Performance of genotypic coreceptor measurement using geno2pheno\_[coreceptor] in B- and non-B HIV subtypes in a large cohort of therapy-experienced patients in Germany

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Background:
Coreceptor usage determination is mandatory for prescription of the CCR5-antagonist Maraviroc (MVC). Recently, the clinically validated standard Trofile assay has been replaced by an enhanced version (Trofile-ES) capable of detecting also CXCR4-using minor variants to levels below 0.3%. For the fast and inexpensive genotypic approaches which can also be used in case of low viral loads below 1000 cp/ml or below 50 cp/ml using proviral DNA from PBMCs, no prospective clinical validation is available yet. In this work, we compare results of both Trofile assays with predictions from geno2pheno[coreceptor] (g2p) in a large German cohort of treatment-experienced patients and analyze the effects of different subtypes.

Materials & Methods:
HIV coreceptor usage was determined with the Trofile test (Trofile® or Trofile-ES® (ESTA) by Monogram), which was used in the maraviroc approval studies. In addition, the V3-loop of gp120 was sequenced using standard bulk-sequencing techniques and tropism as well as subtype inferred from genotype-studies. In addition, the V3-loop of gp120 was sequenced using standard bulk-sequencing techniques and tropism as well as subtype inferred from genotype-studies. Methods to determine HIV-1 coreceptor tropism
Trofile® by Monogram – Used in approval studies – Clinically validated phenotypic assay
Trofile-ES® (ESTA) – "enhanced-sensitivity" – Minority detection improved from 10% to 0.3%
Sequence analyses – RT-PCR from plasma viral RNA and Sanger sequencing – Interpretation by geno2pheno[coreceptor] – Subtyping by geno2pheno[coreceptor]

Results:
Subtyping
<table>
<thead>
<tr>
<th></th>
<th>Geno2pheno[coreceptor]</th>
<th>Rega subtyping tool</th>
</tr>
</thead>
<tbody>
<tr>
<td>No result</td>
<td>2 (0.5%)</td>
<td>387 (49.7%)</td>
</tr>
<tr>
<td>Subtype B</td>
<td>642 (86.7%)</td>
<td>314 (84.6%)</td>
</tr>
<tr>
<td>Non-B</td>
<td>92 (12.4%)</td>
<td>57 (15.4%)</td>
</tr>
</tbody>
</table>

* Of successful predictions
Overall concordance between both subtyping systems: 96.2%

Short sequence length was the most frequent reason for unsuccessful subtyping.

Tropism testing comparisons
738 genotype-phenotype pairs
- 619 standard Trofile®
- 119 ESTA

standard Trofile®
- 414 (66.9%) R5
- 205 (33.1%) D/M

ESTA
- 72 (60.5%) R5
- 47 (39.5%) D/M

Genotype vs TROFILE® vs ESTA (N=119)

Conclusions:
This analysis shows that with the enhanced assay more samples were detected to be D/M-tropic. In the context of the most recently presented clinical data comparing responders and non-responders to MVC-use in patients harboring minority CXCR4-variants, the question arises whether more people suitable for MVC will be screened out when using the enhanced assay.

In contrast to other studies, these results show very good agreements between Trofile and geno2pheno[coreceptor] for non-B isolates suggesting to use the subtype-predictions as an additional confidence measure.