changing regimens during the observation period compared to only 25% for the other regimens ($p=0.01$).

Patients with T CD4 counts below 200 cells/μL tended to modify their ART more frequently than those with higher T CD4 counts (32% vs. 20%, $p=0.04$).

Virological Outcome

The VL results after 48 weeks of ART were available in public databases for 421 (75%) of the 566 treated patients. Viral suppression below 1,000 c/mL was attained by 96.4%, below 200 c/mL in 94.8%, and below LoD (40 c/mL) in 91.7% of the patients. Target RNA not detected (TND), the most stringent metric used, suggesting full suppression, was documented in 65.3% of the cases. In a missing-equals-failure analysis, 72% of the 566 treated cases had documented viremia below 1,000 c/mL and 70.5% below 200 c/mL. At more stringent metrics (< LoD or TND), suppression levels were associated with CD4 > 500 cells/μL (TND, $p<0.001$ and < 40 c/mL, $p=0.014$), VL < 100,000 c/mL (TND, $p<0.001$ and < 40 c/mL, $p=0.02$), absence of mutations associated with NNRTI resistance (TND, $p=0.013$ and < 40 c/mL, $p=0.012$), and a younger age (≤ 25 years old) (TND, $p=0.007$ and < 40 c/mL, $p=0.059$). There was no significant association with gender, ART change, type of first-line regimen, non-B subtype, non-NNRTI TDR, or the combination of all TDR and non-TDR mutations associated with NNRTI resistance. To explore predictors of viral suppression, we performed multivariable unadjusted and adjusted analyses using age, CD4 count, VL at sample collection, subtype, and presence of mutations associated with NRTI resistance, NNRTI, or PI. After adjustment, CD4 > 500 cells/μL, the absence of TDR mutations associated with NNRTI resistance, VL < 100,000 c/mL, and age < 26 years old remained independently associated with TND (full suppression) (Table 3).

Discussion

Transmitted drug resistance mutations (TDR) are potential obstacles to therapy success. Some studies report a deleterious impact on the response to first-line therapy [22], while others report limited or no impact [23, 24] in treatment response, especially if patients used non-NNRTI-based regimens [25]. TDR has been observed worldwide [1], and the rates of prevalence are trending down from the relatively high rates of the late 1990s, as documented recently by the CASCADE collaboration in Europe [26], with stable rates in recent years in developed nations [1]. This scenario contrasts with the increasing rates, especially of mutations conferring

Table 3 Influence of baseline factors and treatment modification on the ability to achieve full viral suppression (RNA target not detected) after week 48 of ART

	Unadjusted			Adjusted		
	Odds ratio	*p*	95% CI	Odds radio	*p*	95% CI
Age over 25 years old	1.93	0.007	1.20-3.12	2.04	0.005	1.24-3.37
Male sex	1.04	0.907	0.57-1.90			
CD4 T counts < 500 cell/mm^3	2.15	<0.001	1.41-3.26	1.81	0.008	1.17-2.81
Viral load > 100,000 copies/ml	2.93	<0.001	1.91-4.51	2.60	<0.001	1.66-4.07
Use of more than one regimen	0.92	0.720	0.56-1.49			
Non-NNRTI-based first-line	1.12	0.628	0.71-1.77			
NRTI CPR mutation	0.67	0.509	0.21-2.16			
NNRTI CPR mutation	2.89	0.017	1.20-6.93	2.64	0.045	1.02-6.83
PI CPR mutation	2.40	0.197	0.64-9.09	2.10	0.473	0.47-9.32
Non-B subtype	1.08	0.736	0.69-1.69			

Unadjusted and adjusted logistic regression analysis of variables obtained at baseline (enrollment) or during a 48-week observation period (number of regimens used) to evaluate their association with a lack of full virological suppression after week 48. The table shows data relative to the most stringent (RNA target not detected) metric of successful treatment. Using a viral load below 40 copies/mL as criterion, only the presence of NNRTI mutations remained significant ($p = 0.033$) after adjustment. CI, confidence interval; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor

resistance to the NNRTI drug class, in developing countries where ART has been introduced more recently [1, 27]. Due to HIV diversity, the Stanford CPR mutations list, adopted by WHO, allows comparability of estimates from different regions. The first objective of our study was to assess the prevalence of mutations and compare it to previous studies conducted by us using a similar methodology, and with the literature, to provide a reliable estimate of mutation prevalence (Table 2). In line with the current recommendation for immediate treatment, this study included mostly recently diagnosed patients. Rates of TDR are comparable to those observed in most studies in the region. This finding strongly suggests a stable, or slowly increasing, intermediate level of resistance, mostly to the NNRTI class. A recent meta-analysis of studies in Latin America estimates similar rates and suggests an increasing level of NNRTI TDR [28].

Brazil has changed the recommended first regimen in April 2017 from an efavirenz-based regimen to incorporating dolutegravir as the preferred drug for use with TDF and 3TC. Both of the currently recommended NRTIs were predicted to be fully active against most of the variants (> 97%) evaluated in this study. As the prevalence of resistance to integrase inhibitors among untreated individuals has been shown to be low and the impact of circulating TDR to NRTI antiretroviral drugs used in the dolutegravir-based regimens is low, resistance to this combination should not be a concern at the moment. However, about half a million people were already using ART in Brazil before the new recommendations were issued. Moreover, in many parts of the world, especially in resource-limited settings, NNRTI-based regimens are allowing an impressive scaling up of treatment in the region. The recent finding associating dolutegravir use with neural birth defects [29] resulted in the return to NNRTI-based regimens for women in some places.

Another objective of this study was to address the impact of TDR on virological outcome after one year of ART. This evaluation must consider that test results were sent to clinical services upon availability, and therapy was instituted or changed at the physicians' discretion. Accordingly, the use of NNRTI-based regimens was significantly less frequent in cases with TDR to NNRTI, and most changed their regimen after receiving the genotype test results. Rates of suppression after 48 weeks of therapy were high overall: over 90% for most metrics. This percentage is higher than that reported at the national level [11], where pre-treatment genotype testing is not available, but this comparison cannot be used directly to support the indication of pre-treatment genotyping.

Presence of TDR to NNRTI, lower T CD4 counts, and higher viremia at entry were significantly associated with virological failure applying the LoD (40 copies/mL) VL threshold, but only TDR to NNRTI remained significant in adjusted analysis. As some studies suggest that full viral suppression may be beneficial to patients [14, 15], we also evaluated the impact of TDR and other variables on the RNA target-not-detected (TND) rate, which is a more stringent metric that is not commonly used in clinical settings. With this criterion, we observed significantly lower rates of viral suppression in adjusted analysis in the presence of TDR to NNRTI, T CD4 cell counts lower than 500 cells/μL, viremia above 100,000 c/mL, and older age (Table 3).

Many patients (22%) changed their antiretroviral regimen in the first year. The reasons for the changes were not documented, and although some seemed to have changed

due to the genotype report, adverse effects appears to be common factors [30]. Although we could not evaluate this issue in this study, the lopinavir-based regimen, which is no longer recommended in Brazil, was the most common ART associated with regimen change, suggesting intolerance as a major issue. A third point to highlight in the study is the increase in subtype C in the region. The current study detected 14.3% (IQR: 11.3-16.8) HIV-1 C in the *pol* region (15.5% if C mosaics are considered), which is twice the proportion observed in a previous study (7.1% CI 95%: 4.2-11.1) [18], with C genomes replacing HIV-1 F as the second most prevalent subtype in this area. The fact that fewer mutations were observed in subtype-C-infected individuals might have contributed to the relative stability in TDR estimates in the region.

There are many limitations to our study. Although genotype testing was provided to all public services within the state, logistical issues limited the access to many services, especially in more remote areas. Moreover, even within a given service, some physicians may order tests more often than others, reflecting different clinical care standards. The use of genotype testing to support therapy allows the overall impact on the virological suppression rate in treated individuals to be evaluated, but not the specific impact of TDR in treatment response; therefore, the actual direct impact of the test in the success of therapy cannot be inferred from our study.

Conclusion

TDR rates are stable in the region and may potentially compromise NNRTI-based regimens. Genotype testing may not be needed before the current integrase-based combination but may be important to patients considering NNRTI-based regimens. High rates of suppression were attained after one year of therapy in this setting with the availability of alternative regimens that allowed ART switching to circumvent the potential deleterious effect of TDR or intolerance.

As viral suppression defined by the 200 c/mL threshold has been correlated to transmission protection (https://www.preventionaccess.org/ consensus) it may be a more appropriate goal for therapy than the 1,000 c/mL standard. The use of more-stringent metrics suggests that high viremia, low CD4 T cell counts, older age and, even with genotype testing, mutations conferring resistance to the NNRTI antiretroviral class may be associated with lower rates of suppression at week 48, but longer follow-up is necessary to assess its relevance for long-term clinical outcomes.

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Compliance with ethical standards

Conflict of interest No potential conflicts of interest were identified by the authors.

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