The Genotypic False Positive Rate Determined by Population V3-Sequencing Predicts the Burden of X4 Minority Quasispecies


Presented at the 9th Eu. Workshop on HIV & Hepatitis – 25 – 27 March 2011, Paphos, Cyprus
Background

• CXCR4-using species can hamper virological response to a maraviroc-containing regimen.

• However, a recent study shows that, among patients with D/M-tropic viruses, those with X4-viruses at a level <10% can respond to a maraviroc-containing regimen.

Swenson et al, CROI 2009, Abstract 680

Change in viral load

>30% X4
10-30% X4
<10% X4

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Background

• Among different assays for tropism assessment, genotypic tropism testing is so far widely used in different European Countries.

• It based on population-based sequencing of the patient-derived HIV-1 gp120 V3 domain, which is the major determinant for co-receptor binding.

• The genetic information contained in the V3 sequence is used to infer HIV-1 tropism by using web-based bioinformatic interpretation tools, such as Geno2Pheno (G2P) and PSSM.
Background

• Along with tropism prediction, Geno2Pheno provides a score, called false-positive rate (FPR). **FPR is a so far qualitative percentage score (range 0-100) that positively predicts the use of the CCR5-coreceptor.**

The higher the FPR is, the higher HIV-1 ability to use CCR5 co-receptor is

<table>
<thead>
<tr>
<th>Model</th>
<th>Prediction</th>
<th>FPR</th>
<th>Remarks</th>
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</thead>
<tbody>
<tr>
<td>Clonal</td>
<td>CCR5-antagonists like Maraviroc or Vicriviroc are likely to be effective.</td>
<td>15.4%</td>
<td></td>
</tr>
</tbody>
</table>

This prediction is based on clonal training data and V3-sequences alone.

**False Positive Rate Range**

0

100

**Increased CCR5 co-receptor usage**
Background

- Along with tropism prediction, Geno2Pheno provides a score, called false-positive rate (FPR). FPR is a so far qualitative percentage score (range 0-100) that positively predicts the use of the CCR5-coreceptor.

The higher the FPR is, the higher HIV-1 ability to use CCR5 co-receptor is increased.

However, **the diagnostic significance of FPR has not yet been evaluated**

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Objective

- To define the potential correlation between FPR by population V3-genotyping and the burden of X4-species, detected by both the ultra-deep V3-pyrosequencing (UDPS) and the enhanced-Sensitivity Trofile phenotypic assay (ESTA).
Methods

• This study included 46 HIV-1 infected (all B-subtype) patients, naïve to maraviroc, with viremia >10,000 copies/ml. Among them, 36 were drug-experienced, and 10 were drug-naïve patients.

HIV tropism was assessed by:

- Population V3-sequencing
- Ultra-deep V3 pyrosequencing (UDPS) with GS-FLX Roche
- Enhanced sensitivity version of Trofile (ESTA)

• For UDPS, only V3 coding sequences detected above 0.5% of viral-species were analyzed. Total number of viral variants obtained per patient at UDPS varied from 618 to 6728.

• Geno2pheno set at cut-off 5.75 was used to predict viral tropism.
R5 variants are detected by UDPS in all patients, irrespective of FPR values at population V3-sequencing, thus confirming the greater pathogenetic relevance of such strains compared to X4 variants.

The graph reports the proportion of R5 variants per patient according to the FPR at population V3 sequencing.

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By contrast......
The graph reports the proportion of X4 variants per patient according to the FPR at population V3 sequencing.

X4 variants progressively decrease by increasing the FPR by population V3 sequencing.

\[ \text{Rho} = -0.59 \]
\[ P = 7.65 \times 10^{-5} \]
In particular......
V3 quasispecies composition at UDPS according to the false positive rate determined by population V3 sequencing

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No X4 variants are detected by both UDPS and ESTA in all patients with FPR >60

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The majority of patients with FPR ranging from 20 to 60 at population V3 sequencing harbour R5-species.
In patients with FPR ranging from 20 to 60, X4 variants at levels >10% are detected in 2 patients, never exceeding 20% of the entire viral population.
• The proportion of patients with X4 variants at levels >10% increases by decreasing the FPR by population V3-sequencing. X4 species at a level >10% are present in all patients with FPR<5, and reach 90% of the entire population in patients with FPR<2.
• The median FPR of V3 sequences detected by UDPS progressively increases by increasing the FPR at population V3 sequencing.
• This suggests that CCR5 usage potential of the entire viral population progressively increases with the FPR at population V3 sequencing.

The graph reports the median (IQR) FPR of V3 sequences detected by UDPS according to different ranges of FPR at population V3 sequencing.

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Conclusions

• FPR determined by population V3-sequencing can predict the burden of X4 variants.

• **No X4 variants** are detected by both UDPS and ESTA in patients with FPR>60, thus suggesting that patients with higher FPR have even greater chances of increased activity of CCR5 inhibitors.

• In addition, the presence of R5 viruses in all patients, including those with very low FPR, confirms the greater pathogenetic relevance of such strains compared to X4.
Conclusions

• Overall, these results suggest that genotypic tropism testing may provide further information (beyond the classical R5 versus X4 tropism definition) for an optimal positioning of CCR5-antagonists in clinical practice.

• Further clinical validation of the role of FPR is necessary.
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