Factors associated with virological success with raltegravir-containing regimens and prevalence of raltegravir-resistance-associated mutations at failure in the ARCA database†


Abstract

Raltegravir (RAL) is the only licensed human immunodeficiency virus (HIV) integrase inhibitor. The factors associated with the virological response to RAL-containing regimens and the prevalence of integrase mutations associated with RAL failure deserve further investigation. From the Antiretroviral Resistance Cohort Analysis database, we selected triple-class-experienced subjects failing their current treatment with complete treatment history available. Selection criteria included HIV-RNA, CD4 count and HIV genotype within 3 months of RAL initiation. Factors associated with 24-week response were analysed; genotypic sensitivity scores (GSS) and weighted-GSS were evaluated. Virological response was achieved in 74.3% of 105 subjects. Mutations associated with RAL failure were detected in 12/24 subjects with the prevalence of Q148H + G140S. Each extra unit of GSS (p 0.05, OR 2.62; 95% CI 1.00–6.87) was found to be associated with response. Weighted-GSS had borderline statistical significance (p 0.063, OR 2.04; 95% CI 0.96–4.33) when stratifying for different cut-offs (<1 as reference, 1–1.49, >1.5), a borderline significant increase in the probability of response appeared for GSS ≥ 1.5 (p 0.053, OR 4.00; 95% CI 0.98–16.25). GSS ≥ 1 showed the highest sensitivity, 82.6%. Receiver operating characteristic curves depicted the widest area under the curve (0.663, p 0.054) of GSS ≥ 1. Unresponsiveness to RAL-containing regimens among triple-class-experienced subjects was low. The activity of the background regimen was strongly associated with response. Although few integrase genotypes were available at failure, half of these were without integrase resistance mutations. The substantial rate of RAL failure in the absence of known RAL-resistance mutations may be associated with adherence issues and this issue warrants further analysis in longer observations.

Keywords: Drug resistance, genotype, human immunodeficiency virus type 1-1, raltegravir, virological response

Original Submission: 28 June 2012; Revised Submission: 27 September 2012; Accepted: 4 November 2012

Editor: G. Antonelli

Article published online: 4 January 2013


10.1111/1469-0691.12100

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†This paper is dedicated to the memory of our dear friend and colleague Raffaella Rosso.

Introduction

The viral integrase (IN) is an important target for treating human immunodeficiency virus type 1 (HIV-1) infection and preventing clinical progression to AIDS [1]. Raltegravir (RAL) is the only licensed HIV IN inhibitor so far [2]. It was approved by the US Food and Drug Administration in October 2007 as
part of antiretroviral therapy in drug-experienced subjects with virological failure—e.g. BENCHMRK trials [3]. Due to an excellent safety profile and potency the use of RAL has been extended (July 2009) to drug-naive individuals—e.g. STARTMRK trial [4].

Resistance to RAL has been extensively studied with regard to major IN resistance and IN polymorphisms in naive and triple-class experienced subjects [5,6]. As described by Margeridon-Theret and Shafer [7], mutations at nine positions (T66I/A/K, E92Q/V, F121Y, Y143C/R, P145S, Q146P, S147G, Q148H/R/Q and N155H/S) are selected by RAL or elvitegravir and reduce susceptibility to either one or both of these drugs [8,9]. Major signature mutations associated with RAL failure are Y143R/H/C, Q148H/K/R, N155H and E92Q. Minor mutations in the I48 pathway are L74M plus E138A, E138K or G140S. Mutations described in the I55 pathway are L74M, E92Q, T97A, Y143H, V151I, G163K/R or D232N. The Y143R/H/C is less common than the other signature mutations. The Q148K/R is mostly detected after short exposure to RAL, whereas Y143R/H/C is observed only after prolonged RAL exposure and usually replaces N155H. A switch from N155H to Q148H can also occur. An exhaustive list of mutations in the IN region is: H51Y, T66I/A/K, V72I, L74I/A/M, E92Q, T97A, T112I, F121Y, T125K, A128T, E138K/A/D, G140R/C/H, Q146K/P, S147G, Q148K/R/H, V151I, S153Y/A, M154I, N155S/H, K156N, E157Q, K160D/N, G163R/K, V1651, V201I, I203M, T206S, S230N/R, V249I, R263K and C280Y [10]. The most important of these are G140S/A/C and E138K/A, which increase the fitness of viruses with Q148H/R/K and lead to high-level resistance to all IN inhibitors, and T97A, which causes high-level resistance to RAL in the presence of Y143C/R, although the most commonly observed is the N155H pathway [11]. Of note, other than resistance, several factors can influence the response to RAL, e.g. adherence, pharmacological profile and drug–drug interactions. Dolutegravir is a second-generation IN inhibitor with an increased genetic barrier to resistance compared with RAL and elvitegravir, which is potentially useful in subjects experiencing virological failure to RAL or elvitegravir [12,13]. Further investigations would be useful to detect the determinants of the virological response to RAL-containing regimens and the prevalence of IN mutations associated with RAL failure in clinical practice. With this aim, we queried the Antiretroviral Resistance Cohort Analysis (ARCA) resistance database (i) to analyse the virological response after 24 weeks of a RAL-containing highly active antiretroviral therapy regimen, (ii) to define factors associated with response at week 24, and (iii) to investigate IN mutations at virological failure.

Materials and Methods

From the ARCA (www.hivarca.net) database, we conducted a retrospective study and selected 685 RAL-containing regimens from 526 triple-class-experienced subjects failing their current treatment and having complete treatment history available. Our patients failing the RAL-containing regimen did experience a real viral rebound (two consecutive HIV-RNA determinations above the limit of quantification) and the genotypes were analysed at the time of virological failure when patients were still taking RAL. Only the initial RAL-containing regimens for each subject were analysed. The genotypic susceptibility score (GSS) and weighted-GSS of the RAL-containing regimen were obtained according to the ARCA built-in algorithm AntiretroRoscan [14] and were available for 105/526 subjects. We used the standard susceptible/intermediate/resistant categorization for all GSS, as given by the output of the HIVDB WEB-SERVICE (Stanford HIV drug resistance database. http://hivdb.stanford.edu/index.html), which were assigned the numerical values of 1.0/0.5/0.0, respectively. Each combination regimen was then given a GSS based on the sum of the (weighted) scores coded for the individual drugs included in the regimen. Further inclusion criteria were (i) detectable HIV-1 RNA at the start of treatment, (ii) availability of CD4 counts, (iii) HIV reverse transcriptase (RT)/protease (PRO) genotype within 3 months before RAL initiation, and (iv) availability of follow-up HIV-RNA at 24 weeks. Different GSS and weighted-GSS cutoffs were evaluated for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under the curve (AUC) in a receiver operating characteristic (ROC) curve analysis. Drug resistance mutations were interpreted following the IAS-USA panel list proposed as RAL-specific (www.iasusa.org, update in November 2011) [15]: L74M, E92Q, T97A, E138A/K, G140S, Y143H/C, Q148H/K/R, V151I, N155H, G163K/R, and D232N. The probability of a virological response at 24 weeks to a RAL-containing therapy was assessed by means of binary logistic regression models. From these models we obtained estimates of relative risks (expressed as OR and 95% CI) for gender, age, HIV-1 RNA, peak HIV-1 RNA, CD4 cell counts, CD4 cell nadir, number of RT mutations, and number of PRO mutations. All of these variables were considered for the multivariate analysis. Kaplan–Meier curves were designed to describe the probability of virological success after starting a RAL-containing regimen. Moreover, we considered the duration of antiretroviral therapy and the duration of nucleoside RT inhibitor/non-nucleoside RT inhibitor/protease inhibitor exposure, GSS (absolute and stratified), and weighted-GSS (absolute and stratified). Different thresholds
for weighted-GSS and unweighted-GSS were evaluated for sensitivity, specificity, PPV, NPV and were plotted on ROC curves. The two-sided statistical significance was set at p 0.05. SPSS 15 for Windows (Chicago, IL, USA) was the statistical software package used for the analyses.

**Results**

**Descriptive analysis**

In all, 105 subjects met all the inclusion criteria. The median (interquartile range; IQR) antiretroviral exposure at the time of RAL initiation was 12.3 (IQR 10.0–15.0) years. Virological response at week 24, defined as HIV-1 RNA <50 copies/mL was achieved in 78 (74.3%) subjects. Virological failure was found in 27 subjects and the mean level ± standard deviation of HIV-RNA was 3.4 log_{10} ± 1.4 copies/mL. At failure of the RAL-containing regimen, 19 subjects had a further RT/PRO genotypic test, whereas IN sequences were documented for 24 patients. Table 1 shows the description of subjects with or without virological control at 24 weeks after RAL initiation.

**Analysis of baseline GSS of RAL companion drugs**

The GSS of RAL companion drugs, i.e. not including RAL, was analysed through a 0.25-step stratification using ANTIRETROSCAN in 105 subjects with available resistance data (RT+PRO) at RAL initiation. The majority of the individuals had a GSS of 1 (25.7%); however, 49.5% of the subjects had a GSS ≤ 1. The most frequent protease inhibitor was darunavir, few enfuvirtide treatments were observed, and etravirine was present in around 20% of subjects without a difference in the virological outcome.

**Drug resistance at baseline and at failure**

The median number of PRO and RT mutations at failure was lower than at baseline: 8 (IQR 2–15) versus 11 (IQR 5–16) and 5 (IQR 2–12) versus 8 (IQR 3–11), respectively. As expected, week-24 non-responders had a higher number of mutations at failure compared with responders, both in PRO (12; IQR 5–19, versus 7; IQR 2–13) and RT (9; IQR 3–19, versus 4; IQR 2–10), without showing any statistical significance. Of the 24 RAL-failing subjects with available IN genotype, 12 (50.0%) did not show any mutation or just had natural IN polymorphisms (H51Y, V72I, G140S, K156N, E157Q and V165I). Conversely, resistance mutations associated with RAL failure were detected in the other 12 subjects; five Q148H + G140S pathway, four N155H pathway, two Y143C/R pathway, and one subject with mixed Y143R + N155H.

**Factors associated with response to RAL-containing therapy at 24 weeks**

Kaplan–Meier estimation showed a 74.3% virological response after initiating a RAL-containing regimen in the entire cohort. GSS > 1.5 had the highest effect with approximately 85% of a virological response. In parallel, weighted-GSS > 1.5 showed 82% of a virological response compared with other weighted-GSS strata (Fig. 1a,b).

Univariate analysis (Table 2) revealed an increased probability of virological response for older subjects: the OR for each extra-year was 1.09, 95% CI 1.02–1.77 (p 0.016) counts, whereas nadir CD4, baseline CD4 counts, peak HIV-1 RNA, and baseline HIV-1 RNA were not associated with virological suppression. Each extra unit of GSS (p 0.05, OR 2.62; 95% CI 1.00–6.87) was found to be a factor associated with response. Weighted-GSS had borderline statistical significance (p 0.063, OR 2.04; 95% CI 0.96–4.33). When stratifying for different cut-offs (<1 as reference, 1–1.49, ≥ 1.5), a borderline significant increase in the probability of response appeared for GSS ≥ 1.5 (p 0.053, OR 4.00; 95% CI 0.98–16.25).

**Multivariate analysis of factors associated with response**

Multivariate analysis found an increased probability of virological response for every extra-year in our cohort of subjects. The adjusted OR were between 1.09 and 1.11 when consid-
TABLE 2. Factors associated with virological response at 6 months according to univariate and multivariate analyses

<table>
<thead>
<tr>
<th>Factor</th>
<th>p</th>
<th>OR [95% CI]</th>
<th>p</th>
<th>OR [95% CI]</th>
<th>p</th>
<th>OR [95% CI]</th>
<th>p</th>
<th>OR [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>x year</td>
<td>0.016</td>
<td>1.09 [1.02-1.17]</td>
<td>0.044</td>
<td>1.10 [1.00-1.20]</td>
<td>0.047</td>
<td>1.09 [1.00-1.19]</td>
<td>0.026</td>
</tr>
<tr>
<td>ARV</td>
<td>x year</td>
<td>0.427</td>
<td>1.06 [0.92-1.21]</td>
<td>0.617</td>
<td>0.66 [0.13-3.36]</td>
<td>0.737</td>
<td>0.75 [0.15-3.91]</td>
<td>0.461</td>
</tr>
<tr>
<td>NNRTI</td>
<td>x year</td>
<td>0.530</td>
<td>1.10 [0.82-1.49]</td>
<td>0.644</td>
<td>0.90 [0.56-1.43]</td>
<td>0.635</td>
<td>0.89 [0.57-1.42]</td>
<td>0.682</td>
</tr>
<tr>
<td>NRTI</td>
<td>x year</td>
<td>0.312</td>
<td>1.07 [0.94-1.23]</td>
<td>0.485</td>
<td>1.78 [0.35-8.99]</td>
<td>0.579</td>
<td>1.59 [0.81-3.08]</td>
<td>0.393</td>
</tr>
<tr>
<td>PI</td>
<td>x year</td>
<td>0.440</td>
<td>0.94 [0.80-1.10]</td>
<td>0.267</td>
<td>0.79 [0.64-1.13]</td>
<td>0.172</td>
<td>0.82 [0.61-1.09]</td>
<td>0.431</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>0.981</td>
<td>0.99 [0.99-1.01]</td>
<td>0.152</td>
<td>0.99 [0.96-1.02]</td>
<td>0.671</td>
<td>0.99 [0.96-1.02]</td>
<td>0.384</td>
</tr>
<tr>
<td>CD4</td>
<td>x10 unit</td>
<td>0.068</td>
<td>1.00 [0.98-1.03]</td>
<td>0.677</td>
<td>0.99 [0.96-1.02]</td>
<td>0.671</td>
<td>0.99 [0.96-1.02]</td>
<td>0.384</td>
</tr>
<tr>
<td>Nadir CD4</td>
<td>x10 unit</td>
<td>0.106</td>
<td>1.05 [0.99-1.13]</td>
<td>0.392</td>
<td>1.04 [0.95-1.13]</td>
<td>0.395</td>
<td>1.04 [0.95-1.13]</td>
<td>0.272</td>
</tr>
<tr>
<td>Log VL</td>
<td>x unit</td>
<td>0.092</td>
<td>0.59 [0.31-1.09]</td>
<td>0.102</td>
<td>0.46 [0.18-1.17]</td>
<td>0.082</td>
<td>0.43 [0.17-1.11]</td>
<td>0.097</td>
</tr>
<tr>
<td>Log peak VL</td>
<td>x unit</td>
<td>0.456</td>
<td>0.72 [0.31-1.70]</td>
<td>0.180</td>
<td>2.72 [0.63-12.02]</td>
<td>0.181</td>
<td>2.68 [0.63-11.33]</td>
<td>0.143</td>
</tr>
<tr>
<td>N Mutations PR</td>
<td>x unit</td>
<td>0.481</td>
<td>0.97 [0.88-1.06]</td>
<td>0.959</td>
<td>1.00 [0.84-1.19]</td>
<td>0.633</td>
<td>1.05 [0.87-1.27]</td>
<td>0.977</td>
</tr>
<tr>
<td>N Mutations RT</td>
<td>x unit</td>
<td>0.300</td>
<td>1.07 [0.94-1.21]</td>
<td>0.286</td>
<td>1.12 [0.91-1.36]</td>
<td>0.274</td>
<td>1.12 [0.91-1.37]</td>
<td>0.318</td>
</tr>
<tr>
<td>GSS</td>
<td>x unit</td>
<td>0.050</td>
<td>2.62 [1.00-6.87]</td>
<td>0.050</td>
<td>4.61 [1.00-21.21]</td>
<td>0.042</td>
<td>4.20 [1.06-16.71]</td>
<td>0.097</td>
</tr>
<tr>
<td>GSS Weighted</td>
<td>x unit</td>
<td>0.063</td>
<td>2.04 [0.96-4.33]</td>
<td>0.050</td>
<td>4.61 [1.00-21.21]</td>
<td>0.042</td>
<td>4.20 [1.06-16.71]</td>
<td>0.097</td>
</tr>
<tr>
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<td>0.042</td>
<td>4.20 [1.06-16.71]</td>
<td>0.097</td>
</tr>
</tbody>
</table>

Virological response was obtained in 74.3% of subjects. Statistically significant *p* values are shown in italics. ARV, antiretroviral drugs; NNRTI, non-nucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; VL, viral load; N, number; GSS, genotypic susceptibility score; AOR, adjusted odds ratio.

Sensitivity, specificity and ROC curve analyses

The GSS cut-off ≥ 1 showed the highest sensitivity, 82.6%, with specificity = 50.0%, PPV = 82.6%, NPV = 50.0%, whereas weighted-GSS cut-off ≥ 1.5 had lower sensitivity and specificity, with PPV = 83.8% and NPV = 40.0%. ROC curves depicted the widest AUC (0.663, p 0.054) of GSS ≥ 1 versus other GSS cut-offs; weighted-GSS cut-off ≥ 1.5 had the widest although not statistically significant AUC (0.649, p 0.081) compared with the other two weighted-GSS cut-offs (Fig. 2a,b).

Discussion

In this cohort analysis, unresponsiveness to RAL-containing regimens among multi-failing subjects with a triple-class experience was relatively low (25.7%). The activity of the background regimen was a factor associated with response, as shown by GSS and weighted-GSS. However, it must be noted that almost half of regimens (49.5%) had a low GSS for RAL companion drugs. Baseline CD4 counts and baseline HIV-1 RNA were not associated with the virological response. A
possible explanation of this finding is the different setting of clinical trials, e.g. BENCHMRK and STARTMRK, compared with cohort observational studies. One possible explanation of the statistical association between age and response to therapy is that older patients in our cohort were more adherent (both in dosing and timing) to treatment.

Although few IN genotypes were available at failure, notably half of these cases were without IN resistance mutations. Finding IN mutations in approximately half of the successfully genotyped subjects has been very common in most RAL trials, both in naive subjects (STARTMRK, QDMRK [16]) and experienced subjects (SWITCHMRK [17], BENCHMRK). This finding represents a common underlying driving force in all virological failures. The relationship between adherence levels and the risk of development of resistance is differently shaped with different drug classes and can be explained by drug pharmacokinetics and fitness costs of resistant virus [18]. The relatively low genetic barrier of RAL suggests the need for optimal regimen adherence to obtain virological success. Using ten-fold cross-validation, the averaged area under the ROC curve for all algorithms increased from 0.76 with unweighted regimen genotypic sensitivity score (rGSS) to 0.80 with weighted rGSS. In our study, each extra unit of GSS and weighted-GSS was associated with a higher probability of response, the level needed for virological success being $\geq 1.5$ for GSS. In parallel, GSS cut-off $\geq 1$ showed the highest sensitivity as pointed out by ROC curve analysis. In our study, the decrease in the median number of PRO and RT mutations at failure was not significant and could be related to genetic adaptation driven by fitness issues following treatment switch or to adherence issues.

The availability of compounds, such as RAL, belonging to new drug classes is crucial for achieving virological success in patients harbouring extensively drug-resistant viruses. Zaccarrelli et al. [23] described no significant association between extended resistance and HIV progression in patients who failed between 2004 and 2008, contrary to what happened to patients who failed between 1999 and 2003. In our study, IN resistance analysis is underestimated because of at least two caveats. First, a potential role of compensatory mutations could not be analysed in the absence of a baseline IN genotype. Second, analysis of the IN genotype was not always performed at failure. Other studies showed the appearance of these mutations at virological failure or at viral rebound following RAL withdrawal [24]. On the other hand, no major IN mutation had been detected by standard sequencing in IN-naive subjects [25], although RAL-resistant minority species could be present before RAL therapy [26–29].

Malet et al. [22] demonstrated that a GSS $< 2$ in the current antiretroviral regimen and HIV-RNA $> 200$ copies/mL at failure were independently associated with the development of RAL resistance. The relationship GSS and treatment outcomes was analysed in a recent report by the research group in Stanford and by the ARCA database [14,21]. Moreover, a recent paper by
Unresponsiveness to RAL-containing regimens among subjects with a triple-class experience was relatively low in this cohort analysis. The activity of the background regimen was strongly associated with response as shown by GSS and less so by weighted-GSS. The substantial rate of RAL failure in the absence of known RAL-resistance mutations may be associated with adherence issues and this issue warrants further analysis in longer observations.

Acknowledgements

The authors acknowledge many patients and colleagues who have been a persistent source of inspiration, Mrs Simonetta Terazzi, Mrs Bianca M. Ghisi and Mrs Elizabeth L. Kaplan, MSW for helping with the manuscript preparation, and Prof. Massimo Galli for helpful advice. This work was partly supported by the FP7 EU-funded CHAIN project (223131) and by the Italian AIDS Research Programme (40h81). This work has been partially presented at the 8th European HIV Drug Resistance Workshop, Sorrento, Italy, 17–19 March 2010. Abstract 27.

Transparency Declaration

SR received research grants and have been involved in advisory boards or educational courses supported by the following companies: Abbott, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead, GlaxoSmithKline, now ViiV Healthcare, Merck, and Janssen-Cilag.

ADL received speakers’ honoraria, served as consultant or participated in advisory boards for GlaxoSmithKline, Gilead, Bristol-Myers Squibb, Abbott Virology, Tibotec-Janssen, Siemens Diagnostics, and Monogram Biosciences.

MZ received research grants and have been involved in advisory boards or educational courses supported by the following companies: Abbott, Abbott Molecular, Boehringer Ingelheim, Gilead Sciences, Janssen-Cilag, Merck, and ViiV Healthcare.

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