Predicted residual activity of rilpivirine in HIV-1 infected patients failing therapy including NNRTIs efavirenz or nevirapine

K. Theys¹, R. J. Camacho¹,², P. Gomes²,³,⁴, A. M. Vandamme¹,² and S. Y. Rhee¹,⁵, on behalf of the Portuguese HIV-1 Resistance Study Group

¹) KU Leuven, University of Leuven, Department Microbiology and Immunology, Rega Institute for Medical Research, Leuven, Belgium, 2) Centro de Malária e outras Doenças Tropicais, Instituto de Higiene e Medicina Tropical e Unidade de Microbiologia, Universidade Nova de Lisboa, 3) Centro Hospitalar de Lisboa Ocidental, Lisbon, 4) Centro de Investigação Interdisciplinar Egas Moniz (CiEIM), Instituto Superior de Ciências da Saúde Sul, Caparica, Portugal and 5) Department of Medicine, Stanford University, Stanford, CA, USA

Abstract
Rilpivirine is a second-generation nonnucleoside reverse-transcriptase inhibitor (NNRTI) currently indicated for first-line therapy, but its clinical benefit for HIV-1 infected patients failing first-generation NNRTIs is largely undefined. This study quantified the extent of genotypic rilpivirine resistance in viral isolates from 1212 patients upon failure of efavirenz- or nevirapine-containing antiretroviral treatment, of whom more than respectively 80% and 90% showed high-level genotypic resistance to the failing NNRTI. Of all study patients, 47% showed a rilpivirine resistance-associated mutation (RPV-RAM), whereas preserved residual rilpivirine activity was predicted in half of the patients by three genotypic drug resistance interpretation algorithms. An NNRTI-dependent impact on rilpivirine resistance was detected. Compared with the use of nevirapine, the use of efavirenz was associated with a 32% lower risk of having a RPV-RAM and a 50% lower risk of predicted reduced rilpivirine susceptibility. Most prevalent RPV-RAMs after nevirapine experience were Y181C and H221Y, whereas L100I+K103N, Y188L and K101E occurred most in efavirenz-experienced patients. Predicted rilpivirine activity was not affected by HIV-1 subtype, although frequency of individual mutations differed across subtypes. In conclusion, this genotypic resistance analysis strongly suggests that the latest NNRTI, rilpivirine, may retain activity in a large proportion of HIV-1 patients in whom resistance failed while they were on an efavirenz- or nevirapine-containing regimen, and may present an attractive option for second-line treatment given its safety profile and dosing convenience. However, prospective clinical studies assessing the effectiveness of rilpivirine for NNRTI-experienced patients are warranted to validate knowledge derived from genotypic and phenotypic drug resistance studies.

Clinical Microbiology and Infection © 2015 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases.

Keywords: Activity, HIV, mutation, resistance, therapy

Original Submission: 11 September 2014; Revised Submission: 12 January 2015; Accepted: 8 February 2015

Editor: G. Antonelli

Article published online: 19 February 2015

Introduction
In the last 25 years, treatment options for HIV type 1 infection strongly expanded in response to a persistent need for viral inhibitors demonstrating higher potency, better tolerability and more favourable resistance profiles. The first-generation non-nucleoside reverse-transcriptase (RT) inhibitors (NNRTIs) efavirenz and nevirapine are confronted with adverse events and a low genetic barrier to resistance development, and second-generation NNRTIs etravirine and the latest approved, rilpivirine, have become available for use in clinical practice. Based on phase III clinical trials ECHO and THRIVE that demonstrated noninferiority compared with standard-care recommended efavirenz in treatment-naive patients with a viral load of <100 000 copies/mL, rilpivirine is currently recommended for first-line therapy [1–4].

Viral escape from drug pressure through resistance development is a major challenge for durable HIV-1 treatment success, and particularly an issue for the NNRTI class due to largely overlapping resistance profiles within and between
different generations. Phenotypic analyses of the rilpivirine trials showed that 90% of patients experiencing rilpivirine resistance-associated virological failure were resistant to etravirine, 87% to efavirenz and 45% to nevirapine, indicating that rilpivirine resistance precludes further NNRTI use [5]. Patients experiencing efavirenz resistance-associated virological failure largely remained susceptible to etravirine and rilpivirine [5]. Rilpivirine was designed to overcome viral variants resistant to first-generation NNRTIs [6], and in vitro sensitivity to rilpivirine was shown in 62% of first-generation NNRTI resistant isolates [7].

To date, a benefit of rilpivirine in clinical settings for patients who acquired NNRTI resistance is largely undefined, although supported by reports of in vivo activity [8,9] and a low prevalence of rilpivirine resistance—associated mutations (RAMs) reported in treatment- and NNRTI-experienced patients [10]. NNRTI sequencing strategies are also of relevance for the current treatment-experienced patient population as efavirenz and nevirapine constitute historically widely used components of cART. The resistance profile of rilpivirine recently has been refined, and clinically widely used resistance interpretation algorithms have incorporated rilpivirine resistance scores [5,7,11]. Considering this updated information, we estimated the expected reduced susceptibility to rilpivirine in HIV-1 patients who failed cART including first-generation NNRTIs, and evaluated a differential impact of efavirenz or nevirapine experience.

Methods

Clinical data were retrieved from a drug resistance database of HIV-1 patients followed up in 22 hospitals located in Portugal and undergoing routine genotypic resistance testing in case of NNRTI-containing therapy failure [12,13]. Eligible patients were naive to second-generation NNRTIs, experienced with either only efavirenz or only nevirapine and on an NNRTI regimen at least 24 weeks in length to avoid failing therapies due to reasons other than antiviral resistance. The most recent viral isolate of each patient spanning RT was collected.

We evaluated the prevalence of the following mutations as markers of (potential) rilpivirine resistance. 1) Sixteen individual mutations K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, Y188L, H221Y, F227C, M230I/L were defined as rilpivirine RAMs (RPV-RAMs), based on the ECHO and THRIVE trials and phenotypic analyses of rilpivirine resistance [4–7]. The combination L100I+K103N confers high-level resistance to rilpivirine and was counted as a 17th RPV-RAM [6,7]. 3) Four mutational patterns of K101E+M184I/V or E138K+M184I/V also were monitored, because NRTI RAMs M184I/V decrease rilpivirine susceptibility compared with the RPV-RAM alone [5]. 4) Individual mutations V90I, K101T, V106I/A, V108I, E138S, V179D/I/F, V189L, G190A/E/S and M230V were considered potential rilpivirine RAMs (pRPV-RAM), according to in vitro or in vivo selection studies or genotypic resistance interpretation algorithms [5,7,8,10,14–17].

Residual activity of rilpivirine was estimated on the distribution of rilpivirine RAMs and the genotypic susceptibility predicted by three interpretation algorithms (ANRS V22, Rega 9.1.0 and HIVdb V6.3.1) [15–17]. Viral isolates were classified as susceptible, intermediate resistant or high-level resistant by Rega and HIVdb, and susceptible or resistant by ANRS. An NNRTI- or subtype-dependent impact was evaluated by comparing mutation prevalence and predicted susceptibility between patient groups. A zero-inflated negative binomial regression model, taking into account the presence of dispersed count data and excess of zero values, was used to model mutation count data in the different groups. Pearson χ² test with Yates continuity correction was used to identify different proportions of patients displaying ≥1 rilpivirine mutations. Logistic regression was used to compare mutation prevalence according to HIV-1 subtype [18]. Data were analysed using the statistical package R with a significance level of 5% [19].

Results

The study included 1212 HIV-1 infected patients failing cART containing a first-generation NNRTI, of which 813 patients (67%) received efavirenz-containing cART and 399 patients (33%) received nevirapine-containing cART. At the start of the current failing regimen, 37% of the 813 efavirenz-experienced patients were treatment-naïve, 40% were NNRTI-naïve and 23% received the same NNRTI previously. Of the 399 nevirapine-experienced patients, these proportions were 31%, 45% and 24%, respectively. The median time on an NNRTI-containing regimen was 27.3 months (interquartile range: 12.0 to 49.7) for the nevirapine group and 22.3 months (interquartile range: 12.2 to 42.6) for the efavirenz group (p 0.04, Mann-Whitney test). The mean viral load (log) was 4.13 RNA copies/mL, with a viral load lower than 1000 copies/mL in 71 patients (7.5%) and above 100 000 copies/mL in 201 patients (16.6%), with comparable proportions in the efavirenz and nevirapine groups (data not shown). A majority of patients accumulated NNRTI resistance at failure, with interpretation algorithms scoring high-level genotypic efavirenz resistance in 83.9% (HIVdb) to 84.5% (ANRS) of efavirenz-experienced patients and high-level nevirapine resistance in 91.0% (HIVdb, Rega) to 94.5% (ANRS) of nevirapine-experienced patients.
At least 1 of 17 defined RPV-RAMS was present in 574 (47.3%) patients: 424 (35.0%) had one RPV-RAM, 141 (11.6%) had two RPV-RAMs and 9 (0.7%) had three or more RPV-RAMs. With a total count of 734 RPV-RAM occurrences, the NNRTI-experienced patient population was characterized by a mean number and variance of 0.61 ± 0.52 RPV-RAMs. Most prevalent RPV-RAMs were Y181C (n = 223, 18.4%), H221Y (n = 121, 9.9%), L100I+K103N (n = 111, 9.2%) and K101E (n = 95, 7.8%) (Fig. 1). L100I was largely (n = 111, 93%) found in the presence of K103N. Most frequent combinations, irrespective of accompanying RPV-RAMs, were Y181C+H221Y (n = 67, 5.5%), K101E+M184V (n = 67, 5.5%), Y181C+K101E (n = 29, 2.4%), H221Y+L100I+K103N (n = 15, 1.3%) and K101E+E138A (n = 14, 1.2%). Common pRPV-RAMs were G190A (n = 169, 13.9%), V106I (n = 103, 8.5%) and V108I (n = 103, 8.5%). Mutations E138R/S, V179F and M230L/I were not detected. The mean and variance of pRPV-RAMs was 0.55 ± 0.54, with 42% of study patients harbouring one or more pRPV-RAMs. A pRPV-RAM was more frequent in patients with RPV-RAMs (49.8%) than without any RPV-RAM (34.3%, p < 0.01). Overall, 793 patients (65.4%) carried at least one RPV-RAM or pRPV-RAM.

An RPV-RAM was present in 60% of the 399 nevirapine-experienced patients compared with 41% of the 813 efavirenz-experienced patients (relative risk [RR] = 1.46, p < 0.001). Taken together, with respective counts of 323 and 411 RPV-RAMs, the mean number of RPV-RAMs per patient was higher in the nevirapine-experienced population (0.81 ± 0.61 per patient) than in the efavirenz-experienced population (0.51 ± 0.45, p < 0.001). Most common RPV-RAMs after efavirenz use were L100I+K103N (13.4%), Y188L (7.6%), K101E (7.1%), H221Y (6.6%) and Y181C (5.6%), accounting for 26.5%, 15.1%, 14.1%, 13.1% and 11.2% of RPV-RAMs detected in efavirenz-experienced patients. H221Y with L100I+K103N was the most frequent RPV-RAM combination (1.8%). Most prevalent RPV-RAMs after nevirapine use were Y181C (44.4%), H221Y (16.8%) and K101E (9.3%), accounting for respectively 54.8%, 20.7%, and 11.5% of RPV-RAMs detected in this population. RPV-RAMs co-occurring most frequently were Y181C+H221Y (14.2%) and K101E+Y181C (4.0%). Similarly, nevirapine-experienced patients had a higher

**FIG. 1.** Prevalence of mutational patterns affecting rilpivirine susceptibility. RAM, resistance associated mutation (left); mutations affecting rilpivirine susceptibility only when in the specified combination (middle); pRAM, potential resistance associated mutation (right). Prevalence in efavirenz-experienced patients (dark grey), in nevirapine-experienced patients (light grey) and in all patients (horizontal bars).
mean number of pRPV-RAMs (0.64 ± 0.57 vs. 0.50 ± 0.52, p < 0.001) and a higher proportion of patients with pRPV-RAMs than efavirenz-experienced patients (48% vs. 38%, p < 0.001, RR = 1.2). RPV-RAMs Y181C/I and H221Y and pRAMs V106A, V179I and G190A were more prevalent after nevirapine failure, whereas RPV-RAMs K101P, Y188L and L100I+K103N, and pRPV-RAMs V179D and G190E/S were more common after efavirenz failure (Fig. 1). When considering viremia levels of <1000, 1000 to 100 000 or >100 000 copies/mL, the proportion of patients with at least one RPV-RAM was 24.6%, 42.2% and 43.8% respectively for the efavirenz group, and 50.0%, 60.9% and 61.1% respectively for the nevirapine group.

Patients were mainly infected with subtype B (40.6%) and G (35.4%) viruses, followed by CRF02_AG (4.6%) and subtype C (3.0%) viruses. The subtype B infected group showed a similar mean number of RPV-RAMs per patient and proportion of patients with RPV-RAMs compared with the subtype G group (0.65, 50.8%) or all pooled patients infected with non-B subtypes (0.63, 47.6%). This was due in part to E138A with a prevalence of 1.6% in subtype B, 2.6% in subtype G and 8.3% in subtype C viruses. However, pRPV-RAM prevalence was higher in subtype B infected patients (0.74, 54.8%) than in subtype G (0.28, 23.8%) or non-B subtype infected patients (0.42, 32.6%, p < 0.01). Specifically, V106I, V108I, V179D/I and G190A occurred significantly more often in subtype B patients, even after correction for a higher use of nevirapine in subtype B infected patients (35%) than in non-B subtype patients (32%).

The proportion of patients fully susceptible to rilpivirine was 52.6% (638 of 1212) by ANRS, 52.2% (633 of 1212) by Rega and 51.9% (629 of 1212) by HIVdb (Fig. 2), with a concordance of 49.7% among all three systems. Efavirenz-experienced patients were significantly more susceptible than nevirapine-experienced patients by Rega (58.3% vs. 39.8%) (Table 1), HIVdb (58.3% vs. 38.8%) and ANRS (58.7% vs. 40.1%), with concordant estimates of respectively 55.7% and 37.3%. Although high-level resistance was predicted more often by Rega than by HIVdb, the proportion of high-level resistance by each algorithm was comparable between efavirenz-experienced and nevirapine-experienced patients (Fig. 2). Concordant estimates of rilpivirine susceptibility across the viremia groups were 72.2%, 55.1% and 51.7% for efavirenz-experienced patients, and 50.0%, 35.5% and 38.9% for nevirapine-experienced patients. HIV-1 subtype did not impact proportions of susceptible patients for any of the algorithms (data not shown).

FIG. 2. Genotypic resistance scores for rilpivirine in different patient populations. ALL, all patients (n = 1212); EFV, efavirenz-experienced (n = 813); NVP, nevirapine-experienced (n = 399). For each interpretation algorithm, proportion of patients scored as high-level resistant (dark grey), intermediate resistant (grey) and susceptible (light grey).
Fifty-three unique RPV-RAMs patterns were detected. Table 1 shows 23 mutational patterns that occurred in two or more patients, accounting for a cumulative 95% of all patients displaying RPV-RAMs, and the impact on rilpivirine susceptibility by Rega. In nevirapine-experienced patients, Y181C occurred at a rate of 25% and was predominantly associated with intermediate resistance to rilpivirine. Mutation Y181C combined with H221Y (13.5%) was the second most prevalent mutational pattern and associated with high-level resistance. In efavirenz-experienced patients, the most common patterns L100I + K103N (10.6%) and Y188L (6.5%) resulted in high-level resistance. RPV-RAMs E138A/Q/G, associated with intermediate resistance, were observed at a low prevalence while E138K/R were absent. Respectively in combination or as a single RPV-RAM, E138A was present in 2.8% or 1.1%, E138Q in 0.8% or 0.6% and E138G in 0.5% or 0.2% of study patients. All 53 patterns and activity estimates by HIVdb and ANRS are available as supplementary material.

### Discussion

The management of HIV-1 infection often is confronted with the administration of consecutive drug regimens due to selection of drug resistance, emphasizing the importance of optimal treatment-sequencing strategies. In this study, we evaluated the potential clinical benefit of the latest approved NNRTI rilpivirine upon virological failure of nevirapine- or efavirenz-containing CART. The therapeutic indication for rilpivirine is currently limited to treatment-naive patients, but its use in second-line or salvage regimens could be clinically relevant given that the rilpivirine trials indicated extensive loss of future NNRTI options after resistance-associated virological failure of rilpivirine [4,5]. Rilpivirine is a second-generation NNRTI with some activity against first-generation NNRTI-resistant isolates, and can address the need for fully active regimens for the treated patient population widely experienced with efavirenz or nevirapine. Although overlapping mutation profiles between all NNRTIs have been reported [4,10,11], it is not well defined to what extent rilpivirine mutations are selected by first-generation NNRTIs and whether susceptibility to rilpivirine would be retained upon treatment failure.

Routine resistance testing of a large representative population revealed that 50% of NNRTI-experienced patients harboured a RPV-RAM, mostly one (74%) or two (25%) mutations, and up to 65% of these patients harboured any mutation associated with rilpivirine use. Three expert-based algorithms estimated preserved rilpivirine activity in approximately 50% of patients. Nevirapine-experienced patients were 46% (RR = 1.46) more at risk of having an RPV-RAM and on average...
less susceptible to rilpivirine than efavirenz-experienced patients. This discrepancy is largely explained by the efavirenz signature mutation K103N (67%), which only reduces rilpivirine susceptibility in the presence of L100I (13.4%), whereas nevirapine signature mutation Y181C (44.4%) confers partial rilpivirine resistance [20]. HIV-1 subtype did not impact estimated activity, but pRPV-RAMs at positions 106, 108, 179 and 190 were more common in subtype B patients, suggesting a subtype influence for these mutations [21,22]. A longer duration of exposure to NNRTIs in the nevirapine group than in the efavirenz group (27.6 vs. 22.3 months) could have affected the estimates of residual rilpivirine activity, especially if duration of treatment failure under NNRTI selection pressure were different [23]. Additionally, other factors such as the potency of the entire regimen and presence of baseline TDR could influence the genetic barrier to and the development of NNRTI resistance.

Anta et al. previously described a varying rilpivirine RAM prevalence and expected activity according to NNRTI use in 1006 patients upon first-generation NNRTI-including therapy failure [10]. This Spanish study predicted that 81% of the patients retained rilpivirine activity, which strongly contrasts with the 50% estimated in our study. However, 27% of the Spanish patients did not have any NNRTI RAM, whereas around 90% of our patient population were scored high-level resistant to nevirapine or efavirenz, which stresses the importance of ana-

Substitutions at position 138 have been associated with rilpivirine use and reduced viral susceptibility to rilpivirine, and are therefore included in resistance interpretation algorithms [4,5,7,15–17]. E138A occurs naturally in treatment-naive patients, ranging from 1.8% in subtype B to 5.9% in subtype C [22], and any evidence of NNRTI selection at this position should be carefully considered in the context of its polymorphic nature [25]. At baseline, E138A is likely to decrease rilpivirine antiviral activity [3]. In our patient population, E138A was prevalent in 1.6% of subtype B, 2.6% of subtype G and 8.3% of subtype C viruses. Studies have described varying rates of E138A upon NNRTI failure depending on the subtype distribution [24–26], and a higher frequency of E138A in specific non-B subtypes could have implications for rilpivirine use. In the study by Lambert-Niclot et al., a significantly higher proportion of non-B subtype infected patients (60%) were scored rilpivirine resistant compared with subtype B infected patients (57%) [24], with a higher prevalence of E138A in non-B viruses (18% vs. 11%). Nonpolymorphic mutations E138G/K/Q/R were either absent or rare in our study, consistent with other studies [10,24].

Despite the limited number of studies investigating a clinical benefit of rilpivirine for patients failing first-generation NNRTI-including therapy, our findings and other findings suggest residual viral susceptibility to rilpivirine in a large subset of patients [10,14]. In vivo virological efficacy of rilpivirine use in second-line regimens has been demonstrated in two pilot studies. After 7 days, a significant decline in HIV-1 RNA levels was observed in patients failing an NNRTI-containing regimen or harbouring NNRTI-resistant virus [8]. Long-term treatment success was achieved in patients who acquired an isolated K103N mutation during prior NNRTI treatment and switched from a boosted protease inhibitor–containing second-line regimen to a rilpivirine-containing regimen [9]. Furthermore, its availability as a single component, its once-daily use and a good safety profile make rilpivirine an attractive option to combine into a second-line regimen.

To date, however, clinical studies on rilpivirine efficacy have been limited to HIV-1 viremic patients without NNRTI resistance mutations [1–4], and knowledge on in vivo activity after treatment failure with NNRTI resistance is largely lacking. Although expert-based interpretation systems also incorporate resistance information obtained from in vitro susceptibility analyses, inferences on residual rilpivirine activity based on existing rules should be clinically validated. Discordances between genotypic and phenotypic resistance testing may exist due to past treatment history, minority resistant variants or the presence of unidentified resistance mutations [27]. Furthermore, rilpivirine should be administered in a fully active regimen, but it is not clear how the presence of rilpivirine RAMs would impact a second- or third-line regimen containing rilpivirine in the presence of other HIV-1 drugs including boosted-protease or integrase inhibitors. Rilpivirine should also be used with caution when HIV RNA levels are above 100 000 copies/mL due to increased risk for virological failure [1–4]. However, only 16% of our patients showed HIV RNA levels above this threshold at failure. Finally, a clinical evaluation of a possible benefit for rilpivirine beyond first-line therapy should acknowledge the availability of etravirine, a second-generation NNRTI already approved for treatment-experienced patients, thereby considering differences in potency, tolerability, cross-resistance, genetic barrier to resistance and dosing convenience in the context of an active regimen [23,28,29].
In the end, prospective clinical studies assessing rilpivirine effectiveness for NNRTI-experienced patients are warranted to validate knowledge derived from genotypic and phenotypic drug resistance studies and to support a more expanded use of rilpivirine in clinical practice.

Transparency declaration

Kristof Thys received funding from the Research Foundation—Flanders (FWO). This work was supported in part by the European Community’s Seventh Framework Programme (FP7/2007–2013) under the project "Collaborative HIV and Anti-HIV Drug Resistance Network (CHAIN)”, grant agreement nr 223131, EURESIST 6FP (IST-2004-027173), by the AIDS Reference Laboratory of Leuven that receives support from the Belgian Ministry of Social Affairs through a fund within the Health Insurance System, European Commission (grant QLKCT200101344, fifth framework; grant LSHPCT2006518211, sixth framework); Belgian AIDS Reference Laboratory Fund, the Research Foundation—Flanders (FWO)—Flanders (grant G.0.692.14N). The authors declare that they have no conflicting interests.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2015.02.011.

References


