Impact of baseline HIV-1 integrase mutations with virological outcome in patients starting a Raltegravir-containing regimen

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Background

• Raltegravir is a very potent and effective strand-transfer integrase-inhibitor (INSTI), recently FDA-approved for use also in first-line HAART regimen.

• Previously, analyses in small cohorts of HAART multi-experienced patients suggest that some integrase polymorphisms at baseline would be associated with different virologic response to Raltegravir.
The role of integrase polymorphisms in Integrase strand-transfer inhibitor naïve patients is still unclear

- **50 patients study:**
  "The presence of novel mutation **K156N** at baseline was significantly associated with high baseline viremia and HIV-RNA >50 copies/ml during RAL treatment”

- **51 patients study:**
  "**T206S** mutation associated with a lower response ”
  (D Da Silva et al, Antivir Ther. 2008; 13(Suppl. 3):A14 (abstract no. 12);17th International HIV Drug Resistance Workshop, 10-14 June 2008, Sitges, Spain)

- **139 patients study:**
  "With the exception of the natural polymorphism **V201I**, baseline integrase mutations were not statistical relevant as independent predictors of virologic success”
  Ceccherini-Silberstein F et al. Impact of baseline HIV-1 integrase mutations with virological outcome in patients starting a raltegravir-containing regimen.

- **189 patients study:**
  "Frequency of **S17N, M50I, and D256E** were statistically different between treatment successes and virologic failures– these need to be characterized further…Additional analyses needed on polymorphism frequency by subtype, association with RAL resistance pathways”
  Miller et al. (Croi 2009)

- **10 patients study (Phenotypic Data):**
  "**E157Q** was the only naturally occurring mutation thought to contribute to resistance to elvitegravir, raltegravir and L-870,810”
  (Low et al, AAC 2009)

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Aim of this study

• Therefore, the aim is to evaluate the impact of baseline HIV-1 integrase polymorphisms with virological outcome in a larger cohort of patients starting a Raltegravir-containing regimen.
Methods

We analyzed 206 HIV-1 infected treatment-experienced patients with triple-class resistant virus who received Raltegravir plus optimized-background-therapy (OBT) from eight clinical centres within Italy and France.

Patients with baseline HIV RNA> 400 copies/mL were enrolled in the study.

**HIV-1-RNA and integrase-genotyping** were assessed at baseline and during Raltegravir treatment. In particular, for 177 patients, viremia values at 24-week were available.

The prevalence of all baseline integrase mutations (frequency > 5%) was calculated in the overall population, and in the responding and not responding patients (Fisher exact test). Specific codon usage for each mutation was considered when its prevalence was >5% in overall. Benjamini-Hochberg method was used to correct for multiple comparisons (False Discovery Rate=0.1)

For subtyping Maximum-Likelihood phylogenetic analyses (PAUP*4.0b10) were performed on integrase sequences.

**Logistic regression analyses** (uni- and multivariate) were performed to investigate if baseline integrase polymorphisms and other variables (such as: baseline HIV-1-RNA, co-administered drugs and subtype [B vs non-B]) were independent predictors of virological success.
## Patients Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age, Years (N=206)</td>
<td>46.2</td>
</tr>
<tr>
<td>% Male</td>
<td>78.2</td>
</tr>
<tr>
<td>Baseline Median HIV RNA (IQR)</td>
<td>4.5 (3.7-5.2)</td>
</tr>
<tr>
<td>Median CD4 Cell Count (IQR)</td>
<td>197 (95-315)</td>
</tr>
</tbody>
</table>

### Subtype % (N)

- B 90.3 (186)
- Non-B 9.7 (20)
- A 1.9 (4)
- C 0.5 (1)
- CRF02AG 2.4 (5)
- CRF12BF 0.5 (1)
- D 1.0 (2)
- F 2.4 (5)
- G 1.0 (2)

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Drugs co-administered with Raltegravir

*PI are RTV-boosted in 90.5% of patients

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The 68% of overall population achieved virological success at 24 weeks after Raltegravir starting (71% infected by B subtype vs 59% infected by non-B subtype)

The efficacy of Raltegravir containing regimen was confirmed in both B and non-B infected patients (p<0.05; Chi Square for trend test). No significant differences (P=0.2, Fisher test) between B and non-B subtype groups in percentage of success at 4-8-12-24 weeks were observed.

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Primary resistance mutations were completely absent at baseline of Raltegravir regimen

Patients achieving virological success (N=124)

Patients not achieving virological success (N=53)

Primary raltegravir resistance mutations are underlined

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Differently, some secondary resistance mutations were present at low frequency

- V151I and S230N polymorphisms were associated with worse and better virological response respectively, but not after multiple comparison test correction.
All integrase polymorphisms described in previous studies were not associated with virologic response at 24 weeks in this cohort.

Only K156N mutation was associated with worse virologic response at 24 weeks, but not after multiple comparison test correction.

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...A novel mutation was found negatively associated with virologic success at 24 weeks of Raltegravir treatment.

<table>
<thead>
<tr>
<th>Position</th>
<th>Codon change</th>
<th>AA change</th>
<th>Failing Patients (N=53)</th>
<th>%</th>
<th>Responding Patients (N=124)</th>
<th>%</th>
<th>P (Fisher test)</th>
<th>Overall N</th>
<th>% (N=177)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>All Codons</td>
<td>T125A</td>
<td>25</td>
<td>47.2</td>
<td>40</td>
<td>32.2</td>
<td>0.068</td>
<td>65</td>
<td>40.1</td>
</tr>
</tbody>
</table>
A novel mutation was found negatively associated with virologic success at 24 weeks of Raltegravir treatment. Only the mutation T125A with specific GCA codon was associated with worse virologic response with statistical significance among 53 polymorphisms analyzed (p=0.002, Benjamini-Hockberg multiple comparison correction method FDR=0.1).
By multivariate logistic regression, the independent predictors of worse virologic response were: *baseline HIV-1 RNA, AZT or D4T co-administration and presence of T125A(GCA) mutation*.

<table>
<thead>
<tr>
<th>Variables</th>
<th>P value</th>
<th>Odd Ratio</th>
<th>95.0% C.I. for Odd Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<tr>
<td>Baseline HIV-1 RNA</td>
<td>0.0003</td>
<td>0.42</td>
<td>0.3</td>
</tr>
<tr>
<td>SubType (B vs NotB)</td>
<td>0.995</td>
<td>1.00</td>
<td>0.3</td>
</tr>
<tr>
<td>DRV Co-administered</td>
<td>0.938</td>
<td>1.03</td>
<td>0.5</td>
</tr>
<tr>
<td>T20 Co-administered</td>
<td>0.900</td>
<td>1.05</td>
<td>0.5</td>
</tr>
<tr>
<td>MVC Co-administered</td>
<td>0.505</td>
<td>0.58</td>
<td>0.1</td>
</tr>
<tr>
<td>ETR Co-administered</td>
<td>0.896</td>
<td>0.95</td>
<td>0.4</td>
</tr>
<tr>
<td>TDF Co-administered</td>
<td>0.536</td>
<td>1.27</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>AZT or D4T Co-administered</strong></td>
<td><strong>0.040</strong></td>
<td><strong>0.31</strong></td>
<td><strong>0.1</strong></td>
</tr>
<tr>
<td>DDI or DDC or ABC Co-administered</td>
<td>0.853</td>
<td>0.92</td>
<td>0.4</td>
</tr>
<tr>
<td>FTC or 3TC Co-administered</td>
<td>0.704</td>
<td>0.82</td>
<td>0.3</td>
</tr>
<tr>
<td>T125A(GCA)</td>
<td>0.006</td>
<td>0.30</td>
<td>0.1</td>
</tr>
</tbody>
</table>
The majority of failing patients with T125A(GCA) were infected by non-B subtype virus

The prevalence of T125A (specific GCA codon) was higher in patients infected with non-B subtype (13/20 [65%]) vs B subtype (35/186 [19%]) (OR=0.12 [CI:0.05-0.33], P=0.00003), with a greater consistence among failing patients (6/7 [86%] non-B subtype vs 14/46 [30%] B subtype, OR=0.07 [CI:0.01-0.52], p=0.009).
125A, specific GCA codon, is the consensus sequence for subtypes A, C, D, G and for CRF02_Ag

While 125T specific codon ACG is the consensus sequence only for the subtype B
T125A(GCA) mutation was associated with Raltegravir failure in another group of patients

- The analysis was performed on 492 Ral naïve and 66 Ral treated patients (36 from our resistance database and 30 from Stanford resistance Database, http://hivdb.stanford.edu/)

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T125A(GCA) mutation was not directly associated with phenotypic resistance

Of note, in 58 HXB2-clonal recombinant viruses with RT-IN region from INI naïve patients, carrying T125 (GCA) mutation, no phenotypic resistance to Raltegravir was observed (all FC values < Biological cutoff).

(Ceccherini et al, 2010, Submitted)
Summary & Conclusions

• Among all integrase polymorphisms analyzed, only the mutation T125A (GCA) was statistically associated with worse virologic response after Benjamini-Hockberg correction after 24 weeks of Raltegravir treatment.

• T125A (GCA) was more prevalent in non-B failing patients and its prevalence gained in Raltegravir treated patients.

• By univariate and multivariate logistic regression analyses, baseline HIV-1 RNA, the co-usage of AZT or D4T and the mutation T125A (GCA) were independent predictors of worse virologic response.

• This finding in non-B subtypes is intriguing and further research is warranted. Currently, in vitro experiments are in progress. A larger cohort of patients should be analyzed to confirm this observation.

• The clinical implications and relevance of this polymorphism is still to be determined.
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