Rilpivirine, emtricitabine and tenofovir resistance in HIV-1-infected rilpivirine-naive patients failing antiretroviral therapy

S. Lambert-Niclot1*, C. Charpentier2, A. Storto2, D. Fofana1, C. Soulie1, S. Fourati1, M. Wirden1, L. Morand-Joubert3, B. Masquelier4, P. Flandre1, V. Calvez1, D. Descamps2 and A. G. Marcelin1

1APHP, Hôpital Pitié-Salpêtrière, Laboratoire de Virologie, UPMC Univ Paris 06, INSERM U 943, F75013 Paris, France; 2APHP, Hôpital Bichat-Claude Bernard, Laboratoire de Virologie, HUPNVS, Université Paris Diderot, Paris 7, PRES Sorbonne Paris Cité, EA4409, F75018 Paris, France; 3APHP, Saint Antoine Hospital, Laboratoire de Virologie, UPMC Univ Paris 06, INSERM U 943, F75011 Paris, France; 4Laboratoire de Virologie, CHU de Bordeaux and UMR5234, Université de Bordeaux, F33100 Bordeaux, France

*Corresponding author. Department of Virology, Pitié-Salpêtrière Hospital, 83 Boulevard de l’Hôpital, 75013 Paris, France. Tel: +33-142175842; Fax: +33-142177411; E-mail: sidonie.lambert@psl.aphp.fr

Received 15 July 2013; returned 5 September 2013; revised 18 October 2013; accepted 28 October 2013

Objectives: In the context of simplification strategies, it is essential to know the feasibility of a switch to a rilpivirine-based therapy. The aim of this study was to describe rilpivirine, tenofovir and emtricitabine resistance in HIV-1-infected patients who experienced virological failure during their previous antiretroviral treatment.

Patients and methods: The studied population included two groups of patients, all rilpivirine naive, tested for resistance by bulk sequencing from 2008 to 2011: the first group (n = 998) failing a nucleoside reverse transcriptase inhibitor (NRTI) plus boosted protease inhibitor (PI)-based regimen and the second group (n = 3733) failing an NRTI plus non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen.

Results: In the first group, the frequency of rilpivirine mutations and resistance to rilpivirine (5.1%) was similar to that in antiretroviral-naive HIV-1-infected patients. Among the 1605 patients from the second group with at least one NNRTI mutation in their HIV, the prevalence of viruses ‘resistant’ or ‘possibly resistant’ to efavirenz, nevirapine and etravirine was 78%, 79% and 74%, respectively, while 59% were resistant to rilpivirine. Resistance to rilpivirine was significantly more frequent in non-B subtype versus B subtype viruses. Among pretreated patients with viruses with at least one NNRTI mutation (other than for rilpivirine), 22% of sequences were susceptible to the combination rilpivirine/emtricitabine/tenofovir disoproxil fumarate.

Conclusions: In patients failing an NNRTI plus NNRTI-based regimen, to know the feasibility of a switch to rilpivirine/emtricitabine/tenofovir disoproxil fumarate, reliable resistance information should be available at the time of use of concurrent NNRTI therapy.

Keywords: retroviruses, non-nucleoside reverse transcriptase inhibitors, resistance mutations

Introduction

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are important and common components of highly active antiretroviral therapy (HAART). Rilpivirine, a second-generation NNRTI, is active against wild-type viruses and retains activity against some NNRTI-resistant HIV-1 strains.1–3 In vivo, virology data are available from the Phase 2 TMC278-C204 study, a dose-ranging study in HIV-1-infected treatment-naive patients,4,5 from two large Phase 3 double-blind studies (ECHO and THRIVE)6,7 and from one Phase 3 unblinded study (STaR).8

Rilpivirine is currently indicated in the USA and Europe in combination with other antiretroviral drugs for the treatment of antiretroviral-naive HIV-1-infected patients with HIV-1 RNA ≤100 000 copies/mL.9 Rilpivirine has also been marketed as a coformulation with emtricitabine and tenofovir disoproxil fumarate to reduce pill burden.10 It is essential to characterize the frequency of resistance to rilpivirine in a population of pretreated HIV-1-infected patients to determine the feasibility of switching to a rilpivirine-based therapy in the context of simplification strategies or rescue interventions. The SPIRIT study evaluated a switch from a boosted protease inhibitor (PI)-based HAART to a simplified regimen of rilpivirine/emtricitabine/tenofovir disoproxil fumarate in virologically suppressed HIV-infected subjects and showed maintenance of virological suppression and a low rate of resistance development.11

The aim of this study was to describe rilpivirine, emtricitabine and tenofovir resistance in HIV-1-infected patients who
experienced virological failure in their antiretroviral treatment history. This population included patients failing a nucleoside reverse transcriptase inhibitor (NRTI) plus boosted PI-based regimen and patients failing an NRTI plus NNRTI-based regimen.

Patients and methods

Study population
HIV-1 seropositive patients included in this study were exposed to antiretroviral drugs, but were rilpivirine naive before the time of virological failure. This population included two groups: the first group (n=998) was composed of patients failing an NRTI plus boosted PI-based regimen and the second group (n=3733) was composed of patients failing an NRTI plus NNRTI-based regimen. Samples were analysed for resistance testing by bulk sequencing from 2008 to 2011. This study was approved by the Agence Nationale de Recherche sur le SIDA (ANRS) Ethics Committee of Action Coordinatrice 11. Patients followed at the Internal Medicine and Infectious Diseases Departments of the Pitie-Salpêtrière Hospital signed individual consent forms.

Genotypic resistance analyses and interpretation
Rilpivirine resistance-associated mutations (RAMs) were defined as K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, Y188L, H221Y, F227C and M230I/L.9,12 Rilpivirine resistance evaluated according to a genotypic ANRS algorithm was defined as having at least one mutation among K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, Y188L, H221Y and M230I/L or as having L100I+K103N. The NNRTI mutations (mutations to efavirenz, nevirapine or etravirine) were determined according to the March 2013 International AIDS Society (IAS) list and resistance was evaluated according to a genotypic ANRS algorithm. The definition of resistance in our study included ‘resistance’ and ‘possible resistance’. Subtype determination was on the basis of the reverse transcriptase and protease coding regions (SmartGene, Switzerland; www.smartgene.com).

Statistical analyses
Between-group comparisons were carried out using Fisher’s exact test and the $\chi^2$ test for categorical variables.

Results

Patients failing an NRTI plus boosted PI regimen (PI group)
This group was composed of 502 patients with subtype B viruses and 496 patients with subtype non-B viruses.

Prevalence of primary rilpivirine RAMs (PI group)
As defined by the IAS list, the prevalence of primary rilpivirine RAMs was low (5%, 50/998). The most prevalent mutations in this analysis were E138A (32 cases, 3.2%), E138K (6 cases, 0.6%), H221Y (6 cases, 0.6%), E138G (4 cases, 0.4%), Y181C (4 cases, 0.4%) (a) 3.5 3 2.5 2 1.5 1 0.5 0.5 Frequency (%) Frequency (%) RPV RAMs RPV RAMs (b) 25 20 15 10 5 0 25 20 15 10 5 0 (a) Patients failing on an NRTI plus PI-based regimen (n = 998). (b) Patients failing on NRTI plus NNRTI with at least one NNRTI RAM (n = 1605). RPV, rilpivirine.
and Y188L (4 cases, 0.4%). The frequencies of rilpivirine RAMs are depicted in Figure 1(a).

Using the IAS list for the analysis, most viruses with a primary rilpivirine RAM had only one: 42 sequences (4.2%) had one rilpivirine mutation and 8 sequences (0.8%) had two rilpivirine mutations. No patient had a sequence with three or more rilpivirine RAMs. The distribution of the number of rilpivirine RAMs was significantly different between B and non-B subtype viruses (Fisher’s exact test, P = 0.02). Indeed, non-B subtype viruses had more rilpivirine RAMs than B subtype viruses. This difference is due mainly to the mutation E138A, which was twice as common among non-B subtype viruses.

**Resistance (PI group)**

According to the ANRS algorithm, 5.1% of samples were resistant to rilpivirine: 3.8% of B subtype viruses versus 6.4% of non-B subtype viruses (P = 0.02, χ² test). The prevalence of the tenofovir mutation K65R and the emtricitabine/lamivudine mutations M184I/V was 0.5% (5/998) and 3% (30/998), respectively, and there was no difference between B and non-B subtypes. According to the ANRS algorithm, predicted resistance to efavirenz and nevirapine in our population was 4% and 3.9%, respectively.

**Patients failing an NRTI plus NNRTI-based regimen (NNRTI group)**

This group was composed of 3733 patients failing an NRTI plus NNRTI-based regimen. Of these patients, 2128 (57%) had viruses without any NNRTI resistance mutations (efavirenz, nevirapine and etravirine). We then more extensively studied the 1605 patients with viruses with at least one NNRTI mutation, composed of subtype B (n = 780) and non-B subtype (n = 825) viruses.

**Prevalence of rilpivirine RAMs (NNRTI group)**

Among the 1605 sequences studied, as defined by the IAS list, the rilpivirine RAMs found to be most prevalent in this analysis were Y181C (363 cases, 22.6%), E138A (225 cases, 14.0%), H221Y (130 cases, 8.1%), K101E (120 cases, 7.5%) and Y188L (107 cases, 6.7%). All other rilpivirine RAMs examined were present at rates <5% and mutation E138R was totally absent in our study population. The frequencies of rilpivirine RAMs are depicted in Figure 1(b). The E138A mutation was found to be more prevalent in non-B subtype than B subtype viruses, with 137 cases (18%) versus 88 cases (11%), respectively (Fisher’s exact test, P = 0.0025).

According to the IAS list, 807 of 1605 (50.3%) sequences harboured at least one rilpivirine RAM: 596 (37.1%) had one mutation, 191 (11.9%) had two mutations and 20 (1.2%) had three mutations. No patient had a sequence with four or more rilpivirine RAMs. The distribution of the number of rilpivirine RAMs was not significantly different between B and non-B subtype viruses (Fisher’s exact test, P = 0.68).

**Resistance (NNRTI group)**

According to the ANRS algorithm, 58.5% (939/1605) of samples were resistant to rilpivirine: 57% of B subtype viruses versus 60% of non-B subtype viruses (Fisher’s exact test, P = 0.001). The prevalence of resistance and possible resistance to efavirenz, nevirapine and etravirine was 78%, 79% and 73.5%, respectively (Figure S1, available as Supplementary data at JAC Online).

Using the ANRS algorithm, among sequences resistant and possibly resistant to efavirenz, nevirapine and etravirine, 59.6%, 59.6% and 61.1%, respectively, were predicted to be resistant to rilpivirine. The prevalence of the associated mutations E138K and M184I was 0.1% (n = 2). The prevalence of K65R and M184I/V was 3.8% (61/1605) and 49% (790/1605), respectively. Only 22% of sequences were predicted to be susceptible to all of the components of rilpivirine/emtricitabine/tenofovir disoproxil fumarate in this NNRTI resistance subgroup.

In the total group (n = 3733) of experienced patients failing an NRTI plus NNRTI (other than rilpivirine) regimen, 25.6% were resistant to rilpivirine.

**Discussion**

Our study evaluated the resistance to rilpivirine, emtricitabine and tenofovir in 4731 rilpivirine-naive, antiretroviral-experienced HIV-1-infected patients failing HAART. In the patient group failing an NRTI plus boosted PI regimen (n = 998), the frequency of rilpivirine mutations and resistance (5.1%) was similar to that in antiretroviral-naive HIV-1-infected patients. According to our analysis using the genotypic ANRS algorithm, in the group of experienced patients failing an NRTI plus NNRTI (other than rilpivirine) regimen, 25.6% were resistant to rilpivirine and 58.5% of those with an NNRTI RAM in their HIV. This resistance to rilpivirine was significantly more frequent in non-B subtype versus B subtype viruses.

In the NNRTI-resistant group, among sequences resistant to efavirenz or nevirapine, ~60% were predicted to be resistant to rilpivirine according to the ANRS algorithm. Some RAMs were common to the etravirine and rilpivirine algorithms, indeed, 61.1% of patients resistant to etravirine were also resistant to rilpivirine. In our study, this cross-resistance was due to the RAMs Y181C and H221Y. Among pretreated patients with viruses with at least one mutation to NNRTIs other than rilpivirine according to the IAS list, only 22% of sequences were predicted to be susceptible to the combination rilpivirine/emtricitabine/tenofovir disoproxil fumarate.

These results confirmed the SPIRIT study showing a similar low frequency of rilpivirine mutations and rilpivirine resistance between the patient group failing an NRTI plus boosted PI regimen and antiretroviral-naive HIV-1-infected patients. This study showed that after NNRTI failure, 57% of patients did not harbour a NNRTI mutation. However, among 43% of patients with at least one NNRTI mutation, only 22% of patients had rilpivirine/emtricitabine/tenofovir disoproxil fumarate-susceptible virus. Currently, rilpivirine is not recommended in patients failing an NNRTI regimen, but the data from the present study suggest there may be a possibility to use rilpivirine in some patients who have previously failed NNRTI therapy. The condition is reliable resistance information available at the time of use of concurrent NNRTI therapy.

**Acknowledgements**

We thank G. Le Mallier and P. Grange for their technical assistance.
Funding
This work was supported by the Agence Nationale de Recherche sur le SIDA (ANRS), the European Community’s Seventh Framework Programme (FP7/2007–2013) under the project ‘Collaborative HIV and Anti-HIV Drug Resistance Network (CHAIN)’ (grant agreement number 223131) and the Association de Recherche en Virologie et Dermatologie (ARVD).

Transparency declarations
None to declare.

Supplementary data
Figure S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org).

References