High frequency of dolutegravir resistance in patients failing a raltegravir-containing salvage regimen

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Received 28 August 2014; returned 26 September 2014; revised 7 October 2014; accepted 9 October 2014

Objectives: Dolutegravir is a second-generation integrase strand transfer inhibitor (InSTI) that has been recently approved by the FDA to treat antiretroviral therapy-naive as well as treatment-experienced HIV-infected individuals, including those already exposed to the first-generation InSTI. Despite having a different mutational profile, some cross-resistance mutations may influence its susceptibility. The aim of this study was to evaluate the impact of a raltegravir-containing salvage regimen on dolutegravir activity.

Patients and methods: Blood samples of 92 HIV-infected individuals with virological failure (two or more viral loads >50 copies/mL after 6 months of treatment) using raltegravir with optimized background therapy were sequenced and evaluated according to the Stanford University HIV Drug Resistance Database algorithm.

Results: Among the 92 patients analysed, 32 (35%) showed resistance to dolutegravir, in most cases associated with the combination of Q148H/R/K with G140S/A mutations. At genotyping, patients with resistance to dolutegravir had viral load values closer to the highest previously documented viral load.

Conclusions: Changes in viraemia during virological failure may indicate the evolution of raltegravir resistance and may predict the emergence of secondary mutations that are associated with a decrease in dolutegravir susceptibility. Early discontinuation of raltegravir from failing regimens might favour subsequent salvage with dolutegravir, but further studies are necessary to evaluate this issue.

Keywords: HIV, antiretroviral resistance, integrase inhibitors, Brazil

Introduction

Dolutegravir (Tivicay®; GlaxoSmithKline) is a promising, new integrase strand transfer inhibitor (InSTI), recently licensed by the FDA for clinical use in treatment-naive1–3 or treatment-experienced subjects,4 including those exposed to InSTI.5 In contrast to the first-generation integrase inhibitors, dolutegravir has a high genetic barrier,6 and has been proven effective against strains resistant to raltegravir and elvitegravir.5 Resistance mutations to dolutegravir are not yet thoroughly established, but some mutations selected in vitro and in vivo by first-generation InSTI were associated with a decrease in dolutegravir susceptibility.5,7,8 Although dolutegravir may be used in raltegravir-experienced patients, drug activity in this situation is expected to be influenced by intraclass cross-resistance that may compromise its potency. In the VIKING-3 study, patients exposed to raltegravir showed a reduced response to dolutegravir when resistance mutations, especially at codon Q148, were present with two or more other mutations (including L74I, E138A/K or G140S/A).5 We and others8,9 have documented important raltegravir resistance in two distinct clinical settings.5 As could be expected, the time of virological failure correlates to resistance accumulation that may impact further the use of the same class of drugs.9 We evaluated integrase sequences from treatment-experienced patients with advanced disease failing a raltegravir-containing salvage regimen to assess the predicted activity of dolutegravir.

Patients and methods

Patients

Blood samples from 92 HIV-infected patients with confirmed virological failure during the use of a raltegravir-containing regimen (two or more viral loads >50 copies/mL after 6 months of treatment) were included in this study. Samples were collected at clinical sites in São Paulo State from July 2009 to July 2013 and processed in the Retrovirus Laboratory at the Adolfo Lutz Institute.

PCR and genomic sequencing

Sequences were obtained from viral RNA, extracted from plasma using a commercial kit (QIAamp Viral RNA Mini Kit, Qiagen, USA) according to the manufacturer’s instructions. Amplification and sequencing of the complete integrase gene was performed as previously described,7 with an RT–PCR assay using SuperScript® III (Invitrogen, Carlsbad, CA, USA) and
Platinum® Taq DNA Polymerase, High Fidelity with corrective action (Invitrogen, Carlsbad, CA, USA), followed by a nested PCR. The PCR product was then direct-sequenced using a BigDye® v.3.1 Cycle Sequencing Kit (Applied Biosystems®, Austin, TX, USA) and resolved in an ABI 3130XL Genetic Analyzer (Applied Biosystems®, Austin, TX, USA). Sequences were edited using Sequencher 4.7 (Gene Codes Corp., Ann Arbor, MI, USA).

Dolutegravir resistance after raltegravir

Determining genotypic resistance, antiretroviral susceptibility and genotypic susceptibility score (GSS)

The mutations associated with resistance to InSTI were analysed according to the Stanford University HIV Drug Resistance Database algorithm (http://hivdb.stanford.edu/index.html) and the International Antiviral Society–USA (IAS-USA) resistance list.11 The susceptibility to integrase inhibitors was determined according to the Stanford University HIV Drug Resistance Database algorithm and the five interpretations generated were dichotomized in our analyses as: (i) no resistance, for those with susceptible, potential low-level resistance or low-level resistance prediction; and (ii) resistance, for those with intermediate or high resistance prediction. The GSS of the optimized background therapy (OBT) was calculated at the beginning of the raltegravir-containing regimen (GSS1) from previous genotyping tests, and from a subsequent genotypic test of the same sample used to determine raltegravir resistance, collected during salvage therapy failure (GSS2). The OBT GSS was the sum of each individual drug. Activity was predicted according to the Stanford University HIV Drug Resistance Database algorithm; GSS scores were defined as: susceptible or potential low-level resistance = 1; low-level resistance = 0.50; and intermediate resistance or high resistance = 0. Raltegravir susceptibility was not included in the calculation of the GSS.

Statistical analysis

Data were analysed using STATA statistical software version 13.0 (StataCorp LP, College Station, TX, USA) and GraphPad software (GraphPad Software Inc., La Jolla, CA, USA), using a level of statistical significance of 0.05.

Ethical approval

This study was approved by the Ethical and Research Committees of the Adolfo Lutz Institute, Secretary of Health of São Paulo, Brazil and

### Table 1. Demographic, clinical and laboratory data from patients included in the study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total patients (n=92)</th>
<th>DTG susceptible (n=60)</th>
<th>DTG resistant (n=32)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), median (IQR)</td>
<td>44 (23–50)</td>
<td>44 (23–50)</td>
<td>44 (28–49)</td>
<td>0.982</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>62 (67)</td>
<td>41 (68)</td>
<td>21 (66)</td>
<td>0.792</td>
</tr>
<tr>
<td>Nadir CD4 count (cells/mm³), median (IQR)</td>
<td>51 (13–143)</td>
<td>63 (21–143)</td>
<td>20 (11–141)</td>
<td>0.140</td>
</tr>
<tr>
<td>CD4 cell count (cells/mm³), median (IQR) at RAL initiation</td>
<td>181 (49–451)</td>
<td>150 (42–465)</td>
<td>229 (55–449)</td>
<td>0.925</td>
</tr>
<tr>
<td>at weeks 12–24 of RAL</td>
<td>314 (126–515)</td>
<td>282 (118–498)</td>
<td>316 (83–445)</td>
<td>0.544</td>
</tr>
<tr>
<td>at sample collection</td>
<td>221 (89–448)</td>
<td>241 (111–435)</td>
<td>198 (31–448)</td>
<td>0.359</td>
</tr>
<tr>
<td>HIV RNA level (log₁₀), median (IQR)</td>
<td>4.44 (3.72–4.82)</td>
<td>4.41 (3.61–4.77)</td>
<td>4.47 (4.09–4.84)</td>
<td>0.420</td>
</tr>
<tr>
<td>at RAL initiation</td>
<td>3.04 (1.70–4.21)</td>
<td>2.71 (1.70–4.07)</td>
<td>3.83 (1.92–4.27)</td>
<td>0.120</td>
</tr>
<tr>
<td>at sample collection</td>
<td>3.99 (3.21–4.58)</td>
<td>3.82 (3.18–5.54)</td>
<td>4.36 (3.63–7.84)</td>
<td>0.011</td>
</tr>
<tr>
<td>Delta viral load</td>
<td>−1.07 (−0.66 to −1.88)</td>
<td>−1.31 (−0.75 to −2.15)</td>
<td>−0.89 (−0.31 to −1.53)</td>
<td>0.033</td>
</tr>
<tr>
<td>RAL exposure (weeks)</td>
<td>115 (70–161)</td>
<td>114 (62–155)</td>
<td>122 (75–171)</td>
<td>0.794</td>
</tr>
<tr>
<td>Time of viraemia (weeks)</td>
<td>75 (33–132)</td>
<td>71 (29–139)</td>
<td>72 (52–179)</td>
<td>0.655</td>
</tr>
<tr>
<td>Number of regimens</td>
<td>7 (7–9)</td>
<td>7 (6–9)</td>
<td>6 (4–8)</td>
<td>0.279</td>
</tr>
<tr>
<td>GSS1, n (%)</td>
<td>38 (79)</td>
<td>24 (77)</td>
<td>14 (82)</td>
<td>0.923</td>
</tr>
<tr>
<td>&lt;2 drugs</td>
<td>10 (21)</td>
<td>7 (23)</td>
<td>3 (18)</td>
<td></td>
</tr>
<tr>
<td>≥2 drugs</td>
<td>50 (81)</td>
<td>30 (71)</td>
<td>20 (100)</td>
<td>0.391</td>
</tr>
<tr>
<td>GSS2, n (%)</td>
<td>12 (19)</td>
<td>12 (29)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>&lt;2 drugs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥2 drugs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RAL, raltegravir; DTG, dolutegravir.

Patients were grouped according to predicted susceptibility to dolutegravir as resistance (intermediate or high resistance) or susceptible (low or no resistance) according to the Stanford University HIV Drug Resistance Database algorithm (http://hivdb.stanford.edu/index.html). Delta viral load is the difference between the highest documented viral load for each patient minus the viral load at genotyping. GSS corresponds to the sum of the predicted activity of each individual antiretroviral drug used in the salvage therapy excluding raltegravir (OBT). Activity was predicted according to the Stanford University HIV Drug Resistance Database algorithm; GSS scores were defined as: susceptible or potential low-level resistance = 1; low-level resistance = 0.50; and intermediate resistance or high resistance = 0. GSS1 is the OBT GSS at the time of initiation of the raltegravir-containing regimen and GSS2 is the OBT GSS at the time of genotyping. Values highlighted in bold indicate P < 0.05.
participating institutions. All patients signed a written informed consent form at participating clinical sites.

Results and discussion

Among the integrate sequences obtained from 92 patients, 32 (35%) showed an important loss of dolutegravir susceptibility (27 with intermediate resistance and 5 with high-level resistance). All patients had advanced disease, with a low CD4 and with most starting raltegravir-containing salvage therapy with a low optimized background genotypic score, after a median of seven previous regimens (Table 1). Cases with dolutegravir resistance tended to have a decrease in CD4 after initial gain. Moreover, patients with resistance to dolutegravir, as compared with those without resistance, had a higher viral load at the time of collection for genotypic testing ($P = 0.011$). At genotyping, patients with resistance had viral load values closer to the highest previously documented viral load than those with no resistance (delta viral load of $-0.89$ versus $-1.31$, $P = 0.033$), suggesting some recovery of replicative fitness. Moreover, although not statistically significant, patients with viraemia higher than 1000 copies/mL between 12 and 24 weeks after initiation of treatment with raltegravir tended to show resistance to dolutegravir ($P = 0.072$). The time of virological failure correlates with the number of mutations (Spearman correlation $r = 0.57$, $P = 0.0036$); it was observed in both resistance and susceptible cases and, although longer for those with dolutegravir resistance, the difference from those who were susceptible is not significant. All patients with resistance to dolutegravir had amino acid substitution at position 148 (glutamine to histidine, arginine or lysine). It was almost invariably accompanied by G140S/A, present in 94% (30/32) of cases. (glutamine to histidine, arginine or lysine). It was almost invariably accompanied by G140S/A, present in 94% (30/32) of cases. The mutation F121Y was found in a single previously reported case and G118R was not observed. These mutations have been associated in vitro with cross-resistance to all INSTIs. A strong association was observed between the presence of mutations G140S/A ($P < 0.001$) and E138A/K ($P < 0.001$) and dolutegravir resistance. Accordingly, our study found that the number of secondary mutations is associated with a worse predicted response to dolutegravir ($P = 0.006$), a prediction that is supported by the clinical observations in the VIKING-3 study, which showed a worse response for patients with two or more resistance-associated mutations, such as G140S/A, L74I and E138A/K. Saladini et al. documented the presence of variants G140S/Q148H in the integrate sequences obtained from patients using a raltegravir-containing regimen, although at a lower rate (17.5%) than found in this study. Our study documents that an important proportion of patients failing a raltegravir-containing regimen in public services in São Paulo, Brazil, have intermediate or high resistance to dolutegravir. Changes in viraemia during virological failure may indicate the evolution of dolutegravir resistance and may predict the emergence of secondary mutations that are associated with a decrease in dolutegravir susceptibility. As 


Funding

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (grant 2011/21958-2); J. d. S. C. was supported by a student scholarship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (D06/12).

Transparency declarations

None to declare.

References

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