Detection of Minority HIV-1 Drug-Resistant Variants Moderately Improves the Prediction of Salvage Antiretroviral Therapy Outcomes

**Background**

Ultrasensitive HIV-1 drug resistance tests like 454 Sequencing (Roche Diagnostics/454 Life Sciences) allow the detection of low-frequency resistant variants which are often missed by Sanger sequencing genotypic tests. Detection of minority drug-resistant mutants more than doubles the risk of virological failure in antiretroviral naïve patients. However, the clinical relevance of detecting minority HIV-1 drug-resistant variants in antiretroviral treatment (ART) exposed subjects remains uncertain.

**Objective**

- To analyze the association between baseline detection of resistance mutations through 454 sequencing and the virological outcome of salvage antiretroviral therapy in comparison with Sanger sequencing.

**Design**

- Retrospective multicenter cohort study performed in 4 centers from Badalona, Madrid, Terrassa and Granada, Spain. The HIV Drug Resistance Surveillance Center of Catalonia and Madrid (HIVDB/ANRS REGA) algorithms.

**Subjects**

- ART-experienced adults.
- Initiating salvage ART including PI/r, nRTV (RAL) or nNRTI (ERT).
- HIV RNA (VL) > 1000 copies/mL and 1 mL plasma available for testing within 15 months before treatment change (TC).
- Clinical follow-up available through at least 48 weeks after TC.
- Good adherence to therapy in clinical records.

**Results**

- 165 subjects were included in the study (Table 1). Pre-TC genotypes were obtained in a median of 48 (0.15) days before TC. Virological outcomes were evaluable in 138 individuals. 41 developed VF.

**Table 1. Characteristics of the cohort**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis, years (median)</td>
<td>25 (7-42)</td>
<td>40 (6-67)</td>
<td>65 (6-42)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.9 (19-28)</td>
<td>24.0 (17-28)</td>
<td>23.9 (19-28)</td>
</tr>
<tr>
<td>CDC stage</td>
<td>1 (20.8%)</td>
<td>2 (38.5%)</td>
<td>3 (60.5%)</td>
</tr>
<tr>
<td>CD4 count, cells/µL</td>
<td>578 (320-1139)</td>
<td>331 (200-988)</td>
<td>454 (258-1139)</td>
</tr>
<tr>
<td>Type of drugs</td>
<td>1 (1 drug resistant variants in ART)</td>
<td>2 (2 drug resistant variants in ART)</td>
<td>3 (3 drug resistant variants in ART)</td>
</tr>
<tr>
<td>Race</td>
<td>White</td>
<td>Black</td>
<td>Total</td>
</tr>
</tbody>
</table>

**Figure 1. Definition of Treatment Change Episode (TCE) and VF**

- TCE was defined as the change of an antiretroviral drug with an observed or expected failure rate ≥5% in a clinical trial or ≥1% in a clinical practice.
- VF was defined as 2 consecutive measurements of VL >1000 copies/mL and 1 mL plasma available for testing within 15 months after treatment change (TC).

**Figure 2. HIV/HIVdb GSS distribution for Sanger and 454**

**Figure 3. Overall accuracy - ROC curves**

- The ROC curve AUCs were (Delong’s test; p=0.06164):
  - GSS-454: 0.74 (95% CI: 0.64 - 0.85)
  - GSS-Sanger: 0.71 (95% CI: 0.68 - 0.83)

- After checking for significance and colinearity among variables, each regression model included, in addition to the respective GSS, previous AIDS diagnoses and number of previous antiretrovirals.

**Table 2. Univariate and Multivariate Cox Models of Risk of Virological Failure**

- Subjects with GSS-454 > 3 were significantly more likely to develop VF than those with GSS-454 ≤ 3. No differences in VF were observed between different categories of GSS-Sanger. However, when combining the 454 and Sanger GSS score categories, GSS-454 > 3 also seemed to discriminate VF better than Sanger GSS scores.

**Conclusions**

Detection of minority HIV-1 drug-resistant variants moderately improves the prediction of salvage ART outcomes. Subjects with GSS-454 > 3 are more likely to develop virological failure. 454 seems to discriminate outcomes better than Sanger. In the multivariate Cox proportional hazards model, however, the only variables significantly associated with increased risk of virological failure were re-entering CCR5 as the sole third agent, and the number of antiretroviral drugs the subject had been exposed to. Subgroup analyses, as well as those accounting for additional maraviroc or T-Cell Zouse are ongoing. Similar results were obtained when we used the ANRS or REGA rules to calculate GSS scores.

**Acknowledgments**

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