Use of a genotypic assay for the prediction of HIV-1 co-receptor tropism and guiding the use of CCR5 antagonists in clinical practice

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Background

- Co-receptor tropism testing is routinely performed prior to the use of Maraviroc (MVC).
- Trofile, and later Enhanced Trofile (ESTA), use patient gp120 to determine entry into cells, but:
  - technically challenging, time consuming;
  - snapshot of patient at time of sampling, no clinical weighting.
- Genotypic tropism testing (GTT), such as g2p, uses the V3 sequence and can also incorporate clinical parameters to determine tropism.
- We have previously reported a significant positive correlation between ESTA GTT by g2p.
- Recent retrospective analyses of the MOTIVATE and MERIT clinical trials provide clinical support to GTT. Prospective studies of patients treated with MVC based on GTT are limited.
Objective

• Determine the co-receptor tropism of patients with either drug-toxicity issues or therapy failure by GTT and prospectively follow patients classed as R5 or Mixed who were started on a MVC-containing regimen to monitor their clinical outcome including:
  – Viral Load (VL) change, CD4 count change.
Methods

- Study population: we introduced GTT as a routine diagnostic service in April 2009. For this analysis we selected patients who started MVC after April 2009 to ensure a consistent testing approach across the cohort.

- Specimen selection:
  - plasma if VL >500 cps/ml;
  - proviral DNA from PBMCs if VL <500 copies/ml, including <50 cps/ml.

- In-house nested PCR and sequencing on 3 replicates/sample. Sequences were processed using the g2p algorithm.

- Interpretation:
  - clonal model at FPR 6%;
  - Clinical model at FPR 15% was also used if information on the CD4 count, CD4 nadir and current VL was available and reliable.

- Results reported as R5, X4 or Mixed. Mixed results included g2p interpretation prediction of R5 by clonal model and X4 by clinical model, and replicate samples or sequences showing mixtures of R5 & X4 by clonal model.
## Patient parameters

<table>
<thead>
<tr>
<th></th>
<th>Treatment Failures</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td><strong>Median CD4 Nadir (Range)</strong></td>
<td>186 (5 - 559)</td>
<td>253 (53 - 617)</td>
</tr>
<tr>
<td><strong>Median CD4 at switch (Range)</strong></td>
<td>477 (44 - 1056)</td>
<td>523 (214 - 1223)</td>
</tr>
<tr>
<td><em><em>Subtype (B/Non-B</em>)</em>*</td>
<td>8/5</td>
<td>7/8</td>
</tr>
<tr>
<td><strong>Median VL at switch log(_{10}) (Range)</strong></td>
<td>2.26 (1.69 - 4.72)</td>
<td>1.69 (1.69 - 4.42)</td>
</tr>
<tr>
<td><strong>Sample type (PBMC/Plasma)</strong></td>
<td>10/3</td>
<td>12/3</td>
</tr>
</tbody>
</table>

*Non-B subtypes: A (2), C (4), G (1), CRF_02 (3), cpx (3).
Subtypes based on *pol* sequences where available.
For calculations, VL <50cp/ml were given a value of 49 (log\(_{10}\) 1.69).
In the Treatment Failures group 2 patients started on MVC showed a Mixed result; 1 patient with a current R5 result had previously shown a Mixed.
## Results

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<td>477 (44 - 1056)</td>
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</tr>
<tr>
<td><strong>Median CD4 at follow-up (Range)</strong></td>
<td>486 (166 - 894)</td>
<td>617 (278 - 1100)</td>
</tr>
<tr>
<td><strong>Log$_{10}$ median VL at switch (Range)</strong></td>
<td>2.26 (1.69 - 4.72)</td>
<td>1.69 (1.69 - 4.42)</td>
</tr>
<tr>
<td><strong>Log$_{10}$ median VL at follow-up (Range)</strong></td>
<td>1.69 (1.69 - 3.43)</td>
<td>1.69 (1.69 - 2.05)</td>
</tr>
<tr>
<td><strong>Median MVC follow-up (Range)</strong></td>
<td>13 weeks (4 - 35)</td>
<td>19 weeks (4 - 42)</td>
</tr>
</tbody>
</table>

Follow-up period is defined as the period between the switch to a regimen containing MVC and the date of the last VL test while still on MVC.
Toxicity switches (1)

- Overall, 15 patients replaced one or more ARVs with MVC due to toxicity, 12 based upon a proviral DNA GTT.
- 11 of the 15 patients switched therapy with a VL <50cps/ml, and the remaining 4 patients changed HAART before achieving VL suppression.
- Median number of active drugs, including MVC in all patients, was 3 (range 2 – 5); 11 of 16 patients (73%) received MVC without a PI/r.
- At follow-up, 14 of 15 (93%) patients either maintained or achieved a VL <50 cps/ml.
  - no patient who switched with a VL <50 cps/ml rebounded to >50 cps/ml.
  - 1 patient has not yet suppressed but has achieved a 2.5 log_{10} VL drop 4 weeks after switching Truvada/EFV for Truvada/MVC.
Toxicity Switches (2)

In the 15 patients switched due to toxicity, MVC replaced the following drugs:

- Truvada, 2
- EFV, 2
- TDF, 2
- T20, 1
- ATV/r, 1
- LPV/r, 2
- SQV/r, 2
- FPV/r, 1
- ATV/Raltegravir, 1
- EPV, 2
- TDF, 2

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Treatment failures (1)

Of the 13 patients with treatment failure who received a new regimen containing MVC, 7 (54%) showed a VL <50 cps/ml at follow-up.

The patients who achieved <50 cps/ml had a similar median number of active drugs in their regimen compared to patients who failed to achieve <50cps/ml.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Log$_{10}$ VL at switch</th>
<th>Log$_{10}$ VL at follow-up</th>
<th>MVC treatment time (Weeks)</th>
<th>Active drugs in regimen</th>
<th>g2p result</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3.24</td>
<td>2.18</td>
<td>35</td>
<td>3</td>
<td>CCR5</td>
</tr>
<tr>
<td>5</td>
<td>4.64</td>
<td>3.43</td>
<td>12</td>
<td>1.5</td>
<td>Mixed</td>
</tr>
<tr>
<td>23</td>
<td>2.47</td>
<td>2.05</td>
<td>21</td>
<td>4</td>
<td>CCR5</td>
</tr>
<tr>
<td>24</td>
<td>4.72</td>
<td>2.64</td>
<td>8</td>
<td>0.5</td>
<td>Mixed</td>
</tr>
<tr>
<td>25</td>
<td>2.04</td>
<td>2.21</td>
<td>8</td>
<td>4</td>
<td>CCR5*</td>
</tr>
<tr>
<td>26</td>
<td>2.03</td>
<td>2.07</td>
<td>9</td>
<td>2.5</td>
<td>CCR5</td>
</tr>
</tbody>
</table>

*Patient 25 had a previous tropism sample interpreted as Mixed. Active drug scores include MVC.
Treatment failures (2)

- Of the 6 patients who had not achieved a VL of <50cps/ml at the end of follow-up:
  - 2 patients had a Mixed tropism result at testing and received either 1 (pt 5) or 0 (pt 24) other active drugs in their background regimen;
  - Of the 4 remaining patients:
    - 3 had dual-class resistance (pt 2 NRTI & NNRTI; pt 25 NRTI & NNRTI) or triple-class (pt26) resistance;
    - the 4th patient has had a low-level VL, between 50 and 300 cps/ml in the absence of detectable drug resistance, for over 2 years.
Conclusions

• From this limited patient data set it can be seen that a g2p R5 interpretation is highly predictive of successful use of MVC in treating HIV-1, with 21/26 patients (81%) maintaining or achieving and maintaining <50 cps/ml during the follow-up period.

• The use of proviral DNA for GTT appears to be reliable in guiding treatment switches in suppressed patients with toxicity.

• Both patients with a Mixed result achieved a reduction in VL (1.21 and 2.08 log_{10}) while on MVC with 1 or 0 other active drugs in their background regimen. This may suggest that MVC may achieve a degree of virological suppression in some patients classified as Mixed.

• While a longer follow-up period and larger numbers are desirable, these findings provide encouraging evidence of GTT for guiding the use of CCR5 antagonists in clinical practice.