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Prevalence of doravirine-associated resistance mutations in HIV-1-infected antiretroviral-experienced patients from two large databases in France and Italy

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Objectives: Doravirine, a novel NNRTI, selects for specific mutations *in vitro*, including mutations at reverse transcriptase (RT) positions 106, 108, 188, 227, 230 and 234. The aim of this study was to examine the prevalence of doravirine-associated resistance mutations in HIV-1-infected antiretroviral-experienced patients.

Methods: Doravirine-associated resistance mutations identified *in vitro* or *in vivo* were studied in a set of 9199 HIV-1 RT sequences from HIV-1 antiretroviral-experienced patients, including 381 NNRTI-failing patients in France and Italy between 2012 and 2017. The following mutations were considered as resistance mutations: V106A/M, V108I, Y188L, G190S, F227C/L/V, M230I/L, L234I, P236L, K103N + Y181C, K103N + P225H and K103N + L100I.

Results: The frequencies of doravirine-associated resistance mutations (total dataset versus NNRTI-failing patients) were: V106A/M, 0.8% versus 2.6%; V108I, 3.3% versus 9.2%; Y188L, 1.2% versus 2.6%; G190S, 0.3% versus 2.1%; F227C/L/V, 0.5% versus 1.8%; M230I/L, 2.8% versus 0%; L234I, 0.1% versus 0.5%; K103N + Y181C, 3.9% versus 3.9%; K103N + P225H, 2.9% versus 4.7%; and K103N + L100I, 1.7% versus 3.9%, with a significantly higher proportion of these mutations in the NNRTI-failing group ($P < 0.05$), except for M230I/L and K103N + Y181C. The overall prevalence of sequences with at least one doravirine-associated resistance mutation was 12.2% and 34.9% in the total dataset and NNRTI-failing patients ($P < 0.001$), respectively. In comparison, the prevalence of the common NNRTI mutations V90I, K101E/P, K103N/S, E138A/G/K/Q/R/S, Y181C/I/V and G190A/E/S/Q were higher (8.9%, 7.9%, 28.6%, 12.6%, 14.2% and 8.9%, respectively).

Conclusions: These results suggest that doravirine resistance in antiretroviral-experienced patients generally and specifically among NNRTI-failing patients is lower than resistance to other NNRTIs currently used, confirming its distinguishing resistance pattern.

Introduction

NNRTIs are a major component of antiretroviral treatment for HIV patients, as they were the third recommended agent in the WHO and European guidelines and, until recently, in US guidelines.[1–3] First-generation NNRTIs efavirenz and nevirapine have a low-level genetic barrier to resistance and consequently the prevalence of HIV-1 resistance to NNRTIs is the highest of the several classes of antiretrovirals, in antiretroviral-naive as well in treated patients.[4–6] Therefore, new NNRTI drugs retaining antiretroviral activity against viruses with K103N, E138K, Y181C and G190A, the most prevalent NNRTI mutations, are needed.

Two large Phase 3 studies have demonstrated the efficacy of doravirine, a new NNRTI, in a population of antiretroviral-naive HIV patients in comparison with efavirenz (DRIVE-AHEAD) or boosted

darunavir (800/100 mg) (DRIVE-FORWARD) in combination with two NRTIs.[7–9] In the DRIVE-SHIFT trial, the switch to doravirine/lamivudine/tenofovir disoproxil fumarate maintained virological suppression through 48 weeks.[10]

The doravirine resistance profile is distinct from that of other NNRTIs with the *in vitro* selection of mutations at reverse transcriptase (RT) positions 106, 108, 188, 227, 230, 234 and 236.[11–14] *In vivo*, the evidenced resistance mutation profiles were concordant: Y188L; V106I + F227C; V106I/V + H221Y + F227C; F227C; V106A + P225H + Y318Y/F; V106T/M, F227C/R; and Y318F/Y.[7–9]

We aimed to study the prevalence of doravirine-associated resistance mutations in HIV-1-infected antiretroviral-experienced patients and especially in NNRTI-failing patients to investigate whether previous NNRTI use could impair doravirine activity.

Materials and methods

Resistance genotypic tests were performed at five reference laboratories: two in Paris (Pitié-Salpêtrière and Bichat Claude Bernard hospitals) and three in Italy (University/Polyclinic of Rome 'Tor Vergata', INMI Spallanzani-IRCCS and Modena Hospital). A total of 9199 HIV-1 RT sequences obtained between 2012 and 2017 from HIV-1 antiretroviral-experienced patients in routine clinical care were analysed. A follow-up HIV viral load measurement was performed between 3 and 6 months; in cases of two consecutive viral loads >50 copies/mL, resistance genotypic testing was performed on the second viral load sample. Among this set of sequences, 381 sequences originated from a low number of NNRTI failures (efavirenz, $n = 189$; etravirine, $n = 32$; nevirapine, $n = 66$; and rilpivirine, $n = 94$). The following RT mutations identified *in vitro* or *in vivo* were considered as doravirine-associated mutations: V106A/M, V108I, Y188L, F227C/L/V, M230I/L, L234I, P236L, K103N + P225H and K103N + L100I.[8,11–14] K103N + Y181C and G190S were also considered in our analysis, as they are known to confer resistance to other NNRTIs. NNRTI mutations associated with resistance to efavirenz, rilpivirine, nevirapine and etravirine were those listed in the 'Agence Nationale de recherche sur le SIDA et les hépatites virales' (ANRS) algorithm (Table of rules 2018; www.hivfrenchresistance.org), in the IAS–USA list 2018 (www.iasusa.org) and in the Stanford HIV drug resistance database (https://hivdb.stanford.edu/dr-summary/resistance-notes/NNRTI/), namely, efavirenz: L100I, K101E/P, K103N/S, V106A/M, V108I, Y181C/I/V, Y188C/H/L, G190A/E/S, P225H and M230L; etravirine: V90I, A98G, L100I, K101E/H/P, V106I, E138A/G/K/Q, V179D/F/T, Y181C/I/V, G190A/E/S and M230L; nevirapine: L100I, K101E/P, K103N/S, V106A/M, V108I, Y181C/I/V, Y188C/H/L, G190A/E/S and M230L; and rilpivirine: L100I, K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, Y188L, G190A/E/S, H221Y, F227C and M230I/L.

Resistance interpretation was made using the Smartgene® Integrated Database Network System (SmartGene, Switzerland; http://www.smartgene.com) according to the Stanford and ANRS algorithms. Resistance and possible resistance were grouped as resistance.

Subtype was determined on the basis of the RT and protease coding regions by the SmartGene algorithm or by phylogenetic analyses, using reference sequences of HIV-1 subtypes and circulating recombinant forms (CRFs) from the Los Alamos Database (https://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html). Between-group comparisons were carried out using Fisher's exact test.

Results

Distribution of HIV-1 subtypes

Among the 9199 sequences, the distribution of subtypes was: 45.3% B, 27.3% CRF02_AG, 3.7% A1, 2.5% C, 1.7% CRF06_cpx and 19.5% other various non-B subtypes. Among the 381 sequences of NNRTI-failing patients, 252 (66.1%) were infected with a B subtype and 129 (33.9%) with a non-B subtype. The distribution of subtype (B versus non-B) was statistically significant for the NNRTI-failing group ($P < 0.001$).

Prevalence of doravirine and other NNRTI resistance-associated mutations

Analysing the overall dataset of HIV-1 antiretroviral-experienced patients, the most frequent doravirine resistance-associated mutations were: V106A/M, 0.8% ($n = 77$); V108I, 3.3% ($n = 307$); Y188L, 1.2% ($n = 107$); G190S, 0.3% ($n = 24$); F227C/L/V, 0.5% ($n = 49$); M230I/L, 2.8% ($n = 256$); L234I, 0.1% ($n = 13$); P236L, 0% ($n = 0$); K103N + Y181C, 3.9% ($n = 361$); K103N + P225H, 2.9% ($n = 264$); and K103N + L100I, 1.7% ($n = 156$) (Figure 1). The prevalence of M230I/L and K103N + L100I was higher for the HIV-1 B subtype than non-B subtypes (3.3% versus 2.4%, $P = 0.009$ and

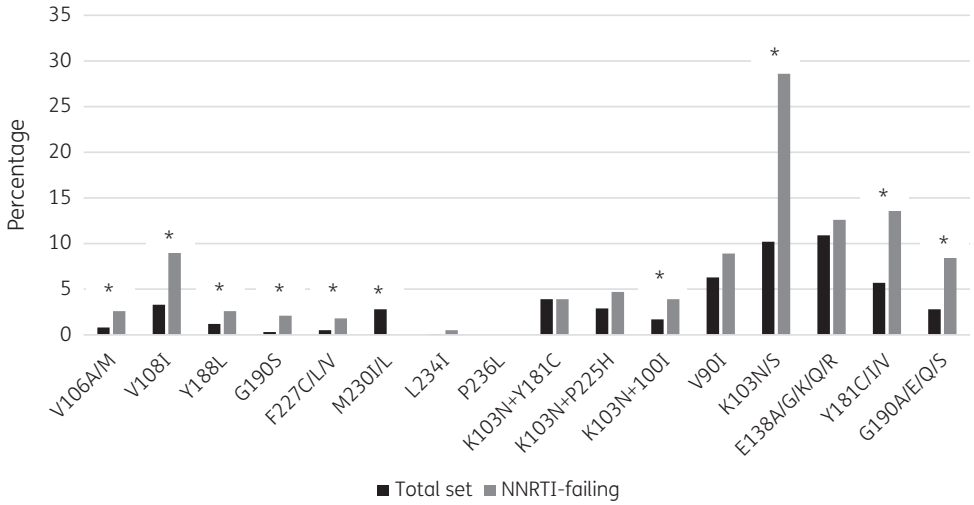

Figure 1. Prevalence of RT sequences with at least one individual doravirine or other NNRTI (>8%) resistance-associated mutation in the total dataset (black) and in the NNRTI-failing group (grey). *$P < 0.05$: statistically significant difference between total dataset and NNRTI-failing group.

2.6% versus 1.0%, $P<0.001$, respectively), in contrast to K103N + P225H (1.8% versus 3.7%, $P<0.001$).

In comparison, the prevalence of the common NNRTI mutations V90I, A98G, L100I, K101E/P, K103N/S, E138A/G/K/Q/R, V179D/F/T, Y181C/I/V, Y188C/H/L, G190A/E/S and T225H was 6.3% (580), 2.5% (231), 1.0% (94), 2.4% (219), 10.2% (934), 10.9% (1001), 1.5% (137), 5.7% (521), 1.7% (153), 2.8% (258) and 1.4% (130), respectively (Figure 1). Some mutations were more frequent in HIV-1 B subtype [L100I (1.6% versus 0.6%, $P<0.001$), E138A/G/K/Q/R (14.3% versus 8.0%, $P<0.001$), V179D/F/T (2.0% versus 1.1%, $P<0.001$), G190A/E/S (2.9% versus 2.7%, $P=0.004$)] or in HIV-1 non-B subtypes [V90I (4.2% versus 8.0%, $P<0.001$), A98G (1.8% versus 3.1%, $P<0.001$), K103N/S (8.7% versus 11.3%, $P<0.001$) and T225H (1.2% versus 1.6%, $P<0.001$)]. There was no difference between B and non-B subtypes for E138K (4.0% versus 3.1%, $P=0.407$).

Resistance to doravirine and other NNRTIs

The overall prevalence of sequences in the total dataset with at least one doravirine resistance-associated mutation was 12.2% ($n=1119$). Considering the ANRS algorithm, 5.6% ($n=512$) of sequences were associated with doravirine resistance. In comparison, the prevalence of sequences associated with resistance was significantly higher for efavirenz (18.8%, $n=1725$), etravirine (8.4%, $n=776$), nevirapine (17.9%, $n=1647$) and rilpivirine (22.3%, $n=2050$) ($P<0.001$) (Figure 2a). Similarly, with the Stanford algorithm, the prevalence of sequences associated with resistance to doravirine was 16.0% ($n=1468$) and lower than for efavirenz 24.8% ($n=2277$), etravirine 24.6% ($n=2267$), nevirapine 24.9% ($n=2294$) and rilpivirine 24.7% ($n=2269$) ($P<0.001$) (Figure 2b).

Prevalence of doravirine and NNRTI resistance-associated mutations in the NNRTI-failing group ($n=381$)

Analysing the NNRTI-failing patients, among the doravirine resistance-associated mutations, the most frequent mutations were: V106A/M, 2.6% (10); V108I, 9.2% (35); Y188L, 2.6% (10); G190S, 2.1% (8); F227C/L/V, 1.8% (7); M230I/L, 0% (0); L234I, 0.5% (2); P236L, 0%; K103N + Y181C, 3.9% (15); K103N + P225H, 4.7% (18); and K103N + L100I, 3.9% (15) (Figure 1). The following mutations are statistically more prevalent ($P<0.05$) in the NNRTI-failing group compared with the whole set of sequences: V106A/M, V108I, Y188L, G190S, F227C/L/V and K103N + L100I. Only M230I/L was statistically more prevalent in the whole group than in the NNRTI-failing group ($P<0.001$) (Figure 1). Furthermore, the mutation F227C/L/V was less frequent in B versus non-B subtypes (0.8% versus 3.9%, $P=0.047$).

In comparison, the prevalence of the common NNRTI mutations V90I, A98G, L100I, K101E/P, K103N/S, E138A/G/K/Q/R, V179D/F/T, Y181C/I/V, Y188C/H/L, G190A/E/Q/S and T225H was 8.9% ($n=34$), 3.4% ($n=13$), 4.2% ($n=16$), 7.9% ($n=30$), 28.6% ($n=109$), 12.6% ($n=48$), 5.2% ($n=20$), 14.2% ($n=54$), 4.7%, ($n=18$), 8.9% ($n=34$) and 5.2% ($n=20$), respectively (Figure 1). No association was observed between these common mutations and HIV subtype.

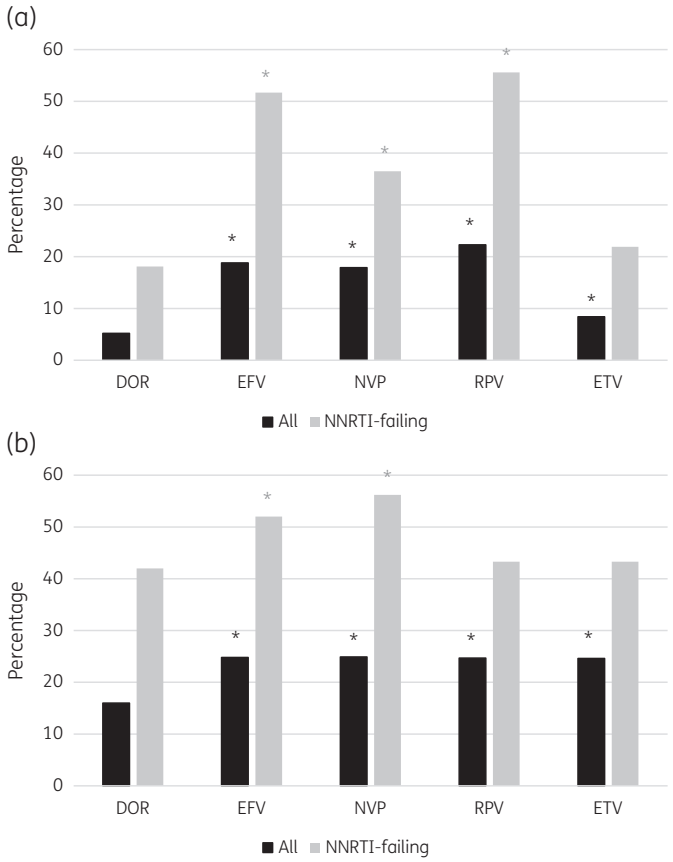

Figure 2. Percentage of RT sequences associated with NNRTI resistance in the whole dataset and in the group of NNRTI-failing patients according to the ANRS (a) or Stanford (b) algorithm. DOR, doravirine; EFV, efavirenz; NVP, nevirapine; RPV, rilpivirine; ETV, etravirine. *$P<0.001$: statistically significant difference from doravirine.

Resistance in the NNRTI-failing group

The overall prevalence of sequences with at least one doravirine resistance-associated mutation in the NNRTI-failing group was 34.9% ($n=133$). Considering the ANRS algorithm, 18.1% ($n=69$) of sequences were associated with resistance to doravirine. This prevalence was significantly lower than the prevalence of sequences associated with resistance to other NNRTIs by the ANRS algorithm: 36.5% ($n=139$) were genotypically resistant to nevirapine ($P<0.001$), 51.7% ($n=197$) to efavirenz ($P<0.001$), 23.1% ($n=88$) to etravirine ($P=0.107$) and 55.6% ($n=212$) to rilpivirine ($P<0.001$) (Figure 1a). With the Stanford algorithm, the resistance to doravirine was 42.0% ($n=160$) and not different from etravirine and rilpivirine resistance, whereas the resistance to the first-generation NNRTIs was higher: efavirenz, 52.0% ($n=209$, $P<0.001$); and nevirapine, 56.2% ($n=214$, $P<0.001$) (Figure 1b).

Discussion

Our study evidenced a low prevalence of doravirine resistance-associated mutations in HIV-1-infected antiretroviral-treated patients in Italy and France. This prevalence was significantly lower than those for other NNRTIs in use, especially first-generation NNRTIs.

In this study, the proportion of non-B subtypes was high (54.6%), with a large variety of subtypes, and slightly higher than in our previous study on doravirine resistance in HIV-1 antiretroviral-naive patients (47.0%).[15] However, it was similar to the prevalence of non-B subtypes in antiretroviral-naive chronically HIV-infected patients in 2015–16 in France (54.8%).[5]

As expected, the prevalence of resistance associated with doravirine and other NNRTIs was higher in the population of HIV-1 antiretroviral-treated patients than in our previous study that showed the rare occurrence of doravirine resistance-associated mutations in HIV-1-infected antiretroviral-naive patients ($n = 137/9764$, 1.4%).[15] For K103N, Y181C and E138A/K mutations, their prevalences observed in this study were more consequential than in the most recent French nationwide study in treated patients with a confirmed viral load >50 copies/mL.[5]

In the DRIVE clinical trials conducted in HIV-1 antiretroviral-naive patients, the evidenced resistance mutation profiles at failure were as follows: Y188L; V016I + F227C; V106I/V + H221Y + F227C; F227C; V106A + P225H + Y318Y/F; V106T/M, F227C/R; and Y318F/Y.[7–9] Globally and except for the single Y318F not studied here, all these doravirine mutations were present at a low percentage, even in the NNRTI-failing patients in our study.

In the DRIVE-SHIFT trial, conducted in virologically suppressed patients, no doravirine resistance-associated mutations were evidenced in patients achieving protocol-defined virological failure. Of note, 24 participants had a virus with baseline NNRTI mutations (K103N, Y181C and G190A) and 23/24 who switched to doravirine/lamivudine/tenofovir disoproxil fumarate remained suppressed at the 48 week follow-up.[10] This suggests that the most frequent NNRTI mutations at RT mutation positions 103, 181 and 190 should probably not impact doravirine activity *in vivo*. K103N + Y181C and G190S, although not specific doravirine-associated resistance substitutions, were included in our analysis as they confer resistance to other NNRTIs. In our study, the prevalence of the K103N + Y181C and G190S mutations was low and did not impact the global resistance of doravirine.

Some small significant differences were observed in the present study for the prevalence of some doravirine mutations, according to the HIV-1 subtype (M230I/L and K103N + L100I more frequent in subtype B and K103N + P225H more frequent in non-B subtypes). In another study, it was shown that Y188L and V106M were more frequent in subtype C while V106A was less frequent in non-B subtypes.[16]

One limitation of the study is the relatively low number of NNRTI failures. However, when the resistance test was performed, more failing patients had previously been exposed to NNRTIs.

The results of interpreting doravirine resistance were different according to the ANRS or Stanford algorithms (18.1% versus 42%) in the NNRTI-failing group. This could be explained by differences in the set mutation list for the same RT position and also in the number of considered positions. For example, the following RT mutations are not taken into account in the ANRS algorithm: L100I, K101E/P, V106I, Y181C/I/V, P225H, F227C and L234I.

According to the Stanford algorithm, our study shows 42% doravirine resistance in the NNRTI-failing group, which was higher than recently evidenced in NNRTI-experienced patients in another study (18.8%).[16] Several factors could explain this difference. The studied doravirine mutations were not strictly similar between the two studies. Indeed, we investigated a larger set of mutations with the inclusion of mutations G190S, F227C/V, M230I, L234I, P236L, K103N + Y181C and K103N + L100I, as well as F227C and L234I alone and not only in association with V106A/M.

In conclusion, these results suggest that doravirine resistance in antiretroviral-experienced patients generally and specifically among NNRTI-failing patients is significantly lower than resistance to other NNRTIs currently used, confirming its distinguishing resistance pattern. In addition, these results are reassuring from the perspective of doravirine use in those previously treated with NNRTIs after genotyping.

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All other authors: none to declare.

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