A 5 amino-acid insertion in the C-terminal region of HIV-2 integrase impacts phenotypic susceptibility to the five integrase inhibitors


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Background

- HIV-2 is defined as an attenuated retroviral infection:
  - Slower progression towards AIDS
  - Spontaneously low to undetectable viral loads
  - Lower rates of transmission

- HIV-2 is naturally resistant to a large number of ARV:
  NNRTI, fusion inhibitor and some PI

- Rapid selection of class-wide resistance
  (i.e. Q151M and K65R for NRTI, I50V/I54M for PI)

- High proportion of therapeutic impasse after second-line treatments

Xiang et al., 1997; Marlink et al. 1994; Popper et al., 1999; Kanki et al., 1994; Matheron et al., 1990; Witvrouw et al., 2004; Descamps et al., 2004; Gottlieb et al., 2009; Descamps et al., 2015
Background

- HIV-2 integrase differs in length, depending on viral group:
  - Group A: 294 AA
  - Group B: 288, 297 or 302 AA

- HIV-2 and integrase inhibitors (INSTI):
  - All HIV-2 groups are susceptible to EVG, RAL and DTG
  - Scarce data on CAB and BIC
  - Described resistance profiles are similar to HIV-1

- Recently, we observed a 5-AA insertion in the C-terminal region of HIV-2 integrase of patients failing a RAL-based regimen

Roquebert et al., 2008; Smith et al., 2015; Smith et al., 2012
OBJECTIVE

Phenotypic characterization of a newly identified genotypic profile of HIV-2 integrase in patients at virological failure of a RAL-based regimen
Material & Methods

• Patients
  – ANRS CO5 HIV-2 cohort
  – Screening of our HIV-2 genotypic database to identify all patients displaying a 5-AA insertion in integrase
  – Plasma samples were retrieved
  → HIV-2 cultures were performed using CD44 MicroBeads® (Miltenyi)

• Retrospective plasma samples to explore dynamics of acquisition of this genotypic profile
  – Measurement of plasma drug concentrations using UPLC-MS/MS
  – Sequencing of integrase region by Sanger method
**Material & Methods**

- **PBMC phenotypic susceptibility assay**
  - Based on ANRS PBMC assay, using 5 INSTI (BIC, CAB, DTG, EVG and RAL)
  - With ARV concentrations ranging from 0 to 1000 nM
  - HIV-2 viral load measured at day 3 or 4 post-infection in supernatant → 50% inhibitory concentrations (IC\textsubscript{50})
  - IC\textsubscript{50} fold-changes obtained by comparing with ROD, HIV-2 reference

Adapted from Brun-Vézinet et al., 1992; Roquebert et al., 2008; Charpentier et al. 2012
Results

- Six patients with an insertion at codon 231 in HIV-2 integrase
  - 6/99 patients experiencing VF under INSTI-regimen (6.1 %)

### Table: Insertion motif at codon 231

<table>
<thead>
<tr>
<th>Patient#</th>
<th>ART</th>
<th>Duration of RAL-based regimen (months)</th>
<th>HIV-2 VL (copies/mL)</th>
<th>Insertion motif at codon 231</th>
<th>RT mutations</th>
<th>PR mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RAL+DRV/r</td>
<td>13</td>
<td>370</td>
<td>G-K</td>
<td>K65R</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>RAL+DRV/r</td>
<td>10</td>
<td>1,460</td>
<td>Y-R-E-G-R</td>
<td>T215L</td>
<td>I50V+I54M</td>
</tr>
<tr>
<td>4</td>
<td>RAL+ABC+3TC</td>
<td>9</td>
<td>570</td>
<td>Y-R-E-G-R</td>
<td>M184V+T215Y</td>
<td>I50V+I54M</td>
</tr>
<tr>
<td>5</td>
<td>RAL+ABC+DRV/r+TDF</td>
<td>6</td>
<td>7,980</td>
<td>S-R-E-G-R</td>
<td>K65R+T69S</td>
<td>I50V+I54M+I84V</td>
</tr>
<tr>
<td>6</td>
<td>RAL+ABC+3TC</td>
<td>10</td>
<td>65,600</td>
<td>S-R-E-G-K</td>
<td>M184V+T215Y</td>
<td>I50V+I54M</td>
</tr>
</tbody>
</table>

- RAL concentrations in the expected therapeutic range
- But sub-optimal ART regimen

Markovicz et al., J AIDS, 2006; Charpentier et al., 2015
Results

• No virus harboured this insertion at baseline of RAL regimen
• Insertion corresponds to a duplication of upstream amino-acids:
  – HIV-1 RKK motif (R231-K258-K266) important for DNA binding
  – Insertion of positively charged amino-acids (K and R)
  – One patient exhibited a transient profile with a 2-AA insertion: Glycine-Lysine
  – « Full » insertion was only selected one year later

→ 2-steps selection ?

Alignment of codons 225 to 240 of integrase sequences obtained prior to and during RAL-based regimen

Eijkelenboom et al., 1999;
Lataillade et al., 2007; Perez-Bercoff et al., 2010
Results

- Four isolates from 3 patients with a 5-AA insertion were assessed
- Insertion impacts susceptibility to all INSTI
  - High-level of resistance for EVG and RAL (20- to >300-fold change in IC<sub>50</sub>)
  - Intermediate resistance for DTG and CAB
    3- to 17-fold change for DTG and 11- to 58-fold changes for CAB
  - Susceptibility to BIC was variable
    - 2 isolates were susceptible (0.4 and 1.1-fold change)
    - Slightly reduced activity for the 2 isolates obtained from the same patient (3- and 5-fold change)
Discussion

• Identification of a new resistance profile
  → 5-AA insertion at codon 231 of HIV-2 integrase
    – Repetition of upstream amino-acids
    – Selected under RAL-based regimen (< 1 year)
    – In heavily pre-treated patients → multi-drug resistant viruses
    – Broad cross-resistance (high-level resistance for EVG and RAL, intermediate resistance for DTG and CAB, slightly decreased susceptibility for BIC)
    – No mutations observed on other residues associated with INSTI-resistance → mutually exclusive mutations ?

• Perspectives:
  – Impact on viral replicative capacity ?
  – Role of the transient 2-AA insertion ?
  – Does it alter integrase function and/or structure ?
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