Archived HIV-1 DNA resistance mutations to rilpivirine and etravirine in successfully treated HIV-1-infected individuals pre-exposed to efavirenz or nevirapine

S. Gallien1,2*, I. Charreau3, M. L. Nere4, N. Mahjoub4, F. Simon2,4, N. de Castro1, J. P. Aboulker3, J. M. Molina1,2 and C. Delaugerre2,4

1Service de Maladies Infectieuses et Tropicales, Hôpital Saint-Louis-APHP, Paris, France; 2INSERM U941, Université Paris Diderot, Sorbonne Paris Cité, Paris, France; 3INSERM SC10, Villejuif, France; 4Laboratoire de Virologie, Hôpital Saint Louis-APHP, Paris, France

*Corresponding author. Service de Maladies Infectieuses et Tropicales, Hôpital Saint-Louis, 1 avenue Claude Vellefaux, 75010 Paris, France. Tel: +33-(0)-142-49-45-72; Fax: +33-(0)-1-42-49-42-83; E-mail: sebastien.gallien@sls.aphp.fr

Received 24 June 2014; returned 20 July 2014; revised 9 September 2014; accepted 10 September 2014

Objectives: Efavirenz and nevirapine failure is associated with a rapid selection of resistance-associated mutations (RAMs), which may impact on etravirine or rilpivirine susceptibility. However, RAMs for rilpivirine and etravirine cannot be reported on previous resistance genotypes because these specific RAMs were not analyzed at that time. Therefore, our objective was to determine, in virologically suppressed HIV-1-infected individuals, the presence of RAMs to rilpivirine, etravirine and the combination of tenofovir/emtricitabine/rilpivirine in HIV-1 DNA from individuals previously exposed to efavirenz and/or nevirapine.

Methods: The studied population included 169 treatment-experienced individuals enrolled in the ANRS 138-EASIER trial who previously failed on and/or were intolerant to efavirenz and/or nevirapine and who had plasma HIV-1 RNA <400 copies/mL. Resistance to rilpivirine, etravirine, tenofovir and emtricitabine by bulk sequencing was performed on extracted HIV-1 DNA from whole blood collected at the time of trial inclusion.

Results: Reverse transcriptase gene amplification was successful in 128/169 (76%) individuals and 95% of HIV-1 were infected with subtype B. Rilpivirine RAMs were detected in 41 (32%) individuals, with highest frequency for the mutations Y181C/I/V (18%), K101E/P (7%) and E138A/G/K/Q/R/S (6%) and the association L100I + K103N/S (5%). Etravirine RAMs were detected in five (4%) individuals. Resistance to emtricitabine, tenofovir and at least one drug included in the combination of tenofovir/emtricitabine/rilpivirine were detected in 72 (56%), 12 (9%) and 88 (69%), respectively.

Conclusions: In individuals with suppressed viraemia under antiretroviral therapy (ART), but who had been previously exposed to an efavirenz and/or nevirapine-based regimen, rilpivirine RAMs are frequent and etravirine RAMs are rare. This finding suggests that the switch to a rilpivirine-based regimen should not be recommended.

Keywords: antiviral, HIV, NNRTIs

Introduction

Treatment failure with the first-generation NNRTIs, efavirenz and nevirapine, is associated with the rapid selection of resistance-associated mutations (RAMs) in plasma. This may impact on susceptibility to the second-generation NNRTIs, etravirine and rilpivirine.1 In individuals previously exposed to the first-generation NNRTIs, and under virological control with antiretroviral therapy (ART) for many years, the switch to second-generation NNRTIs may be considered, especially in simplification therapy, where rilpivirine has been approved as a single-tablet regimen with tenofovir disoproxil fumarate and emtricitabine.12 The use of previous plasma resistance genotypes is recommended to determine rilpivirine susceptibility. However, in virologically suppressed individuals, previous plasma resistance genotypes cannot provide information for rilpivirine and etravirine RAMs because these particular second-generation NNRTI RAMs were unknown at that time and also because sequences could not be stored and therefore not available anymore.

RAMs identified in plasma are theoretically also present in HIV-1 DNA archived as integrated proviruses in HIV-infected cells. PBMCs are a source of archived HIV-1 DNA that can be used for genotypic analyses, acting as a record of past RAMs, from individuals with undetectable plasma HIV-1 RNA.6 The
successful virological response to ART may be impacted using regimens containing drugs for which specific archived resistance mutations in HIV DNA have been detected.\(^5,6\)

The aim of this study was to determine the presence of RAMs to rilpivirine, etravirine and the combination of tenofovir/emtricitabine/rilpivirine in HIV-1 DNA from individuals virologically suppressed and previously exposed to the first-generation NNRTIs.

**Methods**

**Study population**

HIV-1-infected individuals in this study had been enrolled in the EASIER (ANRS 138) trial. The EASIER trial was a multicentre, randomized, comparative, 48 week open-label trial with a primary endpoint at week 24, in which 169 virologically suppressed (<400 HIV-1 copies/mL plasma) treatment-experienced individuals, who had a prior history of triple-class (PI, NRTI and NNRTI) antiretroviral drug failure or intolerance, receiving a stable enfuvirtide-containing regimen, were randomized to switch to raltegravir or remain on enfuvirtide.\(^7\) The protocol was approved by the Ethics Committee CPP Paris I Hôtel-Dieu (trial number NCT00454337). All patients gave written informed consent.

**Genotypic resistance analyses and interpretation**

HIV-1 DNA resistance genotyping was performed in a central laboratory prior to treatment randomization. Viral DNA was extracted from 200 mL of frozen stored whole blood using an automatic nucleic acid extractor (MagnoPure, Roche, Meylan, France). Population sequencing of the reverse transcriptase (RT) gene was performed according to the ANRS consensus method (http://www.hivfrenchresistance.org/ANRS-procedures.pdf). Sequences were aligned with the HIV-1 subtype HXB2 reference strain on the SmartGene HIV-1 Service Module (SmartGene, Zug, Switzerland).

RT sequencing was performed on HIV-1 DNA at the time of inclusion for all individuals. Antiretroviral drug resistance was evaluated using the September 2013 ANRS algorithm (http://www.hivfrenchresistance.org/2013/Algo-sep-2013.pdf) and the March 2013 IAS list (https://www.iiasa.org/sites/default/files/tam/21-1-6.pdf). Resistance to rilpivirine was defined as the presence of at least four mutations among V90I, A98G, L100I, K101E/H/I/P/R, V179L, Y181C/I/V, Y188L, and K221Y. Resistance to tenofovir was defined as the presence of at least one mutation among E138K, Y181C/I/V, E138A/G, K103N/S or L100I, K103R, V179D. Resistance to etravirine was defined as the presence of at least four mutations among V90I, A98G, L100I, K101E/H/I/P/R, V106I, V179D/F/I/L/M/T, Y181C/I, G190A/S and M230L, or as having at least one mutation among E138K, Y181V and Y181C+H221Y. Resistance to tenofovir was defined as the presence of at least six mutations among M41L, E44D, D67N, T69D/N/S, L74V/I, L210W and T275Y/F, or as having K65R/E, an insertion at codon 69 or K70E. Resistance to emtricitabine was defined as the presence of M184V/I or an insertion at codon 69. The definition of resistance in our study included ‘resistance’ and ‘possible resistance’. Subtype determination was on the basis of the RT coding regions using the SmartGene HIV-1 Service Module (SmartGene, Zug, Switzerland).

**Statistical analyses**

All reported values are medians (with IQRs) for continuous variables and frequencies and percentages for categorical variables. Fisher’s exact test or the \(\chi^2\) test was used to compare categorical variables and the Wilcoxon test was used to compare continuous variables.

Univariate logistic regression analyses were used to assess association between baseline parameters and detection of RAMs to emtricitabine, tenofovir and rilpivirine in baseline PBMC DNA. Baseline parameters studied were HIV-1 subtype, nadir and baseline CD4+ T cells, baseline HIV viral load (VL) <50 copies/mL, time since starting the first ART, and NRTI-and/or tenofovir- and/or lamivudine/emtricitabine-containing regimens at the time of sampling.

Comparisons were made using a two-sided \(\alpha\) level of 0.05. Statistical analyses were performed with SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA).

**Results**

RT gene amplification from PBMCs succeeded in 128/169 (76%) individuals with no statistical difference found for the median CD4+ cell count in patients in whom RT amplification was successfully performed or not (395 versus 364 cells/mm\(^3\)).

Table 1 shows baseline characteristics of these subjects. Individuals were mostly men (84%), with a median age of 48.4 years, were highly treated experiencing with a median time (IQR) since commencement of the first ART of 13.6 years (12–15.2), and 49% were classified as CDC stage C. At baseline, 122 individuals had an NRTI included in their ART regimen: tenofovir in 73 (57%), lamivudine or emtricitabine in 97 (76%) and abacavir in 48 (38%). Seven individuals (5%) were receiving NNRTIs (efavirenz or nevirapine) and all but one were receiving a ritonavir-boosted PI.

Baseline plasma RNA HIV-1 was <50 copies/mL in 107 individuals (86%). The baseline median CD4+ T cell count was 395 cells/mm\(^3\) (range 270–513). Overall 122 individuals (95%) were infected with HIV-1 subtype B. Other subtypes detected were CRF01_AE in two (2%) and D in one (1%); subtype was undetermined in three (2%).

**Table 1. Baseline characteristics of 128 individuals enrolled in the EASIER trial (with RT gene amplification)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Min–Max</th>
<th>Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>19.9–70.8</td>
<td>48.4 (43.3–56.2)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>108 (84%)</td>
</tr>
<tr>
<td>CDC classification</td>
<td>C</td>
<td>63 (49%)</td>
</tr>
<tr>
<td>Baseline CD4+ (cells/mm(^3))</td>
<td>113–1254</td>
<td>395 (270–513)</td>
</tr>
<tr>
<td>Nadir CD4+ (cells/mm(^3))</td>
<td>0–404</td>
<td>56 (14–118)</td>
</tr>
<tr>
<td>Time since first ART (years)</td>
<td>4.4–20.1</td>
<td>13.6 (12–15.2)</td>
</tr>
<tr>
<td>ART at baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRTI</td>
<td>all</td>
<td>122 (95%)</td>
</tr>
<tr>
<td>TDF</td>
<td>73 (57%)</td>
<td></td>
</tr>
<tr>
<td>3TC/FTC</td>
<td>97 (76%)</td>
<td></td>
</tr>
<tr>
<td>ABC</td>
<td>48 (38%)</td>
<td></td>
</tr>
<tr>
<td>NNRTI</td>
<td>7 (5%)</td>
<td></td>
</tr>
<tr>
<td>PIs/r</td>
<td>127 (95%)</td>
<td></td>
</tr>
<tr>
<td>HIV-1 RNA (&lt;50 copies/mL)</td>
<td>107 (84%)</td>
<td></td>
</tr>
<tr>
<td>HIV-1 subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>122 (95%)</td>
<td></td>
</tr>
<tr>
<td>non-B</td>
<td>6 (5%)</td>
<td></td>
</tr>
</tbody>
</table>
The prevalence of at least one rilpivirine RAM was 32% (41/128). The most prevalent rilpivirine RAMs were E138A/G/K/Q/R/S (8/128, 6%), Y181C/I/V (23/128, 18%), K101E/P (9/128, 7%) and the association L100I + K103N/S (6/128, 5%). The prevalence of at least one etravirine RAM was 4% (5/128) (Figure 1).

Resistance to emtricitabine and tenofovir was detected in 72/128 (56%; all M184V/I) and 12/128 (9%; K65R in 2, K70E in 3 and at least six mutations among M41L, E44D, D67N, T69D/N/S, L74V/I, L210W and T215Y/F in 10), respectively. No individual had the two RAMs E138K + M184I together, and one had the two mutations E138K + M184V.

Resistance to at least one drug included in the combination of tenofovir/emtricitabine/rilpivirine was observed for 88/128 (69%) individuals, consisting predominately of M184V/I alone (42/88, 48%).

In univariate logistic regression, none of the baseline parameters studied (HIV-1 subtype, nadir and baseline CD4+ T cells, baseline VL <50 copies/mL, time since starting the first ART, and NNRTI- and/or tenofovir- and/or lamivudine/emtricitabine-containing regimens at the time of sampling) was significantly associated with the detection of RAMs to emtricitabine, tenofovir or rilpivirine in baseline PMBC DNA (data not shown).

Discussion

This study evaluated the presence of archived HIV-1 DNA resistance mutations to rilpivirine and etravirine in 128 HIV-1-infected virologically suppressed individuals pretreated with nevirapine and/or efavirenz and triple-class antiretroviral-experienced.

Among the individuals in whom the RT gene was successfully amplified, we found HIV-1 DNA resistance to rilpivirine and etravirine in 32% and 4%, respectively.

These findings confirm the high rate of selected RAMs to rilpivirine following previous use of first-generation NNRTIs, mainly at the RT gene codons 181 (18%), 101 (7%) and 138 (6%). In two retrospective resistance analyses in patients pre-exposed to an NNRTI-based regimen but naïve to rilpivirine, rilpivirine RAMs were similar in prevalence rate (23.5% and 22.6%) and type of rilpivirine RAM (22.5% and 22.6%, 10.5% and 7.5% and 5.3% and 14% at codons 181, 101 and 138, respectively).8,9

Prevalence of etravirine RAMs was low in our cohort (4%) and was similar to the prevalence found in sub-Saharan cohorts of individuals failing efavirenz- or nevirapine-based regimens, ranging between 5% and 30%.10,11

The mutation E138K, which confers cross-resistance to both rilpivirine and etravirine, conversely to other substitutions in RT position 138 (A, G, Q, R and S), was rarely selected after failure on efavirenz and nevirapine (only one patient).

In addition, both rilpivirine and etravirine RAMs were most frequently detected in non-B HIV-1 subtypes, whereas viruses identified in EASIER individuals belonged to subtype B in 95%.

In contrast, potential resistance to the single-tablet combination of tenofovir/emtricitabine/rilpivirine was high (69%) and was mainly due to the detection of the mutation M184V/I alone in 56% of cases, conferring resistance to emtricitabine.

As resistance testing performed on HIV DNA lacks sensitivity compared with previous plasma genotypes,13,14 we could have underestimated the prevalence of rilpivirine and etravirine RAMs. The main limitation of this study is the rate of RT amplification failure in almost a quarter of the EASIER individuals; it was not anticipated that the amplification rate of the RT gene in PBMCs was not linked to the CD4+ T cell count, as previously reported.15

Another limitation was the use of the population-based sequencing approach, which may not detect minor variants and therefore could have also underestimated RAMs. Deep sequencing techniques would improve archived RAM detection,16,17 and the presence of minority resistant quasispecies may predict the later detection by population-based sequencing of clinically significant RAMs.18 The prevalence of RAMs can vary between different PBMC populations and between different body compartments,19,20 and sequencing of DNA from selected memory T cells, the main

Figure 1. Rilpivirine (a) and etravirine (b) RAMs in PBMC DNA at baseline in individuals enrolled in the EASIER trial (percentage and number of individuals with RAMs among the 128 individuals). (a) RAMs M230I/L/V and V179L and the association of L100I + K103N/S were not detected. (b) *Among V90I, A98G, L100I, K101E/H/I/P/R, V106I, V179D/F/I/L/M/T, Y181C/I, G190A/S and M230L.
cellular HIV reservoir, would increase the sensitivity of DNA RAM detection. However, these methods remain too costly and technically complex (and need large sample volumes) compared with our approach of amplifying RT from total DNA extracted from whole blood, to be used in routine clinical practice.

In conclusion, these results confirm that in individuals with suppressed plasma viraemia under ART, but who had been previously exposed to efavirenz and/or nevirapine-based regimens, rilpirivirine RAMs are frequent in archived PBMC DNA. This suggests that the switch to a rilpirivirine-based regimen should not be recommended in this setting.

Acknowledgements

This study was presented in part at the Twenty-first Conference on Retroviruses and Opportunistic Infections, Boston, MA, USA, 2014 (Abstract 591).

We are greatly thankful to Professor Dominic Dwyer for his critical review of the manuscript and English editing prior to submission.

Funding

This work was supported by a grant from the Agence Nationale de Recherches sur le SIDA et les Hépatites Virales (ANRS).

Transparency declarations

None to declare.

Author contributions


References