Residual phenotypic susceptibility to doravirine in multidrug resistant HIV-1 from subjects enrolled in the PRESTIGIO Registry

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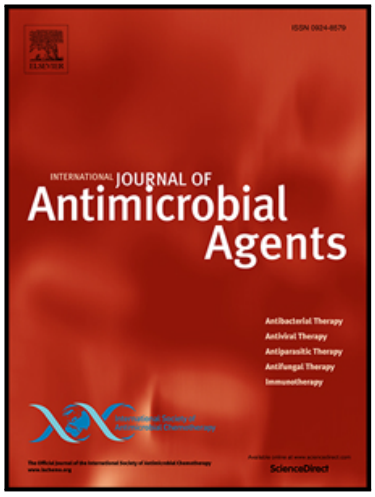

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Residual phenotypic susceptibility to doravirine in multidrug resistant HIV-1 from subjects enrolled in the PRESTIGIO Registry

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Running title: *In vitro* susceptibility to second-generation NNRTI in MDR HIV-1

Keywords: doravirine, rilpivirine, etravirine, *in vitro* susceptibility, multi-drug resistance, HIV-1

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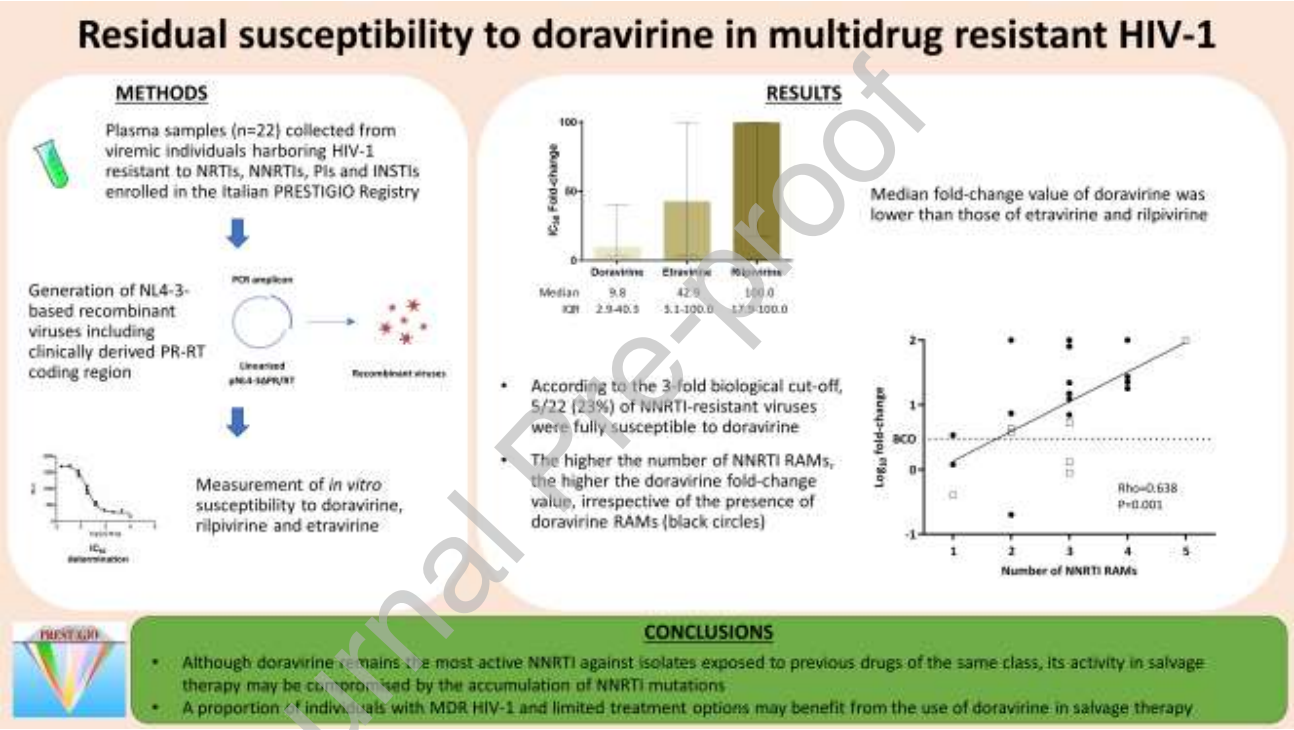
Graphical Abstract



Highlights

- Doravirine showed higher activity compared to etravirine and rilpivirine in MDR HIV-1
- Full susceptibility to doravirine was retained in 23% of NNRTI resistant viruses
- The higher the number of NNRTI mutations, the higher the resistance to doravirine
- Resistance to doravirine has been detected even in the absence of doravirine RAMs
- Stanford HIVdb algorithm predicted doravirine activity with fair accuracy

Abstract

Background

Doravirine shows a rather distinct resistance profile within the NNRTI class. This study aimed to evaluate the phenotypic susceptibility to doravirine, rilpivirine and etravirine in a panel of multidrug-resistant (MDR) HIV-1 isolates collected from people living with HIV (PLWH) enrolled in the PRESTIGIO Registry.

Methods

Recombinant viruses expressing PLWH derived protease-reverse transcriptase coding region were generated from plasma samples at virological failure with documented resistance to PIs, NRTIs, NNRTIs and INSTIs. *In vitro* susceptibility was assessed through a phenotypic assay measuring fold-change values with respect to the reference NL4-3 virus. Genotypic susceptibility was computed by the Stanford HIVdb algorithm 8.9-1.

Results

Plasma samples were collected from 22 PLWH, twenty (91%) were male, median age 55 years (IQR 50-58), time since HIV-1 diagnosis 27 years (23-31), time on ART 23 years (22-26). Median doravirine, etravirine and rilpivirine fold-change values were 9.8 (2.9-40.4), 42.9 (3.1-100.0) and 100.0 (17.9-100.0), respectively. According to the fold-change cut-offs, full susceptibility was observed in 5 (23%), 4 (18%) and 1 (5%) cases with doravirine, etravirine and rilpivirine, respectively. Irrespective of the presence of specific doravirine mutations, higher numbers of NNRTI mutations correlated with higher fold-change values for doravirine. By comparing the distribution of fold-change values with the Stanford HIVdb predicted susceptibility, a significant correlation was detected for doravirine and rilpivirine but not etravirine.

Conclusion

Despite extensive cross-resistance among NNRTIs, doravirine can be a valid option in a proportion of PLWH with MDR HIV-1. Doravirine activity appeared to be inferred with fair accuracy by HIVdb algorithm.

1. Introduction

Doravirine is the latest nonnucleoside reverse transcriptase inhibitor (NNRTI) approved for the treatment of HIV-1-infected therapy naïve people living with HIV (PLWH) or as a switch option in virologically suppressed PLWH without past or present evidence of resistance to the NNRTI class [1,2]. Clinical studies showed that doravirine had non-inferior efficacy, improved pharmacokinetics and/or safety profile both in first-line therapy and as switch option in virologically suppressed PLWH, compared with the standard of care [3-5]. In addition, doravirine efficacy was documented in a small group of therapy naïve individuals with the transmitted NNRTI mutations K103N and G190A [6].

Emergent resistance to doravirine in clinical trials led to different combinations of the mutations A98G, V106A/I/M, V108I, Y188L, H221Y, P225H, F227C, Y318F [7], while the individual NNRTI mutations G190E/S and M230L were found to reduce doravirine activity *in vitro* [8-10]. This pattern is relatively distinct from those involved in resistance to the other NNRTIs. Indeed, doravirine has shown full activity against 92.5% of viruses included in a large panel of clinical isolates, even in presence of the most common single NNRTI mutations except for Y188L and Y318F. In addition, doravirine has shown to retain full activity in presence of multiple NNRTI mutations and in more than half of isolates resistant to the other NNRTIs [11]. Considering the low prevalence of doravirine resistance associated mutations (RAMs) in both treatment naïve and experienced

individuals [12-14], together with the limited cross-resistance with etravirine and rilpivirine [9,11], the use of doravirine in combination with the investigational nucleoside reverse transcriptase translocation inhibitor islatravir and an optimized background therapy is under clinical evaluation in subjects harboring NNRTI and nucleoside reverse transcriptase inhibitor (NRTI) RAMs (NCT04233216). This clinical trial was further supported by *in vitro* experiments where the combination of doravirine and islatravir exhibited a higher genetic barrier to resistance with respect to the combination of doravirine/lamivudine and dolutegravir/lamivudine [15]. However, a previous *in vitro* study on a small panel of NNRTI resistant clones showed that doravirine susceptibility was affected by multiple NNRTI RAMs, suggesting that phenotypic investigation might be needed to support treatment decision with complex resistance patterns [16]. Aiming to add further data on doravirine activity and on the cross-resistance with the other second-generation NNRTIs, we evaluated the phenotypic susceptibility to doravirine, etravirine and rilpivirine in a panel of multidrug resistant HIV-1 isolates collected from heavily treatment experienced individuals enrolled in the Italian PRESTIGIO Registry.

2. Materials and Methods

2.1 Patients and samples

Plasma samples were collected from individuals enrolled in the Italian PRESTIGIO Registry (NCT04098315), which includes PLWH with documented genotypic resistance to NRTIs, NNRTIs and protease inhibitors (PIs) plus either genotypic resistance to integrase strand transfer inhibitors (INSTIs) or virological failure to an INSTI regimen without an integrase genotype. Genotypic resistance to a drug class was defined as at least intermediate resistance to at least one drug in the class, according to the Stanford HIVdb algorithm, version 8.9-1.

The PRESTIGIO Registry was approved by the San Raffaele Scientific Institute Ethical Committee with protocol number 41/int/December_2017 and the use of residual, anonymized clinical samples for research studies was regulated by patient informed consent. The collection of clinical information and biological samples is allowed once the Ethics Committee of each participating centers has approved the participation in the Registry. Demographic, clinical, and virological data of multidrug resistant PLWH were retrieved from the PRESTIGIO Registry database. The Prestigio Registry has generated studies aiming to evaluate the effectiveness of different antiretroviral regimens and the evolution of the genotype and phenotypic susceptibility of antiretroviral drugs used in highly treatment experienced PLWH with virological failure [17-21].

2.2 Cells and reagents

293T Lenti-X cells (Takara Bio, Kusatsu, Japan) were cultured in DMEM high glucose with L-glutamine, supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. The TZM-bl cell line was obtained from the Centre for AIDS Reagent of the National Institute for Biological Standards and Control and cultured in DMEM high glucose with L-glutamine, supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin. All cell culture media and reagents were obtained from EuroClone (Italy).

2.3 Antiviral drugs

The NNRTI etravirine and rilpivirine were obtained through the NIH AIDS Reagent Program, while doravirine was purchased from MedChemExpress (Monmouth Junction, NJ, USA).

2.4 Generation of recombinant viruses

The protocol for the generation of recombinant viruses consisted in a homologous recombination between a modified NL4-3 vector lacking the region encompassing the GAG cleavage sites, the protease and the first 290 aminoacids of reverse transcriptase (pNL4-3ΔPR-RT, HXB2 nucleotide coordinates of deletion 1850-3420) and a clinically derived PCR fragment corresponding to the deletion [22]. The plasmid was generated by reverse PCR using primers including the SacII restriction enzyme sequences, while the PCR fragment had a 109- and 171-base pair overlap with the ends of linearized pNL4-3ΔPR-RT. For the amplification of the target region, viral RNA was extracted from the bottom 0.4 mL of plasma following centrifugation at 20,000 g for 90 minutes, by using the EZ1 automatic system and the DSP Virus Kit (Qiagen) according to the manufacturer's instructions. The reverse transcription and first-round PCR were performed using the SuperScript III One-Step RT-PCR System with Platinum Taq High Fidelity (Invitrogen) using the primers P210 (5'-ACCCTTCAGGAACAAATAGSATGGA-3', HXB2 nucleotide coordinates 1513-1537) and P220 (5'-TTCTGCTATTAAGTCTTTTGMTGGGTCRTA-3', HXB2 3504-3533). Two microliters of the first-round PCR were used as the template for a nested PCR including the Q5 Hot Start High-Fidelity DNA Polymerase (New England Biolabs) and the primers P240 (5'-CAAAGGAACCCTTYAGAGAYTATGT-3', HXB2 1655-1679) and P533 (5'-GCTAYTAARTCTTTTGWTGGGTCATA-3', HXB2 3502-3529). Triplicate nested PCRs of each sample were purified, combined with 10 µg of linearized pNL4-3ΔPR-RT and co-transfected in 293T Lenti-X cells through a calcium phosphate method as previously described [22]. Supernatants harboring recombinant viruses were harvested 48 hours post transfection and expanded in MT-2 cells to increase viral titers. In presence of large cellular syncytia, supernatants were harvested and stored at -80°C.

2.5 Determination of the *in vitro* susceptibility to doravirine, rilpivirine and etravirine

In vitro susceptibility to doravirine, rilpivirine and etravirine was determined in duplicate through a TZM-bl cell-based assay previously shown to correlate well with the reference phenotypic Phenosense Assay in the measurement of susceptibility to HIV-1 protease, reverse transcriptase, and integrase inhibitors [22]. Briefly,

10,000 TZM-bl cells/well were infected with the wild-type NL4-3 strain or NNRTI resistant viruses at multiplicity of infection of 0.03 in the presence of five-fold dilution of doravirine, rilpivirine (range 10 µM – 0.00512 nM) and etravirine (range 5 µM – 0.00256 nM). After 48 hours, cells were treated with the Glo-Lysis buffer (Promega, Madison, WI, USA) and the Bright-Glo Luciferase Assay (Promega), then relative luminescence units were measured through the GloMax Discover instrument (Promega) and elaborated with GraphPad software to calculate half-maximal inhibitory concentration (IC_{50}) values. Fold-change (FC) values were calculated with respect to the IC_{50} value obtained with the NL4-3 wild-type strain. Viruses with FC >100 were considered as FC = 100 for statistical analyses. To infer drug activity based on phenotypic FC values, available drug-specific cut-offs from Monogram Biosciences were considered including 3-fold and 2.5-fold as biological cut-off for doravirine and rilpivirine, respectively, and 2.9-fold and 10-fold as the lower and upper clinical cut-off for etravirine, respectively.

2.6 HIV-1 sequencing, subtyping and genotypic prediction of drug activity

The reverse transcriptase sequences within PCR amplicons generated to produce recombinant viruses were obtained by Sanger population sequencing using primers P214 (5'-TTTGCCAGGAAAATGGAAACCAAAAATGAT-3', HXB2 2363-2392) and P533. The HIV-1 subtype was assigned by using the COMET HIV-1 subtyping tool [23]. According to the rules of Stanford HIVdb algorithm, the following NNRTI mutations with score equal to or higher than 15 were considered as associated with resistance to doravirine: A98G, L100I, K101E, V106A/M, Y181I/V, Y188F/L, G190E/S/Q, P225H, F227C/I/L/V, M230I/L, L234I.

2.7 Statistical analysis

FC values calculated for the three NNRTIs were compared by Friedman test followed by pairwise comparisons by Dunn's test with Bonferroni correction. The Spearman test was used to test the correlation between FC

values for each pair of drugs. The Jonckheere-Terpstra test was used to analyze the association of the phenotypic drug susceptibility with the number of NNRTIs used and with the Stanford HIVdb susceptibility level. The Mann-Whitney test was used to compare phenotypic susceptibility values depending on exposure to the different NNRTIs. All statistical analyses were performed by SPSS (IBM Corporation) version 20.

3. Results

3.1 Characteristics of the study population

Samples were collected from 22 PLWH with a median age of 55 years (IQR 50-58), 20 (91%) males, a median time since HIV-1 diagnosis of 27 years (IQR 23-31) and a median time on antiretroviral therapy of 23 years (IQR 22-26) (Table 1). At sample collection, 9, 5 and 8 PLWH had been exposed to 1, 2 and 3 NNRTIs, respectively, with a median time of cumulative exposure to NNRTIs of 47 months (IQR 10-71). At the time of sampling, 10 and 1 PLWH were on treatment with etravirine and rilpivirine, respectively. Viral sequences were attributed to subtype B in 20 cases and subtype F1 in two cases.

3.2 Phenotypic susceptibility to doravirine, etravirine and rilpivirine

Recombinant viruses had different NNRTI RAM burdens, ranging from one (3/22 cases, 14%), two (5/22, 23%), three (9/22, 41%), four (4/22, 18%) to five (1/22, 5%) mutations, while major Stanford HIVdb doravirine RAMs were detected in 17/22 (77%) viruses (table 2). NRTI and PI RAMs included in the recombinant viruses have been described in the supplementary table 2. Doravirine, etravirine and rilpivirine showed the lowest FC value in 14/22 (64%), 6/22 (27%) and 0/22 (0%) cases, respectively (Figure 1). Indeed, the median doravirine FC value (9.8, IQR 2.9-40.4) was significantly lower than the median rilpivirine FC value (100.0, IQR 17.9-100.0) ($P < 0.001$) but not than the median etravirine FC value (42.9, IQR 3.1-100.0) ($P = 0.211$), while etravirine and

rilpivirine did not differ from each other (P = 0.071). However, there was a significant correlation between the FC values for any pair of drugs (doravirine vs. etravirine: rho = 0.517, P = 0.014; doravirine vs. rilpivirine: rho = 0.762, P <0.001; etravirine vs. rilpivirine: rho = 0.785, P <0.001).

Cases with FC >100 were common for rilpivirine and etravirine but infrequent for doravirine (15, 10 and 4, respectively). In two cases, all the drugs showed an FC value higher than 100, indicating complete lack of NNRTI activity. One of these recombinant viruses (RV-14) had a complex pattern of NNRTI mutations but none of them was considered as a major doravirine RAM, although alternative mutations occurred at positions involved in doravirine resistance such as 100, 101 and 190. The other virus (RV-16) harboured mutations E138K and G190E, the latter being among the individual NNRTI mutations able to cause a substantial reduction of doravirine susceptibility [10].

Despite sharing the same NNRTI RAMs, RV-13 and RV-15 showed substantially different levels of phenotypic resistance to all the drugs, with RV-13 more resistant to doravirine (4.1-fold), etravirine (10.3-fold) and rilpivirine (>6.9-fold) compared with RV-15. These two viruses differed also for the viral subtype (F1 for RV-13 and B for RV-15) and for the accompanying NRTI RAMs (D67N, K70R, T215L, K219E for RV-13; M41L, A62AV, D67N, K70G, V75I, M184MV, L210W, T215Y, K219Q for RV-15).

Based on currently available biological or clinical cut-offs, predicted full in vivo susceptibility was observed in few cases, namely 5 (23%), 4 (18%) and 1 (5%) cases with doravirine, etravirine and rilpivirine, respectively, while an additional 3 cases had intermediate susceptibility to etravirine. Notably, full susceptibility to all the three NNRTIs was predicted only for RV-17, harbouring the singleton K103N mutation. The other cases with predicted susceptibility to multiple NNRTIs included RV-11 and RV-15, both susceptible to doravirine and etravirine. One isolate with the uncommon singleton A98G mutation (RV-9) retained full susceptibility to etravirine and FC values slightly above the biological cut-offs for doravirine and rilpivirine.

The cumulative number of NNRTIs included in the current plus past treatments did not correlate with the FC value measured to any of the three NNRTIs (supplementary table 1). Similarly, FC values calculated for doravirine did not correlate with the time of exposure to NNRTI (rho = 0.082, P = 0.718), with the time elapsed since last exposure to NNRTI (rho = -0.237, P = 0.288). The inclusion of etravirine (n=10) or rilpivirine (n=1) in the failing regimen at sample collection was associated with higher median FC values for etravirine (100.0, IQR 48.0-100.0 with vs. 4.0, IQR 0.5-26.0 without; P = 0.004) and rilpivirine (100.0, IQR 100.0-100.0 with vs. 30.6, IQR 3.9-100.0 without; P = 0.029) but not for doravirine (17.9, IQR 7.4-80.1 with vs. 4.4, IQR 0.9-27.1 without; P = 0.145). Notably, only 2/10 cases where etravirine was included in the failing regimen showed full phenotypic susceptibility to doravirine and all the three cases of exposure to both etravirine and rilpivirine were associated with high levels of phenotypic resistance to doravirine (RV-8, RV-14, RV-16).

3.3 Comparison of genotypic and phenotypic resistance

When analyzing the distribution of FC values according to the predicted susceptibility as determined by Stanford HIVdb, a significant correlation was detected for doravirine and rilpivirine (P <0.001 and P = 0.001, respectively), but not for etravirine (P = 0.131) (Figure 2). Interestingly, higher numbers of Stanford HIVdb major NNRTI RAMs positively correlated with higher FC values calculated for doravirine (P = 0.001), with viruses harboring two or more NNRTI RAMs showing FC values higher than the biological cut-off irrespective of the presence of major doravirine RAMs (figure 3).

4. Discussion

Clinical trials have demonstrated that doravirine may represent a valuable treatment option for both naïve and virologically suppressed PLWH due to improved genetic barrier to resistance compared with past NNRTIs,

excellent tolerability, and low potential for drug-drug interactions [24]. However, clinical studies are still needed to better define the role of doravirine, both in naïve and treatment experienced individuals. Firstly, clinical data are required to compare the efficacy and safety profile of doravirine with respect to second-generation INSTI based regimens, which are mostly recommended as first-line treatment. Second, clinical studies addressing the role of doravirine in the presence of transmitted or acquired resistance to past NNRTIs are eagerly awaited to complete the assessment of drug profile, particularly in low-middle income countries. For example, a recent analysis revealed that the prevalence of predicted doravirine resistance in NNRTI-experienced individuals is higher in a South African cohort than in two European study populations (84.8% vs. 42.0% and 18.8%, respectively) [12,13,25].

As previously reported [11], the improved antiviral activity of doravirine with respect to etravirine and rilpivirine against NNRTI-resistant isolates was confirmed in this study, with a panel of 22 recombinant viruses from PLWH with resistance to the four main antiretroviral classes. When considering the provisional 3-fold biological cut-off, full susceptibility to doravirine was observed in 5 (23%) of NNRTI resistant viruses, as compared with 4 (18%) to etravirine and only 1 (5%) to rilpivirine. Although doravirine had the lowest reduction in FC values compared to the other NNRTIs, it must be noted that the pairwise difference was significant with respect to rilpivirine but not to etravirine. As a further caveat, it must be emphasized that almost all the isolates (19/22) had been exposed to etravirine, including concomitant exposure at the time of sampling in 10 cases, as opposed to none to doravirine. Thus, the sample panel was strongly biased towards selection of RAMs by etravirine which may have favored disproportionally loss of phenotypic activity with etravirine, while saving activity for doravirine. Analysis of a complementary panel of viruses, i.e. isolates with emergent resistance to doravirine and with no exposure to etravirine, is needed to complete the assessment of cross-resistance between doravirine and etravirine. Preliminary data from the few cases of first-line doravirine failures in clinical trials suggest maintenance of full or partial etravirine activity [10]. In addition, the prediction of in vivo activity could be based on clinical cut-offs for etravirine but not for doravirine which is currently

interpreted based on a provisional biological cut-off. Determining a clinical cut-off for doravirine may be helpful to better compare the role of these two NNRTIs in the context of prior exposure and resistance to this class of drugs.

Each isolate had a unique set of NNRTI RAMs, with one exception. RV-13 and RV-15 shared the same RAM pattern, however FC values were significantly different from each other for all the three drugs. This highlights the possibility that additional mutations not currently acknowledged as NNRTI RAMs modulate susceptibility to NNRTIs. Alternatively, the genetic background of the different subtypes involved (B and F1) and/or some effects of NRTI RAMs [11,26] may have played a role.

It must be noted that recombinant viruses harbored a clinically derived fragment including the first 290 aminoacids of the reverse transcriptase, thus excluding mutation Y318F which has been shown to be associated with significant reduction of doravirine susceptibility *in vitro* [11]. However, according to the HIV Stanford database, Y318F mutation has been detected in only 1% of individuals receiving efavirenz or nevirapine.

In agreement with previous studies [11,16], this work showed that the accumulation of NNRTI RAMs due to past or current exposure to NNRTIs decreased doravirine susceptibility, with substantially reduced activity in most viruses harboring ≥3 major NNRTI RAMs. The time of exposure to NNRTI and the number of previously experienced NNRTI did not significantly affect the susceptibility to doravirine, indicating that the previous exposure to NNRTI do not predict the residual activity of doravirine. Importantly, high-level doravirine resistance was detected in viruses without major doravirine resistance mutations, suggesting that cross resistance is quite common among NNRTI resistant strains [27]. By comparing genotypic and phenotypic data, we observed that the activity of doravirine and rilpivirine, but not etravirine, could be predicted with good accuracy by Stanford HIVdb. Indeed, predicted resistance to etravirine was underestimated, particularly in six

cases with predicted intermediate resistance which showed FC values >100. On the other hand, two isolates with FC values below or equal to the lower clinical cut-off, indicating full or partial susceptibility to etravirine, were classified as highly resistant by HIVdb. This highlights the remaining uncertainties in inferring susceptibility to etravirine by genotyping, despite frequent updates of multiple interpretation algorithms [28].

5. Conclusions

Although doravirine remains the most active NNRTI against isolates exposed to previous drugs of the same class, its activity in salvage therapy may be compromised by the accumulation of NNRTI mutations, including cases without major doravirine RAMs. These data suggest that doravirine might be properly considered in salvage regimens following the genotypic resistance testing in a proportion of PLWH with 4-drug class resistant HIV-1 and limited treatment options to achieve the suppression of viral replication. Overall, doravirine may have a significant role in the management of difficult to treat PLWH as a fully active drug or a partially active drug particularly when novel antiretroviral classes are available.

Acknowledgments

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TRIESTE: Roberto Luzzati, Andrea Misin; VERONA: Marina Malena, Marta Fiscon

This work is dedicated to the memory of Giulio Maria Corbelli, a dear friend and a true champion of activism for HIV/AIDS fight, who passed away while this paper was under revision.

DECLARATIONS

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Figure 1. Doravirine (DOR), etravirine (ETR) and rilpivirine (RPV) IC_{50} fold-change values of recombinant viruses harbouring NNRTI RAMs.

Figure 2. Distribution of (A) doravirine (DOR), (B) etravirine (ETR) and (C) rilpivirine (RPV) IC_{50} fold-change values according to the predicted susceptibility levels as determined by the Stanford HIVdb algorithm.

Legend. S = susceptible; PLLR = potential low-level resistance; LLR = low-level resistance; I = intermediate resistance; R = high-level resistance; BCO = biological fold-change cut-off value; LCO = lower clinical fold-change cut-off value; UCO = upper clinical fold-change cut-off value.

Figure 3. Distribution of doravirine fold-change values according to the presence of major NNRTI resistance associated mutations (RAMs) as defined by Stanford HIVdb. Black circles indicate fold-change values associated with viruses harboring doravirine RAMs.

Legend. BCO = doravirine biological fold-change cut-off (= 3-fold). Black circles indicate fold-change values associated with viruses harboring doravirine RAMs.

Table 1. Patients characteristics at the time of sampling. Data are described as median (IQR) or number of cases (%)

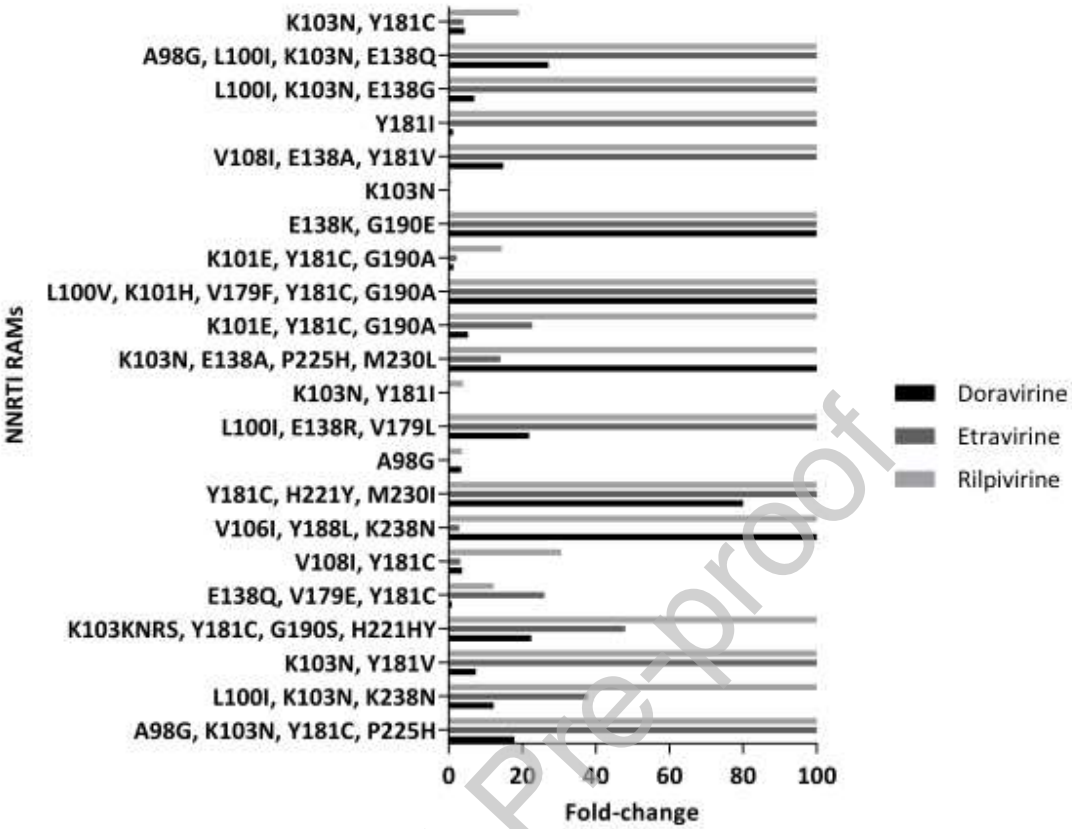
Number of PLWH	22
Male gender	20 (91%)
Age, years	55 (50-58)
Time since HIV-1 diagnosis, years	27 (23-31)
Time on ART, years	23 (22-26)
Occurrence of previous AIDS events	12 (52%)
Nadir CD4+ cell count, cells/mm^3	50 (10-147)
HIV-1 RNA, log_{10} copies/mL	4.30 (3.35-5.14)
CD4+ cell count, cells/mm^3	195 (80-279)
CD8+ cell count, cells/mm^3	1012 (358-1448)
CD4/CD8 ratio	0.2 (0.1-0.5)
Number of drugs included in the current regimen: 2 3 4 5	 4 (18%) 9 (41%) 6 (27%) 3 (14%)
Number of drug resistance mutations for each drug class: PI NRTI NNRTI INSTI	 6 (1-8) 5 (3-7) 3 (2-3) 2 (2-3)

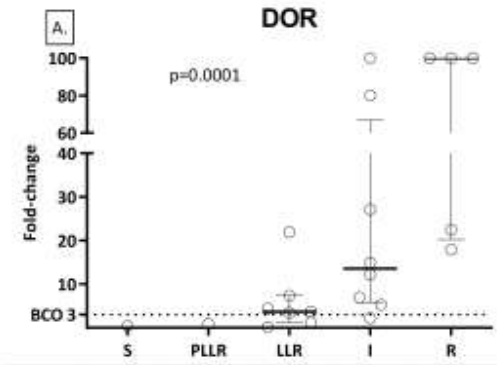
Table 2. Phenotypic and genotypic susceptibility of recombinant viruses (RV) harboring NNRTI resistance associated mutations (RAMs) according to current or past exposure to NNRTIs. Mutations associated with reduced susceptibility to doravirine are in bold.

RV	Major Stanford HIVdb NNRTI RAMs	Subtype	NNRTI exposure at sample collection	Previous exposure to NNRTI	IC_{50} fold-change values			Stanford HIVdb predicted susceptibility		
					Doravirine	Etravirine	Rilpivirine	Doravirine	Etravirine	Rilpivirine
1	**A98G**, K103N, Y181C, **P225H**	B	Etravirine	Efavirenz, nevirapine	17.9	>100	>100	R	I	R
2	**L100I**, K103N, K238N	B	Etravirine	Efavirenz	12.2	37.8	>100	I	I	R
3	K103N, **Y181V**	B	Etravirine	Nevirapine	7.4	>100	>100	LLR	R	R
4	K103KNRS, Y181C, **G190S**, **H221HY**	B	Etravirine	None	22.5	48.0	>100	R	R	R
5	E138Q, V179E, Y181C	B	None	Efavirenz, nevirapine	0.9	26.0	12.2	PLLR	I	R
6	V108I, Y181C	B	None	Efavirenz, etravirine, nevirapine	3.7	3.2	30.6	LLR	I	I
7	V106I, **Y188L**, K238N	B	None	Efavirenz	>100	2.9	>100	R	LLR	R
8	Y181C, **H221Y**, **M230I**	B	Rilpivirine	Etravirine	80.1	>100	>100	I	I	R
9	**A98G**	B	None	Nevirapine	3.4	0.5	3.7	LLR	PLLR	LLR
10	**L100I**, E138R, V179L	B	Etravirine	None	21.9	>100	>100	LLR	I	R
11	K103N, **Y181I**	B	None	Efavirenz, etravirine, nevirapine	0.2	0.4	3.9	LLR	R	R
12	K103N, E138A, **P225H**, **M230L**	B	None	Etravirine	>100	14.1	>100	R	I	R
13	**K101E**, Y181C, G190A	F1	None	Efavirenz, etravirine	5.3	22.7	>100	I	R	R
14	L100V, K101H, V179F, Y181C, G190A	B	Etravirine	Nevirapine, rilpivirine	>100	>100	>100	I	R	R
15	**K101E**, Y181C, G190A	B	Etravirine	None	1.3	2.2	14.4	I	R	R

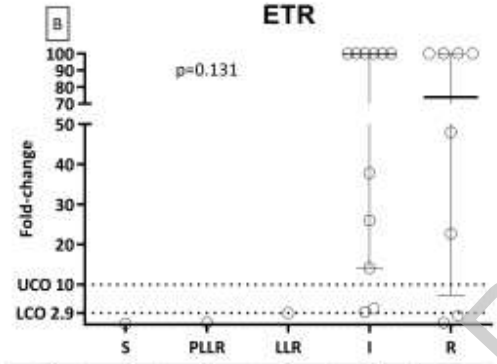
16	E138K, **G190E**	B	Etravirine	Efavirenz, rilpivirine	>100	>100	>100	R	I	R
17	K103N	F1	None	Efavirenz, etravirine, nevirapine	0.4	0.2	0.6	S	S	S
18	V108I, E138A, **Y181V**	B	Etravirine	Efavirenz, nevirapine	14.9	>100	>100	I	R	R
19	**Y181I**	B	Etravirine	None	1.2	>100	>100	LLR	R	R
20	**L100I**, K103N, E138G	B	None	Efavirenz, etravirine	7.0	>100	>100	I	I	R
21	**A98G**, **L100I**, K103N, E 138Q	B	None	Etravirine	27.1	>100	>100	I	I	R
22	K103N, Y181C	B	None	Etravirine	4.4	4.0	19.1	LLR	I	I
			Median IC_{50} fold-change (IQR)		**9.8 (2.9-40.4)**	**42.9 (3.1-100)**	**100 (17.9-100)**			

Legend. S = susceptible; PLLR = potential low-level resistance; LLR = low-level resistance; I = intermediate resistance; R = high-level resistance.

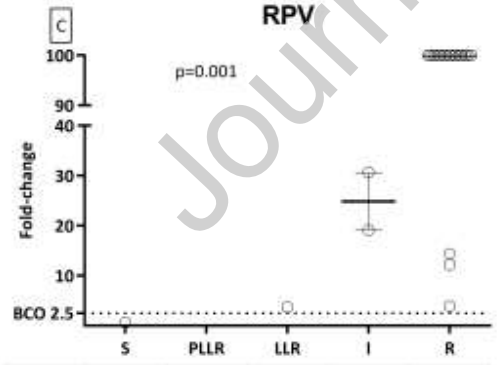




	S	PLLR	LLR	I	R
N	1	1	7	8	5
Median	0.4	0.9	3.7	13.6	100
IQR			1.2-7.4	5.7-66.9	20.2-100



	S	PLLR	LLR	I	R
N	1	1	1	11	8
Median	0.2	0.5	2.9	100	74.0
IQR				14.1-100	7.3-100



	S	PLLR	LLR	I	R
N	1	0	1	2	18
Median	0.6		3.7	24.9	100
IQR				19.1-30.6	100-100