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

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Running title: Primary doravirine HIV-1 resistance

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ABSTRACT

Background: Doravirine is a novel HIV-1 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, 53.0% and 47.0% of patients had B and non-B subtypes, respectively. Overall, the presence of at least one doravirine resistance associated mutation (n=137; 1.4%) or the K103N/Y181C mutations (n=5; 0.05%) was very rare. The most prevalent mutations were V108I (n=62; 0.6%), Y188L (n=18; 0.2%), H221Y (n=18; 0.2%) and Y318F (n=23; 0.2%). 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: 8.5%, 8.1%, 8.3% and 3.9%; Stanford: 9.9%, 10.0%, 7.5%, and 9.4%, 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 acquired and transmitted HIV drug resistance (TDR), mostly driven by NNRTIs. [1]

Doravirine is a novel HIV-1 NNRTI in phase III clinical development with *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] 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 efavirenz in combination with 2 NRTIs 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 across various subtypes and to compare this prevalence to those known for currently available NNRTIs.

MATERIALS AND METHODS

All bulk HIV resistance genotypes performed for routine clinical routine for drug-naïve HIV patients care performed between 2010 and 2016 were retrieved at 6 reference laboratories: 2 in France (Pitié-Salpêtrière and Bichat Claude Bernard hospitals, n=2941), 3 in Italy (University of Rome "Tor Vergata", INMI Spallanzani-IRCCS, Modena Hospital, n=4063) and 1 in Greece (Department of Hygiene Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, n=1230). In addition, sequences data from drug-naïve patients were provided by a number of centers included in the ARCA database (www.dbarca.net, n=1530) in Italy. 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 to doravirine.

NRTIs (zidovudine, emtricitabine/lamivudine, abacavir, tenofovir) and NNRTIs (efavirenz, rilpivirine, nevirapine and etravirine) mutations associated with resistance 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/).

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).

HIV-1 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 with Fisher's exact test using the BiostatTGV web site (https://biostatgv.sentiweb.fr/?module=tests).

RESULTS

Distribution of HIV-1 subtypes in antiretroviral-naïve patients

A total a 9764 RT sequences obtained between 2010 and 2016 for HIV-1 treatment-naïve patients in routine clinical care were analyzed (2010-2012: n=4939; 2013-2016: n=4825). The distribution of subtypes was: 53.0% B subtypes and 47.0% non-B subtypes. Subtypes with prevalence higher than 3.0% included CRF02_AG (14.6%), A (6.3%), C (3.3%) and F (3.2%). There was a significant increase of non-B subtypes in 2013-2016 with respect to 2010-2012 (49.4% versus 42.7%, respectively, $p < 0.001$).

Prevalence of doravirine resistance associated mutations

The overall prevalence of sequences with at least 1 doravirine resistance-associated mutation was 1.4% (n = 137). The number of sequences with 1, 2, 3 and 4 doravirine resistance-associated mutations was 127 (1.3%), 8 (0.1%), 1 (0.01%) and 1 (0.01%), respectively. The presence of the double mutant K103N/Y181C was 0.05% (n=5). This prevalence was significantly lower than the prevalence of sequences with at least 1 resistance-associated

mutations for other NNRTIs: efavirenz (4.3%, n = 421), nevirapine (4.3%, n = 421), rilpivirine (7.7%, n=755) or etravirine (11.7%, n = 1143) ($p < 0.001$) (Figure 1).

Among the doravirine resistance-associated mutations, the most frequent mutations were V108I (0.6%; n=62), Y188L (0.2%; n=18), H221Y (0.2%; n=18) and Y318F (0.2%; n=23) (Figure 2). The other doravirine resistance-associated mutations were very rare: V106A/M (0.1%; n=8), G190S (0.1%; n=5), F227C/L/V (0.1%; 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 (2.1%; n=208), E138A/G/K/Q/R (6.5%; n=637), Y188C/H/L (0.2%; n=22) and G190A/E/S (0.5%; n=51) (Figure 2). Between 2010-2012 and 2013-2016, there was only a significant increase for K103N/S (2.0% versus 3.0%, $p = 0.003$) and in G190A/E/S (0.3% versus 0.7%, $p = 0.003$).

Interpretation of resistance to doravirine, NRTIs and other NNRTIs

The presence of at least one doravirine-associated mutation was interpreted as resistance to doravirine, thus 1.4% (n=142) of sequences were considered resistant to doravirine in comparison with 8.5% (n=833) to rilpivirine, 8.1% (n=788) to etravirine, 8.3% (n=809) to nevirapine and 3.9% (n=348) to efavirenz according to the 2017 ANRS algorithm. Then, 0.8%, 0.5%, 0.9%, 0.9%, of the sequences were both resistant to doravirine and rilpivirine or etravirine or nevirapine or efavirenz, respectively. The results were slightly different according to the Stanford algorithm: 9.9% (n=967) for rilpivirine, 10.0% (n=979) for etravirine, 7.5% (n=730) for nevirapine and 9.4% (n=828) for efavirenz, and 1.0%, 1.0%, 0.6% and 0.6% of the sequences both resistant to doravirine and efavirenz or nevirapine or etravirine or rilpivirine, respectively.

For NRTIs, 3.5%, 1.6%, 1.0% and 0.2% of sequences were resistant to zidovudine, lamivudine/emtricitabine, abacavir and tenofovir with both resistance algorithms, respectively. Few samples were considered resistant to doravirine and also to zidovudine or lamivudine/emtricitabine or abacavir or tenofovir in 0.4%, 0.4%, 0.09% and 0.02% of cases, respectively.

NNRTI resistance according to the subtype

There was no relationship between subtypes and the presence of doravirine-associated mutations (1.6% and 1.3% 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 (11.2% versus 5.12%, p<0.001 and 8.0% versus 6.8%, p=0.025, respectively) and rilpivirine (9.3% versus 7.7%, p=0.006 and 10.7% versus 8.9%, p=0.003, respectively). The resistance to etravirine was also statistically higher for B subtype only with the Stanford algorithm (10.9% versus 9.0%, p=0.002).

DISCUSSION

This is the first study showing that the prevalence of doravirine resistance-associated mutations in HIV-1-infected treatment-naïve patients is very low in a large European database, 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 (47.0%) compared to the continuous HIV drug resistance surveillance program (SPREAD) taking place in 27 countries

in Europe from 2002 to 2007 (32.7%), or to the last studies in France or in Italy (30.8%). [11–13] However, this higher prevalence of non-B subtypes is consistent with their continuous increase in Europe or their high prevalence observed recently in Greece. [13–15] Thus, our study provides a representative view of HIV subtypes circulating in Western Europe and shows that resistance to NNRTIs was higher for B than non-B subtypes, except for doravirine.

In *in vitro* studies, the 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. [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 mutations list. For example, E138A was present in 4.2% of the 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 on doravirine phenotypic susceptibility to and also virological response.

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 observations, particularly because patients harboring NNRTI-resistant virus were deliberately excluded from clinical trials completed so far.

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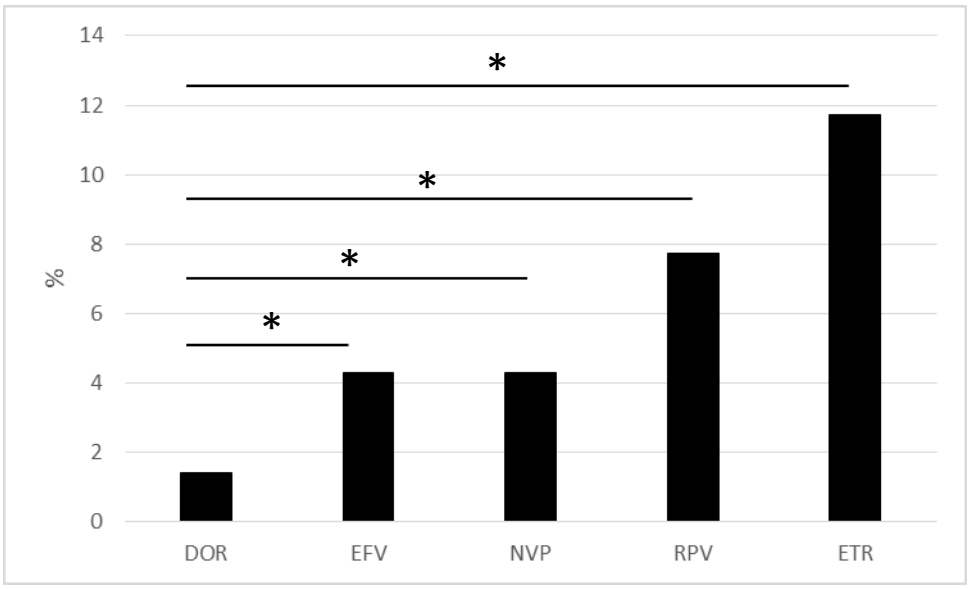

Figure 1: Percent of Reverse Transcriptase sequences with at least one resistance mutation to NNRTI

Doravirine (DOR), Efavirenz (EFV), Rilpivirine (RPV), Nevirapine (NVP) and Etravirine (ETR).

* : $p < 0.0001$

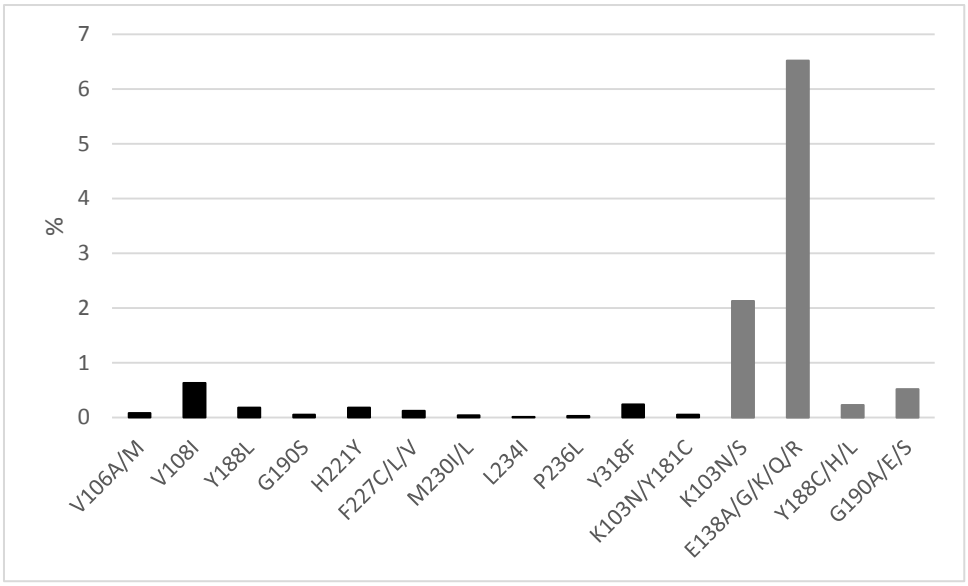

Figure 2: Prevalence of Reverse Transcriptase sequences with at least one individual resistance mutation to Doravirine or other NNRTI

In black: mutations associated with resistance to doravirine, in grey: mutations associated to other NNRTIs