SHORT COMMUNICATION

Drug-resistance development differs between HIV-1-infected patients failing first-line antiretroviral therapy containing nonnucleoside reverse transcriptase inhibitors with and without thymidine analogues*

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Objectives

We evaluated the emergence of drug resistance in patients failing first-line regimens containing one nonnucleoside reverse transcriptase inhibitor (NNRTI) administered with zidovudine (ZDV) + lamivudine (the ZDV group) or non-thymidine analogues (non-TAs) (tenofovir or abacavir, + lamivudine or emtricitabine; the non-TA group).

Methods

Three hundred HIV-1-infected patients failing a first-line NNRTI-containing regimen (nevirapine, $n = 148$; efavirenz, $n = 152$) were included in the analysis. Virological failure was defined as viraemia ≥ 400 HIV-1 RNA copies/mL for the first time at least 6 months after starting the NNRTI-based regimen. For each patient, a genotypic resistance test at failure was available. The presence of drug-resistance mutations in HIV-1 reverse transcriptase was evaluated by comparing patients treated with NNRTI + zidovudine + lamivudine *vs.* those treated with NNRTI + non-TA.

Results

A total of 208 patients were failing with NNRTI + zidovudine + lamivudine and 92 with NNRTI + non-TA. No significant differences were observed between the non-TA group and the ZDV group regarding the time of virological failure [median (interquartile range): 12 (8–25) *vs.* 13 (9–32) months, respectively; $P = 0.119$] and viraemia [median (interquartile range): 4.0 (3.2–4.9) *vs.* 4.0 (3.3–4.7) $\log_{10}$ copies/mL, respectively; $P = 0.894$]. Resistance to reverse transcriptase inhibitors (RTIs) occurred at a significant lower frequency in the non-TA group than in the ZDV group (54.3 *vs.* 75.5%, respectively; $P = 0.001$). This difference was mainly attributable to a significantly lower prevalence of NNRTI resistance (54.3 *vs.* 74.0%, respectively; $P = 0.002$) and of the nucleoside reverse transcriptase inhibitor (NRTI) mutation M184V (23.9 *vs.* 63.5%, respectively; $P < 0.001$) in the non-TA group compared with the ZDV group. As expected, the mutation K65R was found only in the non-TA group (18.5%; $P < 0.001$).

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Conclusions

At first-line regimen failure, a lower prevalence of RTI resistance was found in patients treated with NNRTI + non-TA compared with those treated with NNRTI + zidovudine + lamivudine. These results confirm that the choice of backbone may influence the prevalence of drug resistance at virological failure.

Introduction

Highly active antiretroviral therapy (HAART) has resulted in a reduction in morbidity and mortality among those with HIV infection since its introduction into clinical use [1]. Moreover, use of effective HAART is thought to limit the transmission of HIV, and, as a consequence, of drug-resistant viruses. Emergence of drug resistance has been associated with poorer virological outcomes and increased mortality in patients who receive first-line HAART [2,3].

For this reason, genotypic resistance testing (GRT) has become an important component in the management of HIV infection and is now recommended for both antiretroviral-naïve and drug-experienced patients [4–6].

Currently, regimens containing two nucleos(t)ide reverse transcriptase inhibitors (NRTIs) plus a nonnucleos(t)ide reverse transcriptase inhibitor (NNRTI) as the third agent are among the options strongly recommended by guidelines for first-line therapy [4–7]. In Western countries, the NRTIs generally recommended as a component of first-line regimens are lamivudine (3TC) or emtricitabine (FTC) together with tenofovir (TDF), abacavir (ABC), zidovudine (ZDV) or didanosine (ddI) [4–6], with the choice of NRTI based on individual patient characteristics. The World Health Organization (WHO) currently recommends starting antiretroviral combination regimens with an NNRTI such as nevirapine (NVP) or efavirenz (EFV), in combination with 3TC or FTC and either ZDV [a thymidine analogue (TA) inhibitor] or TDF (a non-TA) [7]. It is well recognized that failure of a first-line regimen that includes two drugs with low genetic barriers to resistance, such as EFV/NVP and 3TC or FTC, is associated with an increased risk of accumulation of resistance mutations [8–11]. This can, in turn, limit therapeutic drug options for second-line regimens. However, the emergence of drug resistance after failure of a first-line regimen containing an NNRTI with 3TC/FTC and either ZDV or a non-TA such as TDF or ABC has not been well characterized. The possibility should be considered that the selection of resistance under ZDV pressure may be intrinsically different from that under non-TA pressure as a result of different genetic barriers to resistance to these drugs [12], differences in penetra-tion into target cells and/or different pharmacokinetics/pharmacodynamics [13]. A recent study showed that patients who experienced virological failure on regimens containing TDF had lower rates of resistance emergence than those who experienced virological failure on regimens containing ZDV [14], probably because of the improved tolerability, adherence and pharmacokinetics of TDF resulting from its once-daily dosage.

In this study we evaluated whether the emergence of drug resistance in patients failing first-line regimens containing an NNRTI administered with ZDV was comparable to that of patients treated with a non-TA, such as TDF or ABC.

Methods

Study population and data collection

HIV-1-infected patients included in this study were selected from two large Italian resistance databases [a database collecting data for HIV-1-infected patients followed at several clinical centres in Rome and the surrounding area, and the multicentre Antiretroviral Resistance Cohort Analysis (ARCA) database; https://www.hivarca.net/] and from the UK Collaborative HIV Cohort (UK CHIC; http://www.ukchic.org.uk/) with linked resistance data from the UK HIV Drug Resistance Database (http://www.ctu.mrc.ac.uk/hivrdb) (see Appendix S1). Eligible individuals were those who experienced virological failure on a first-line regimen that contained one NNRTI administered with either ZDV or a non-TA. Virological failure was defined as a plasma HIV RNA $\geq$ 400 HIV-1 RNA copies/ml for the first time after at least 6 months of uninterrupted use of the NNRTI. Patient information, including details of antiretroviral treatment, viro-immunological values and the results of any resistance tests performed at the time of virological failure, was retrieved from the individual databases and merged centrally.

Resistance analysis

The presence of mutations in the HIV-1 reverse transcriptase (RT) was evaluated by comparing patients treated with ZDV + 3TC *vs.* those treated with the non-TA + 3TC (or

FTC). Drug-resistance mutations of interest were taken from the latest International AIDS Society-USA report [15] and the Stanford database (http://hivdb.stanford.edu). Specifically, resistance to RT inhibitors (RTIs) was considered to exist if at least one NRTI mutation (M41L, A62V, K65N/R, D67N, K70E/G/R, L74I/V, V75I, F77L, Y115F, F116Y, Q151M, M184I/V, L210W, T215F/Y or K219E/Q) or major EFV/NVP mutation (L100I, K101E/P, K103N/S, V106A/M, V108I, Y181C/I/V, Y188C/H/L, G190A/E/S, P225H, M230L or K238T) was present.

Statistical analysis

χ^2 tests (Pearson or Fisher's exact test, where appropriate) for categorical variables and the Mann–Whitney test for continuous variables were used to compare the groups receiving ZDV and a non-TA. The Benjamini–Hochberg method was used to identify results that were statistically significant in the presence of multiple-hypothesis testing [16].

Logistic regression models were fitted to assess whether the emergence of resistance to RTIs (at least one NRTI mutation and/or at least one major EFV/NVP mutation), NRTIs (at least one NRTI mutation) or NNRTIs (at least one major EFV/NVP mutation) was associated with the type of treatment received (ZDV vs. non-TA and NVP vs. EFV), baseline viraemia, baseline CD4 cell count, and the time elapsed from starting the first-line regimen to GRT at failure.

The statistical program used for analyses was SPSS for Windows (version 17.0.1; SPSS, Chicago, IL).

Results

Patient characteristics and virological failure

Three hundred HIV-1-infected patients with failure of a first-line NNRTI-containing regimen were included in the analyses. Of these, 144 patients were Italian (74 were followed in Rome and the surrounding area, and 70 were from ARCA), and 156 were from UK CHIC. Of the 300 patients included in the analysis, 152 (50.7%) were treated with EFV, while the remaining 148 (49.3%) were treated with NVP. Two-hundred and eight patients had experienced virological failure while on a regimen containing ZDV + 3TC, while 92 had experienced virological failure on a regimen containing a non-TA (TDF + 3TC: 36 patients; TDF + FTC: 26 patients; ABC + 3TC: 30 patients). NVP was mainly administered with ZDV + 3TC [ZDV group: 128 (61.5%); non-TA group: 20 (21.7%)], while EFV was mainly administered with a non-TA [ZDV group: 80 (38.5%); non-TA group: 72 (78.3%)] ($P < 0.001$). The characteristics of patients according to treatment are summarized in Table 1. In particular, as expected, patients treated with a non-TA had started their first-line regimen more recently than those treated with ZDV. In particular, the use of a non-TA as part of the first-line regimen in this population

Table 1 Patients' characteristics according to the treatment administered as the first-line regimen

Baseline characteristics	NRTI administered			NNRTI administered		
	ZDV	Non-TA	*P*-value	EFV	NVP	*P*-value
Number of patients	208	92		152	148	
Male (%)	70.6	67.4	0.586	**76.0**	**62.9**	**0.016**
Age (years) [median (IQR)]	33 (26–41)	38 (29–44)	0.103	35 (29–43)	33 (26–41)	0.322
Risk factor (%)						
Heterosexual	42.8	37.0	0.344	**33.6**	**48.6**	**0.008**
Homosexual	**23.6**	**34.8**	**0.043**	**35.5**	**18.2**	**0.001**
Sexual	1.4	4.3	0.124	2.6	2.0	0.729
Drug addiction	9.6	8.7	0.801	7.9	10.8	0.385
Other/unknown	22.6	15.2	0.143	20.4	20.4	0.437
BL viraemia (log copies/mL) [median (IQR)]	5.0 (4.4–5.3)	5.0 (4.1–5.6)	0.599	5.0 (4.2–5.4)	5.0 (4.3–5.4)	0.932
Subtype B* (%)	75.2	70.6	0.590	72.6	75.7	0.590
BL CD4 count (cells/μL) [median (IQR)]	224 (130–348)	150 (59–230)	**< 0.001**	186 (86–253)	222 (104–325)	0.069
Year of therapy start [median (IQR)]	2002 (2000–2003)	2005 (2004–2007)	**< 0.001**	2004 (2001–2006)	2002 (2000–2004)	**< 0.001**
Time from starting first-line regimen to GRT at failure (months) [median (IQR)]	**18 (11–36)**	**15 (9–28)**	**0.011**	18 (10–36)	15 (10–34)	0.613
Time of virological failure (months) [median (IQR)]	13 (9–32)	12 (8–25)	0.119	**14 (9–34)**	**12 (8–25)**	**0.040**

BL, baseline (the time of starting the first-line regimen); EFV, efavirenz; GRT, genotypic resistance test; IQR, interquartile range; NNRTI, nonnucleos(t)ide reverse transcriptase inhibitor; NRTI, nucleos(t)ide reverse transcriptase inhibitor; non-TA, non-thymidine analogue; NVP, nevirapine; ZDV, zidovudine.
*Subtype information was available for about 50% of the population. Pearson χ^2 (for categorical variables) and the Mann–Whitney test (for continuous variables) were used, where appropriate, to compare the following groups: (1) patients treated with a zidovudine (ZDV) regimen and those treated with a non-thymidine analogue (non-TA) regimen (columns 2–4); (2) patients treated with an efavirenz (EFV) regimen and those treated with a nevirapine (NVP) regimen (columns 5–7). In bold are indicated statistically significant differences ($P < 0.05$) in clinical and demographic parameters between the two groups.

significantly increased over the years (from about 4% before 2001 to 100% in 2009; $P < 0.001$ by χ^2 test for trend; data not shown).

At the time of virological failure, no significant differences were observed between the ZDV and non-TA groups regarding the time of virological failure [median (interquartile range (IQR)) for the non-TA group: 12 (8–25) months; for the ZDV group: 13 (9–32) months; $P = 0.119$] or the viral load at failure [median (IQR) for the non-TA group: 4.0 (3.2–4.9) $\log_{10}$ copies/mL; for the ZDV group: 4.0 (3.3–4.7) $\log_{10}$ copies/mL; $P = 0.894$]. However, a significant difference was observed between the two groups in terms of the duration of therapy at the time of the GRT [median (IQR) for the non-TA group: 15 (9–28) months; for the ZDV group: 19 (11–37) months; $P = 0.011$] (Table 1).

Of note, in the non-TA group no significant differences in the patients' characteristics were observed between patients treated with TDF and those treated with ABC (data not shown).

Genotypic resistance

In general, RTI resistance occurred at a lower frequency in the non-TA group compared with the ZDV group (54.3 *vs.* 75.5%, respectively; $P = 0.001$) (Fig. 1). This difference was mainly attributable to a significantly lower prevalence of NNRTI resistance (54.3 *vs.* 74.0%, respectively; $P = 0.002$)

and of the 3TC/FTC mutation M184V (23.9 *vs.* 63.5%, respectively; $P < 0.001$) in the non-TA group. In contrast, M184I was found more frequently in the non-TA group (3.3 *vs.* 1.0% in the ZDV group; $P = 0.12$).

Of note, a smaller proportion of patients with at least one TA mutation (M41L, D67N, K70R, L210W, T215F/Y or K219E/Q) was observed in the non-TA group than in the ZDV group (8.7 *vs.* 14.4%, respectively; $P = 0.126$). The K103N mutation was the most common major EFV/NVP mutation detected in both groups (non-TA group: 22.8%; ZDV group: 38.9%; $P = 0.010$). Regarding ABC/TDF resistance mutations, as expected, the mutations K65R and Y115F were found only in the non-TA group (K65R: 18.5%; $P < 0.001$; Y115F: 3.3%; $P = 0.031$), while the ABC resistance mutation L74V was found almost exclusively in the non-TA group (L74V: 4.3 *vs.* 0.5% in the ZDV group; $P = 0.032$), in particular only in patients treated with ABC (13.3 *vs.* 0% in TDF-treated patients; $P = 0.010$).

No other significant differences in the mutation prevalence were observed between patients treated with TDF and those treated with ABC (data not shown).

Interesting findings were obtained for the NNRTI mutations at RT positions 106, 181 and 190. In particular, regarding the position 106, the V106M was the only EFV/NVP mutation observed in both AZT-group (3.8%) and non-TA-group (9.8%, $P = 0.048$). The mutation Y181I was completely absent in the overall population, while the
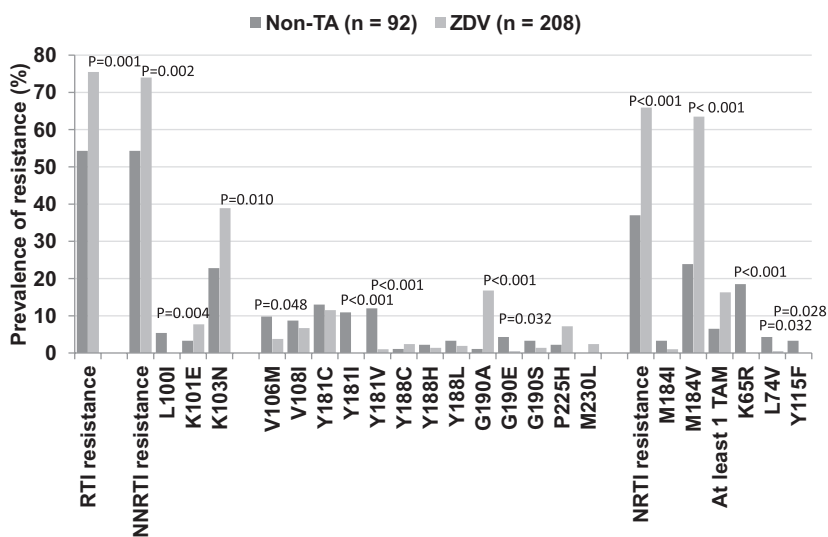

Fig. 1 Comparison of the prevalence of resistance mutations at first failure between the group of patients treated with a nonnucleos(t)ide reverse transcriptase inhibitor (NNRTI) + zidovudine (ZDV) + lamivudine (3TC) and the group treated with an NNRTI + a non-thymidine analogue (non-TA) + 3TC/emtricitabine (FTC). Statistically significant differences in the mutation prevalence were assessed using Fisher's exact test. All *P*-values in the figure are for differences that were also significant after correction for multiple tests. The NNRTI mutation K101P and the nucleos(t)ide reverse transcriptase inhibitor (NRTI) mutations K70G and L74I were completely absent in the analysed population. The following reverse transcriptase inhibitor (RTI) mutations, with an overall prevalence of < 1.7% ($n = 5$ patients), are not shown in the figure: the NNRTI K103S, V106A and K238T and the NRTIs K65N and K70E. TAM, thymidine analogue mutation.

Table 2 Multivariable logistic regression models of factors predictive of the occurrence of drug resistance at failure of a first-line regimen containing a nonnucleos(t)ide reverse transcriptase inhibitor (NNRTI) + zidovudine (ZDV) + lamivudine (3TC) or an NNRTI + a non-thymidine analogue (non-TA) + 3TC/emtricitabine (FTC)

Factor	RTI resistance		NRTI resistance		NNRTI resistance	
	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
ZDV vs. non-TA	**2.12 (1.03–4.34)**	**0.039**	**2.92 (1.46–5.84)**	**0.002**	2.02 (0.98–4.14)	0.055
NVP vs. EFV	**2.15 (1.08–4.28)**	**0.029**	1.71 (0.90–3.21)	0.099	**2.23 (1.12–4.45)**	**0.023**
Baseline viraemia (per 1 log_{10} copies/mL increase)	**1.39 (1.00–1.91)**	**0.046**	**1.49 (1.07–2.06)**	**0.017**	**1.45 (1.04–2.00)**	**0.025**
Baseline CD4 count (per 50 cells/μL increase)	0.95 (0.89–1.02)	0.143	0.98 (0.92–1.05)	0.625	0.93 (0.87–1.00)	0.064
Time from starting first-line regimen to GRT at failure (per 6 months more)	**0.90 (0.82–0.98)**	**0.018**	0.93 (0.86–1.02)	0.127	**0.89 (0.82–0.98)**	**0.015**

Three logistic regression models were built to evaluate factors independently associated with reverse transcriptase inhibitor (RTI), nucleos(t)ide reverse transcriptase inhibitor (NRTI) or nonnucleos(t)ide reverse transcriptase inhibitor (NNRTI) resistance. Factors statistically significant in multivariable analyses ($P < 0.05$) are indicated in bold.
CI, confidence interval; EFV, efavirenz; GRT, genotypic resistance testing; NRTI, nucleos(-t)ide reverse transcriptase inhibitor; NVP, nevirapine; RTI, reverse transcriptase inhibitor.

mutation Y181V was nearly absent in the non-TA group (1.0 vs. 12.0% in ZDV group; $P < 0.001$). The mutation G190A was found more frequently in the ZDV group (16.8 vs. 1.1% in the non-TA group; $P < 0.001$), while G190E was found more frequently in the non-TA group (4.3 vs. 0.5% in the ZDV group; $P = 0.032$).

Results from the multivariable logistic regression (after adjusting for the type of treatment, baseline viraemia, baseline CD4 cell count and the time elapsed from starting the first-line regimen to GRT at failure) confirmed that RTI resistance was more common in patients failing regimens containing ZDV than in those failing regimens containing a non-TA [adjusted odds ratio (AOR) 2.12; 95% confidence interval (CI) 1.03–4.34; $P = 0.039$] and more common in those failing a regimen containing NVP than in those failing a regimen containing EFV (AOR 2.19; 95% CI 1.11–4.33; $P = 0.002$). As expected, ZDV use was an independent predictor of NRTI resistance (AOR 2.62; 95% CI 1.34–5.14; $P = 0.01$), while NVP use was associated with NNRTI resistance (AOR 2.28; 95% CI 1.15–4.39; $P = 0.017$) (Table 2). The occurrence of both NRTI and NNRTI resistance was also associated with higher baseline viraemia. Moreover, a longer time from starting the treatment to GRT at failure was associated with a reduced risk of RTI resistance, in particular NNRTI resistance. No effect of subtype on the occurrence of resistance was found in the subgroup of patients for whom this variable was available (data not shown).

Discussion

Analysing a cohort of patients failing a first-line NNRTI-containing regimen, treatment with regimens that included a non-TA (with 3TC/FTC) was associated with a reduced risk of occurrence of drug resistance at the time of failure

compared with treatment with ZDV. This difference was mainly attributable to a significantly lower prevalence of NNRTI resistance and of the NRTI mutation M184V in the non-TA group. To our knowledge, few data are available for the comparison of the emergence of resistance after ZDV and non-TA failure [14]. This information is important for clinicians and patients when choosing a new therapy after first-line regimen failure, particularly in resource-limited settings, where drug choices for second-line regimens are limited and GRTs at failure are not always available.

As expected, we found that the ABC/TDF-associated mutation K65R was present exclusively in patients failing regimens that included a non-TA, and the ABC mutation L74V was exclusively present in ABC-treated patients, while a higher proportion of TA mutations was found in patients failing regimens that contained ZDV.

It is noteworthy that our resistance results are very similar to those found in a study conducted in the Swiss HIV Cohort, where 45 NNRTI failures were analysed (prevalences of K65R, K103N + Y181C, M184IV and at least one TA mutation were 8, 39, 58 and 13%, respectively) [17]. The overall prevalence of RTI resistance observed in our study (69.3%) is also similar to that observed in a meta-analysis of clinical trials comparing several regimens with NNRTI + different backbones, performed by Gupta et al. (65.7%), with a similar prevalence of K65R (5.7% in our study vs. 5.3% in Gupta et al.) [10]. However, the prevalence of other mutations in our study was slightly higher compared with that found in Gupta et al.'s meta-analysis. Indeed, in our study the prevalences of NNRTI resistance, M184IV, and at least one TA mutation were 68.0, 52.7 and 13.3%, respectively, compared with 53.0, 35.3 and 1.5%, respectively, in Gupta et al.'s study [10]. The lower prevalence of resistance found in clinical trials may be a consequence of the fact

that studies of this type often do not adequately reflect the 'real life' of clinical practice. For instance, very frequently in clinical trials, patients with specific characteristics are excluded, such as those infected with a resistant virus or patients with unstable lifestyles (e.g. injecting drug users), which may have a behavioural effect that cannot be adequately controlled for (e.g. nonadherence). Moreover, our study is a retrospective study, and GRT was not always immediately performed at virological failure or at the same time for all the patients (as is generally the case in clinical trials).

Multivariable analysis confirmed the association between an increased likelihood of RTI resistance and the use of ZDV, with ZDV use being an independent predictor of NRTI resistance. Moreover, multivariable analysis also highlighted the greater prevalence of NNRTI resistance in individuals with failure of regimens containing NVP. Both NRTI and NNRTI resistance was associated with higher baseline viraemia. We did not find an effect of subtype on the prevalence of RTI resistance, although this information was only available in around 50% of patients, of whom around 70% were infected with subtype B, and thus these analyses may have been under-powered. Thus, further studies are required to better elucidate the role of subtype in the emergence of resistance after first-line failure. Of note, while our high prevalence of subtype B is consistent with other studies which have reported a very high prevalence of B subtype in Western and Central Europe [18], most HIV-1 infections world-wide, particularly in resource-limited settings, are caused by other non-B subtypes [18,19]. Our study has other limitations. An important limitation of our analysis is the lack of information regarding patients' adherence to antiretroviral therapy, a crucial factor in the achievement and maintenance of virological success. Another limitation is the lack of primary resistance information; we could not exclude the possibility that some of the resistance mutations found at failure may already have been present at baseline, prior to treatment. For example, this was probably the case for the eight patients harbouring a virus with at least one TA mutation (T215 revertants, two patients; D67N, three patients; T1215F, one patient; M41L, one patient; M41L + D67N + L210W + T215F, one patient) and treated with a non-TA, as TA mutations are generally not selected during the use of non-TA-containing regimens. Moreover, as patients were selected with no randomization, we cannot exclude the possibility of unmeasured confounding. The retrospective design did not permit a detailed description of the emergence of mutations over time, as the GRT was performed at varying times after virological failure. This may have led to the inclusion of patients with different mutation patterns as a result of different durations of

selective pressure exerted by antiretroviral drugs on viraemic individuals. However, when the time from starting the first-line regimen to GRT at failure was included among the covariates in the multivariable analysis, ZDV use (as well NVP use) still remained independently associated with RTI resistance development. Moreover, the number of patients treated with non-TAs was small in comparison to those treated with ZDV.

Nevertheless, our findings strongly suggest that the use of a non-TA in the first-line regimen reduces the probability of emergence of RTI resistance during failure. In conclusion, the results obtained in this study confirm that the choice of backbone may influence the prevalence of drug resistance at virological failure.

Acknowledgements

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Appendix S1. Antiretroviral Resistance Cohort Analysis (ARCA) group (members and centres).