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Rare occurrence of doravirine resistance associated mutations in HIV-1-infected treatment-naïve patients

Running title: Primary doravirine HIV-1 resistance

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ABSTRACT

Objectives: Doravirine is a novel HIV-1 non-nucleoside reverse transcriptase inhibitor (NNRTIs) recently shown to be non-inferior both to darunavir/ritonavir and efavirenz in combination therapy with two nucleoside reverse transcriptase inhibitor in treatment-naïve patients. Doravirine has an *in vitro* resistance profile that is distinct from other NNRTIs and retains activity against viruses containing the most frequently transmitted NNRTIs mutations. The aim of this study was to examine the prevalence of doravirine associated mutations in HIV-1-infected treatment-naïve patients in Europe.

Patients and methods: From 2010 to 2016, 9764 treatment-naïve patients were tested for NNRTIs antiretroviral drug resistance by bulk sequencing in Greece, Italy and France. We studied the prevalence of doravirine resistance associated mutations previously identified *in vitro*: V106A/M, V108I, Y188L, V190S, H221Y, F227C/L/V, M230I/L, L234I, P236L, Y318F and K103N/Y181C.

Results: Among 9764 sequences, 52.99% and 47.01% of patients had B and non-B subtypes, respectively. Overall, the presence of at least one doravirine resistance associated mutation (n=137; 1.40%) or the K103N/Y181C mutations (n=5; 0.05%) was very rare. The most prevalent mutations were V108I (n=62; 0.63%), Y188L (n=18; 0.18%), H221Y (n=18; 0.18%) and Y318F (n=23; 0.24%). The frequency of doravirine resistance mutations was similar between B and non-B subtypes. In comparison, the prevalence of rilpivirine, etravirine, nevirapine and efavirenz resistance was higher whatever the used algorithm (ANRS: 8.53%, 8.07%, 8.28% and 3.90%; Stanford: 9.90%, 10.02%, 7.47%, and 9.44%, respectively).

Conclusions: The prevalence of doravirine resistance mutations is very low in antiretroviral-naïve patients. These results are very reassuring for doravirine use in naïve patients.

INTRODUCTION

Intensive scale-up of antiretrovirals worldwide has led to a dramatic decrease in HIV-1 related morbidity and mortality. Despite this success, the expansion of treatment has been accompanied by a significant increase in the prevalence of both acquired and transmitted HIV drug resistance (TDR). TDR may impact response to therapy, leading to virologic failure and the evolution of further drug resistance. The increasing prevalence of TDR has been mostly driven by non-nucleoside reverse transcriptase inhibitors (NNRTIs), particularly in sub-Saharan Africa as a result of the extensive use of efavirenz and nevirapine. [1]

Doravirine is a novel HIV-1 NNRTI in phase III clinical development. Doravirine has an *in vitro* resistance profile that is distinct from other NNRTIs, retaining activity against viruses containing the most frequently transmitted NNRTIs mutations, such as K103N, E138K, Y181C and G190A [2]. Doravirine selects for distinct mutations *in vitro*, including mutations at positions 106, 108, 227 and 234 with multiple mutations required for significant levels of resistance [3]. Some studies characterized the *in vitro* phenotypic susceptibility of NNRTI-associated mutant viruses to doravirine. Only few single mutations were associated with >10-fold reduced susceptibility to doravirine, including V106A, Y188L and M230L. [4] Furthermore, the double and triple mutants V106A/F227L, V106/L234I, V106A/F227L/L234I or V106A/G190A/F227L all showed substantial resistance to doravirine. [3–5]

Recent phase III trials showed that doravirine has non-inferior efficacy when compared to darunavir/r (800/100 mg) or to efavirenz in combination with 2 NRTIs (tenofovir and emtricitabine or abacavir and lamivudine) in treatment-naïve patients. [6,7] Data on the

occurrence of doravirine-associated mutations in treatment-naïve patients is crucial to inform the further provision of treatment.

The aim of this study was to examine the prevalence of doravirine-associated mutations in HIV-1-infected treatment-naïve patients in Europe over time (2010-2016) across various subtypes and to compare this prevalence to those known for currently available NNRTIs: efavirenz, rilpivirine, nevirapine and etravirine.

MATERIALS AND METHODS

Bulk resistance genotypic tests were performed between 2010 and 2016 at 6 reference laboratories: 2 in Paris, France (Pitié-Salpêtrière and Bichat Claude Bernard hospitals), 3 in Italy (University of Rome "Tor Vergata", INMI Spallanzani-IRCCS, Modena Hospital) and 1 in Greece (Department of Hygiene Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, Athens, Greece). In addition, HIV-1 RT sequence data from drug-naïve patients were provided by a number of centers included in the ARCA database (www.dbarca.net) in Italy without overlap with the above mentioned reference laboratories. Doravirine-associated mutations identified *in vitro* and used to define doravirine resistance in this study were: V106A/M, V108I, Y188L, V190S, H221Y, F227C/L/V, M230I/L, L234I, P236L, Y318F and K103N/Y181C. [2–5] HIV-1 with at least one of these mutations was considered as resistant.

NNRTIs mutations associated with resistance to efavirenz, rilpivirine, nevirapine and etravirine were those listed in the ANRS algorithm (table of rules 2017; www.hivfrenchresistance.org), in the IAS list 2017 (www.iasusa.org) and in the Stanford HIV drug resistance database (HIVdbversion 8.5; 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, M230L; etravirine: V90I, A98G, L100I, K101E/H/P, V106I, E138A/G/K/Q, V179D/F/T, Y181C/I/V, G190A/E/S, M230L; nevirapine: L100I, K101E/P, K103N/S, V106A/M, V108I, Y181C/I/V, Y188C/H/L, G190A/E/S, M230L; rilpivirine: L100I, K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, Y188L, G190A/E/S, H221Y, F227C, M230I/L.

Resistance interpretation was made using the Smartgene® Integrated Database Network System (SmartGene, Switzerland; http://www.smartgene.com) according to the Stanford University (https://hivdb.stanford.edu) or the ANRS Algorithm (http://www.hivfrenchresistance.org).

Subtype was determined on the basis of the reverse transcriptase (RT) and protease coding regions by Smartgene algorithm (Smartgene®, Switzerland) or by phylogenetic analyses, using reference sequences of HIV-1 subtypes and circulating recombinant forms (CRF) 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 in antiretroviral-naïve patients

A total a 9764 reverse transcriptase sequences obtained between 2010 and 2016 for HIV-1 treatment-naïve patients in routine clinical care were analyzed: 4939 were performed between 2010-2012 and 4825 between 2013 and 2016. The distribution of subtypes was: 52.99% B subtypes and 47.01% non-B subtypes. Subtypes with prevalence higher than 3.00% included

CRF02_AG (14.62%), A (6.28%), C (3.35%) and F (3.19%). There was a significant increase of non-B subtypes in 2013-2016 with respect to 2010-2012 (49.43% vs. 42.68%, respectively, $p < 0.001$).

Prevalence of doravirine resistance associated mutations

The overall prevalence of sequences with at least 1 doravirine resistance associated mutation was 1.40% (n = 137). The number of sequences with 1, 2, 3 and 4 doravirine resistance associated mutations was 127 (1.30%), 8 (0.08%), 1 (0.01%) and 1 (0.01%), respectively. The presence of the double mutant K103N/Y181C was 0.05% (n=5). This overall prevalence was significantly lower than the prevalence of sequences with at least 1 resistance associated mutations for other NNRTIs: efavirenz (4.31%, n = 421), nevirapine (4.31%, n = 421), rilpivirine (7.73%, n=755) or etravirine (11.72%, n = 1143) ($p < 0.001$) (Figure 1).

Among the doravirine resistance associated mutations, the most frequent mutations were V108I (0.63%; n=62), Y188L (0.18%; n=18), H221Y (0.18%; n=18) and Y318F (0.24%; n=23) (Figure 2). The other doravirine resistance associated mutations were very rare: V106A/M (0.08%; n=8), G190S (0.05%; n=5), F227C/L/V (0.12%; n=12), M230I/L (0.04%; n=4), L234I (0.01%; (n=1), P236L (0.03%; n=3), K103N/Y181C (0.05%, n=5). In comparison, the prevalence of common NNRTIs mutations were K103N/S (2.13%; n=208), E138A/G/K/Q/R (6.52%; n=637), Y188C/H/L (0.23%; n=22) and G190A/E/S (0.52%; n=51) (Figure 2). Between 2010-2012 and 2013-2016, there was only a significant increase for K103N/S (2.04% versus 2.98%, p = 0.003) and in G190A/E/S (0.32% versus 0.77%, p = 0.003).

Resistance

As one mutation was considered as resistance to doravirine, 1.45% (n=142) of samples were resistant to doravirine in comparison with 8.53% (n=833) to rilpivirine, 8.07% (n=788) to etravirine, 8.28% (n=809) to nevirapine and 3.90% (n=348) to efavirenz according to the 2017 ANRS algorithm. These results were slightly different according to the Stanford algorithm: 9.90% (n=967) for rilpivirine, 10.02% (n=979) for etravirine, 7.47% (n=730) for nevirapine and 9.44% (n=828) for efavirenz.

There was no relationship between any subtypes and the presence of any doravirine associated mutations. Indeed, the overall prevalence of sequences with at least 1 doravirine resistance associated mutation and considered as resistant variants was 1.59% (n=84) and 1.29% (n=58) in B versus non-B subtypes, respectively (p=0.168). In contrast, according to both ANRS and Stanford algorithms, the prevalence of resistance was statistically higher for B than non-B subtypes for nevirapine and rilpivirine (table 1). The resistance to etravirine was also statistically higher for B subtypes only with the Stanford algorithm (table 1).

DISCUSSION

This is the first study evaluating the prevalence of doravirine resistance associated mutations in a large European database of antiretroviral-naïve HIV-1-infected patients. These results showed that the prevalence of doravirine resistance associated mutations in HIV-1-infected treatment-naïve patients in Greece, Italy and France is very low, significantly lower than other NNRTIs resistance associated mutations, antiretrovirals potentially recommended as first line regimen. [8–10] This occurrence was stable over time and not related to any HIV-1 subtype.

The proportion of non-B subtypes was higher in our study (47.01%) compared to the continuous HIV drug resistance surveillance program (SPREAD) taking place in 27 countries

in Europe from 2002 to 2007 (32.66%), or to the last study in France among 1318 French patients diagnosed at the time of primary HIV-1 infection in 2007–2012 (33.70%) or in Italy in 4323 drug naïve individuals between 2010 and 2014 (30.80%). [11–13] However, this higher prevalence of non B subtypes is consistent with the continuous increase of the non-B subtypes in Europe or the high prevalence of non-B subtypes observed recently in Greece. [13–15] Thus, our study provides a representative view of HIV subtypes circulating in Western Europe.

The resistance to NNRTIs was higher for B than non B subtypes in this study, except for doravirine resistance which was not impacted by HIV subtypes. It is according to several studies showing that transmitted drug resistance was higher in HIV-1 subtype B infected men having sex with men in primary infection or in chronically antiretroviral naïve HIV-1 infected patients in France, as well as in previous European report. [12,16]

In *in vitro* studies, the HIV resistance mutations associated to doravirine with the highest fold change were V106A, Y188L and M230L. [4] In the DRIVE-FORWARD clinical study, resistance to doravirine emerged in one participant as a multiple mutant (V106I, H221Y and F227C) in the context of non-compliance. [6] In DRIVE-AHEAD, in the doravirine group, the NNRTI mutations were for 1.6% of patients: Y188L; V106I, F227C; V106V/I, H221H/Y, F227C; F227C; V106A, P225H, Y318Y/F; V106M/T, F227C/R. [7] In our study, the prevalence of these resistance mutations was very low (<0.2%) and the double or triple HIV mutants showing the highest level of *in vitro* resistance were virtually absent (<0.001%). [3–5]

Overall, our results showed that primary resistance is currently less frequent for doravirine than for other second generation NNRTIs such as etravirine and rilpivirine. This difference could be explained by some resistance mutations associated to etravirine or rilpivirine, like V90I, A98G, V106I, V179D/F/T and especially E138A, which are not included in the

doravirine resistance associated mutation list. For example, E138A was present in 4.2% of sequences in this study. Similarly, the prevalence of the E138A polymorphic substitution which can decrease rilpivirine susceptibility was 3.2% (95% CI 1.9%–4.6%) in 2010/11 in antiretroviral naïve chronically HIV-1 infected patients in France. [16] One limitation of this study is its descriptive aspect. It should be interesting to further study the impact of these studied resistance mutations to doravirine virological response.

According to Lambert-Niclot's analysis using both the IAS and ANRS lists, 5% of the samples from treatment-naive patients had primary rilpivirine resistance associated mutations from 2008 to 2011. [17] Notably, the prevalence of primary resistance to rilpivirine increased over time since it nearly doubled during our study (8.53%). As the doravirine resistance mutations were different from the other NNRTIs, we can expect no or low impact of the prevalence increase of resistance mutations for the other NNRTIs, in the context of transmitted drug resistance.

These results are very reassuring in the perspective of the use of doravirine in naïve patients since doravirine remains active against the commonly transmitted efavirenz and rilpivirine mutations *in vitro.* However, the role of doravirine *in vivo* remains to be confirmed through clinical observation, particularly because patients harboring NNRTI-resistant virus were deliberately excluded from clinical trials completed so far.

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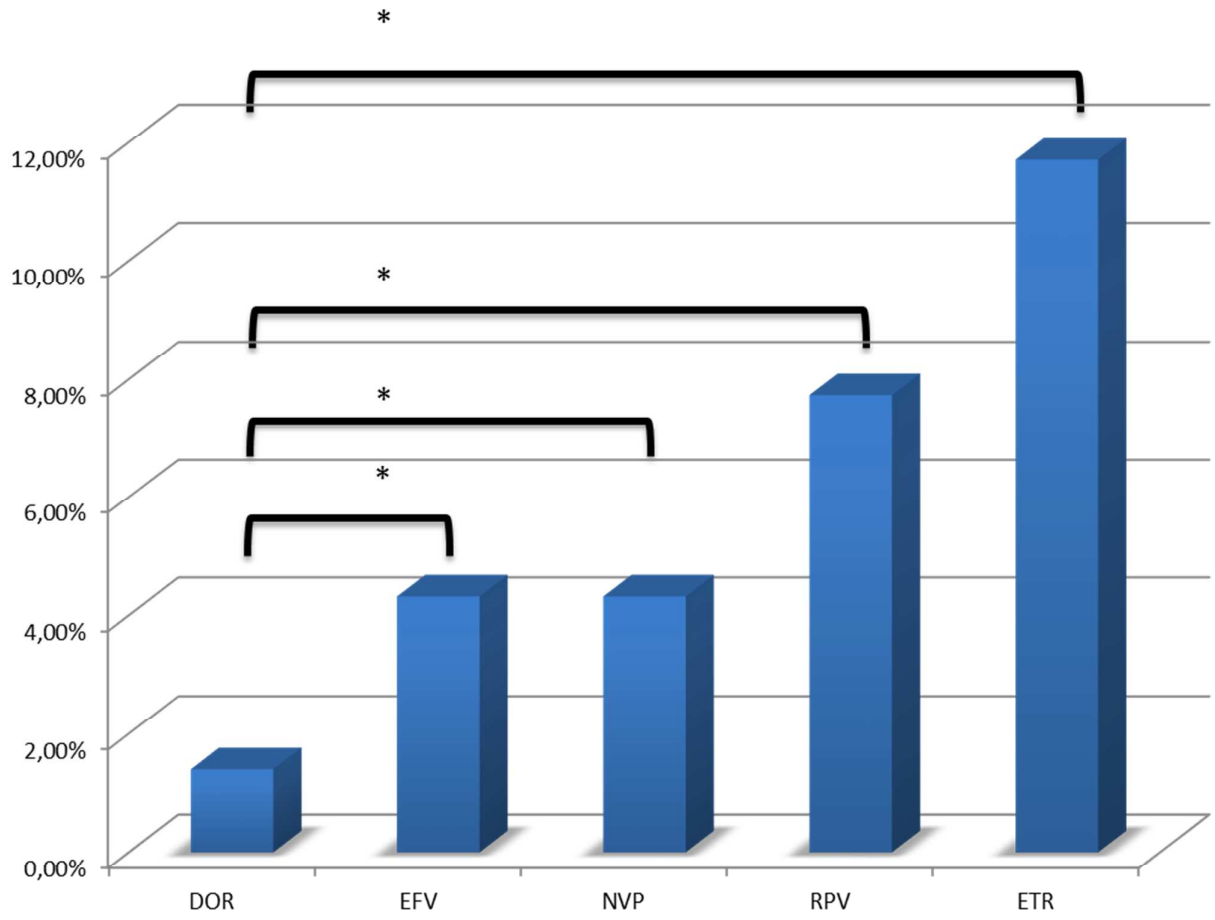

Figure 1: Percent of Reverse Transcriptase sequences with at least one resistance mutation to NNRTI Doravirine (DOR), Efavirenz (EFV), Rilpivirine (RPV), Nevirapine (NVP) and Etravirine (ETR).

* : $p < 0.0001$

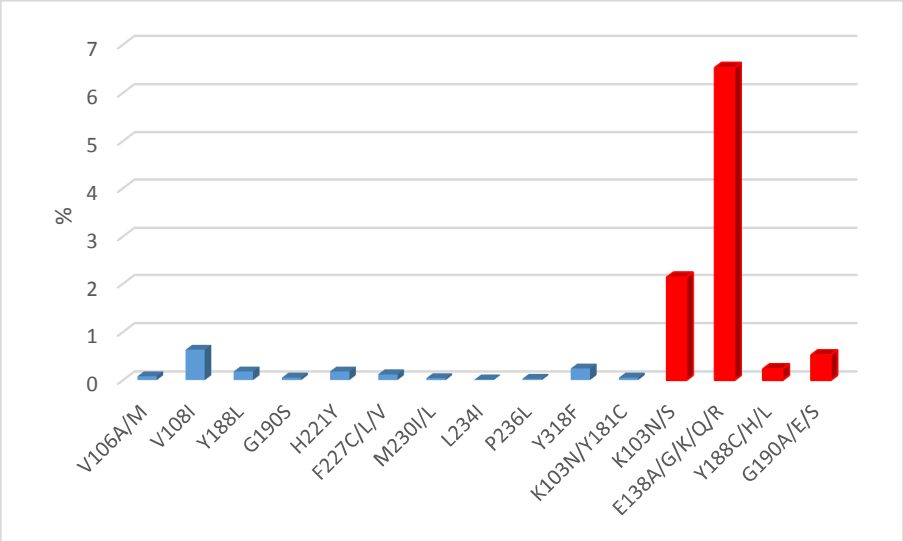

Figure 2: Prevalence of Reverse Transcriptase sequences with at least one resistance mutation to Doravirine or other NNRTI

In blue: mutations associated with resistance to doravirine, in red: mutations associated to other NNRTIs

	ANRS algorithm			Stanford algorithm		
	B	Non-B		B	Non-B	
Efavirenz, n (%)	212 (4.02)	136 (3.72)	P=0.500	485 (9.73)	343 (9.08)	P=0.320
Nevirapine, n (%)	590 (11.19)	219 (5.12)	P<0.001	423 (8.02)	307 (6.83)	P=0.025
Etravirine, n (%)	443 (8.40)	345 (7.68)	P=0.190	574 (10.88)	405 (9.01)	P=0.002
Rilpivirine, n (%)	488 (9.26)	345 (7.68)	P=0.006	565 (10.71)	402 (8.94)	P=0.003

Table 1: HIV resistant variants according to the B and non-B subtypes.