The presence of HIV-1 protease compensatory mutations increases over the years and correlates with a shorter time to virological failure in PI treated patients

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Specific mutations at HIV-1 protease positions 10,13,16,20,36,60,62,63,69,82,89,93 are:

- Wild-type amino acids in several non-B subtypes.

<table>
<thead>
<tr>
<th>Position</th>
<th>cons_B</th>
<th>cons_A</th>
<th>cons_A1</th>
<th>cons_A2</th>
<th>cons_C</th>
<th>cons_F1</th>
<th>cons_F2</th>
<th>cons_F1F2</th>
<th>cons_G</th>
<th>cons_H</th>
<th>cons_AE</th>
<th>cons_AG</th>
<th>cons_DF</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td></td>
<td>M.........</td>
<td>M.........</td>
<td>M.........</td>
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<td>M.........</td>
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<tr>
<td>90</td>
<td></td>
<td>M.........</td>
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</tbody>
</table>

Comparison of protease amino acid sequence among the most frequent HIV-1 subtypes and recombinant forms

Specific mutations at protease positions 10,13,16,20,36,60,62,63,69,82,89,93 are:

- natural polymorphisms in HIV-1 B subtype that increase their prevalence in HIV-1 B infected patients failing protease inhibitors.

Novel Human Immunodeficiency Virus Type 1 Protease Mutations Potentially Involved in Resistance to Protease Inhibitors

Valentina Svicher,1† Francesca Ceccherini-Silberstein,1†† Fulvio Erba,1 Maria Santoro,1 Caterina Gori,2 Maria Concetta Bellocci,2 Sara Giannella,2 Maria Paola Trotta,2 Antonella d’Arminio Monforte,3 Andrea Antinori,2 and Carlo Federico Perno2

Antimicr agents and chem, 2005
Specific mutations at protease positions 10,13,16,20,36,60,62,63,69,82,89,93 are:

✓ also well known to play a compensatory role in PI resistance.

Secondary Mutations in the Protease Region of Human Immunodeficiency Virus and Virologic Failure in Drug-Naive Patients Treated with Protease Inhibitor–Based Therapy

Carlo F. Perno,1,2 Alessandro Cozzi-Lepri,15 Claudia Balotta,4 Federica Forbici,1 Michela Violin,9 Ada Bertoli,1 Guido Facchi,5 Patrizio Pezzotti,3 Gianpiero Cadeo,2 Giulio Tositti,3 Sandro Pasquinucci,6 Sergio Pauluzzi,9 Alfredo Scalzini,10 Bernardino Salassa,11 Antonella Vincenti,13 Andrew N. Phillips,13 Ferdinando Dianzani,4 Amelia Appice,10 Gioacchino Angarano,11 Laura Monno,11 Giuseppe Ippolito,1 Mauro Moroni,2 Antonella d'Arminio Monforte,2 and the Italian Cohort Naive Antiretroviral (I.CO.N.A.) Study Group9

Natural Polymorphisms in HIV-1 Protease: Impact on Effectiveness of a First-Line Lopinavir-Containing Antiretroviral Therapy Regimen

Karen Champenois,16 Sylvie Deuffic-Burban,1,2 Laurent Cotte,3,4 Patrice André,5 Philippe Choisy,6 Faiza Ajana,6 Laurence Bocket,7 and Yazdan Yazdanpanah1,6

J of Infectious Diseases, 2001

J of Medical Virology, 2008
However, their impact on the virological response to PI currently used with RTV boosting has not yet been defined…
Aims

Thus, this study aims at evaluating:

✔ the prevalence of these compensatory mutations over the years in PI-naïve subtype B infected patients;

✔ their relationship with PI virological failure;

✔ their effect on the binding affinity between protease and protease-inhibitors.
Methods (I)

- This study included 2,560 samples from 2,235 HIV-1 B infected patients: 1,953 drug-naïve and 282 PI-naïve (experienced to reverse-transcriptase inhibitors), collected from 1997 to 2010;

- In this subset of patients, the prevalence of the following protease mutations: L10I/V, I13V, G16E, K20I/R, M36I, I62V, L63A/P, H69K/Q, V82I, L89I/M, I93L has been estimated over time;

- Statistical significance was evaluated by chi-square for trend test and Fisher exact test;

- The evolution of the HIV-1 subtype B at protease positions 10, 13, 16, 20, 36, 60, 62, 63, 69, 82, 89, 93 was evaluated by the Shannon entropy analysis.
Methods (II)

- The role of these mutations on the virological outcome was evaluated in 194 HIV-1 B infected patients starting their first PI (LPV/r) containing regimen, and without major drug-resistance mutations at baseline.

- Factors influencing virological failure (at least two determinations of ≥50 copies/ml after virological success) were assessed by multivariable logistic-regression.

- The effect of these mutations on the LPV binding affinity to HIV-1 protease was also evaluated by docking-analysis using the AutoDock Vina approach.
Results
The percentage of HIV-1 B subtype infected patients with at least 1 protease polymorphism remains stable over time.

<table>
<thead>
<tr>
<th>Protease polymorphisms, N%</th>
<th>Prevalence N (%) (^a)</th>
<th>2005 - 2006 (N=531)</th>
<th>2007 - 2008 (N=783)</th>
<th>2009 - 2010 (N=637)</th>
<th>P-value (b)</th>
<th>P-value (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>73 (10.6)</td>
<td>48 (9.0)</td>
<td>69 (8.8)</td>
<td>52 (8.2)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>At least 1</td>
<td>618 (89.4)</td>
<td>483 (91.0)</td>
<td>715 (91.3)</td>
<td>585 (91.8)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>1 or 2</td>
<td>404 (58.5)</td>
<td>273 (51.4)</td>
<td>371 (47.4)</td>
<td>306 (48.0)</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>more than 2</td>
<td>214 (31.0)</td>
<td>210 (39.5)</td>
<td>343 (43.8)</td>
<td>279 (43.8)</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\(a\) Prevalence of protease mutations L10I/V, I13V, G16E, K20I/R, M36I, I62V, L63A/P, H69K/Q, V82I, L89I/M, I93L was evaluated on a total of 2560 samples from 2235 HIV-1 subtype B infected patients naive to PI.

\(b\) P-value was calculated by a chi-square for trend test;

\(c\) Statistically significant difference between samples collected before 2004 and 2009-2010 was calculated by Fisher exact test.
The percentage of patients with >2 polymorphisms increases over time

<table>
<thead>
<tr>
<th>Protease polymorphisms, N%</th>
<th>Prevalence N (%)</th>
<th>P-value b</th>
<th>P-value c</th>
</tr>
</thead>
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<tr>
<td>None</td>
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a Prevalence of protease mutations L10I/V, I13V, G16E, K20I/R, M36I, I62V, L63A/P, H69K/Q, V82I, L89I/M, I93L was evaluated on a total of 2560 samples from 2235 HIV-1 subtype B infected patients naive to PI.
b P-value was calculated by a chi-square for trend;
c Statistically significant difference between samples collected before 2004 and 2009-2010 was calculated by Fisher exact test.
Among all mutations analyzed, only specific polymorphisms significantly increase over time.

Prevalence of protease mutations L10I/V, I13V, G16E, K20I/R, M36I, I62V, L63A/P, H69K/Q, V82I, L89I/M, I93L was evaluated on a total of 2560 samples from 2235 HIV-1 subtype B infected patients naive to PI. Statistically significant difference between samples collected before 2004 and 2009-2010 was calculated by Fisher Exact test.
Evaluation of the protease compensatory mutations impact on the virological response to PI containing regimen
The proportion of patients failing PI-treatment is not influenced by the presence of compensatory mutations. Statistically significant differences between samples were calculated by Fisher exact test and Chi-square for trend test.
Differently, the presence of protease polymorphisms at baseline strongly correlates with a shorter time to virological failure in LPV/r treated patients.

Number of protease polymorphisms at baseline

*Statistically significant difference between samples was calculated by Mann Withney.
Differently, the presence of protease polymorphisms at baseline strongly correlates with a shorter time to virological failure in LPV/r treated patients.

Number of protease polymorphisms at baseline

*Statistically significant difference between samples was calculated by Kruskall Wallis Test.
The high number of baseline protease polymorphisms is a predictor of virological failure within 48 weeks. The analysis was performed on 194 patients. Predictor variables considered are: viral load and CD4 cell count at baseline, drugs co-administered with LPV/r, previous HAART experience, number of baseline protease polymorphisms, time to reach virological success. Only predictors with p < 0.1 in univariate model are reported in the table.

<table>
<thead>
<tr>
<th>Independent predictors of virological failure within 48 weeks</th>
<th>Univariable Analysis</th>
<th>Multivariable Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds Ratio (Low-High)</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>Negatively associated</strong></td>
<td></td>
<td></td>
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<tr>
<td>Time virological success (weeks)</td>
<td>0.89 (0.79-0.99)</td>
<td>0.03</td>
</tr>
<tr>
<td>FTC vs TA</td>
<td>0.04 (0.02-1.06)</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Positively associated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of protease polymorphisms ≥4</td>
<td>5.0 (1.30-7.51)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>2.62 (1.13-6.21)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

The analysis was performed on 194 patients. Predictor variables considered are: viral load and CD4 cell count at baseline, drugs co-administered with LPV/r, previous HAART experience, number of baseline protease polymorphisms, time to reach virological success. Only predictors with p <0.1 in univariate model are reported in the table.
Cluster analysis highlights the **co-presence at baseline of L10V, I13V, and I93L only in patients failing LPV/r**

Dendrogram was obtained using average linkage hierarchical clustering. At each edge bootstrap values are shown.
Localization of L10V, I13V and I93L in the HIV-1 protease

Localization of PR mutations correlated with PI/r failure in the structure of HIV-1 protease, complexed with LPV.
The combination of these mutations widely affects the protease affinity for LPV

The graph reports the variation of the free energy differences ($\Delta G$) in kcal/mol between the wild-type ($\Delta G_{\text{wild-type}}$) and the mutated ($\Delta G_{\text{mutant}}$) HIV-1 B subtype protease complexed with LPV. Docking simulations were performed by using the AutoDock Vina approach.
Differently by other mutational pathways, founded at baseline only in patients successfully treated with LPV.

The graph reports the variation of the free energy differences ($\Delta G$) in kcal/mol between the wild-type ($\Delta G_{\text{wild-type}}$) and the mutated ($\Delta G_{\text{mutant}}$) HIV-1 B subtype protease complexed with LPV. Docking simulations were performed by using the AutoDock Vina approach.
Our study shows that specific HIV-1 B-subtype protease polymorphisms (wild-type amino acids in several HIV-1 non-B subtypes):

- Increase their prevalence in HIV-1 B infected population over the years;
- Correlate with a shorter time to virological failure to LPV/r;
- Affect the binding affinity of the HIV-1 protease for LPV.

This suggests a progressive evolution of the HIV-1 subtype B, that may impact the response to PI containing regimens.
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- M. Andreoni

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**CHAIN**
- collaborative group project