Genotypic tropism testing: use in clinical practice

European Resistance Workshop
Sorrento, March 2010

Dr Nicky Mackie
Genotyping to determine Tropism in Clinical Practice

1. Research applications
   - SMASH
   - Maraviroc, darunavir/r once daily PK Study

2. Routine Clinical applications
   - Prior to commencing 1st line drug therapy
   - Patients experiencing virological failure where use of MVC is considered
   - Patients who have VL<50 copies/ml on treatment who require a switch for toxicity, e.g lipoatrophy
SMASH Study

A prospective, randomised study to assess safety, changes in platelet reactivity, plasma cardiac biomarkers, immunological and metabolic parameters in HIV-1 infected subjects undergoing a switch in antiretroviral therapy.

‘The St. Marys and The Mater Switch Study’

Eligibility criteria:
plasma HIV RNA < 50 copies/mL for at least 24 weeks prior to screening currently receiving a stable antiretroviral regimen comprising of:

- any boosted protease inhibitor
- at least one NRTI including abacavir and / or didanosine
- CCR5 tropic virus (c2V3 loop sequence stored sample)
SMASH Study design

Arm 1 (immediate switch in antiretroviral therapy)
Continue current boosted protease inhibitor
Switch NRTI backbone to maraviroc 150 mg bid

Arm 2 (continue current antiretroviral therapy)
No change to current antiretroviral therapy for twelve weeks
After twelve weeks switch therapy as per Arm 1

Primary endpoint:
Mean change from baseline in platelet reactivity between treatment arms at week 12.

Target number:
n=40
St. Mary’s Hospital, London (n=25)
Mater Hospital, Dublin (n=15)
Genotyping to determine Tropism in Clinical Practice

1. Research applications
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Case Study - Background

33-year old British male

Admission 2007

PC
Left leg weakness
4/7 progressive weakness left leg, ‘gave way’

Feverish and sweaty
Sore throat and dry cough

No sensory / speech / visual / balance disturbance
Upper limbs not affected

No history trauma
No bladder/bowel problems

First episode
Childhood asthma

Left eye strabismus – surgical correction

Idiopathic Thrombocytopenia ~ 1995

Headaches and photophobia 1999
• CSF acellular, resolved

Anxiety – seen Psychology
No recreational drugs / injecting drug use
Smokes 10/day
Alcohol <4u/week

Male partner – USA (6 years)
Negative HIV test 6/12 ago
Temp 37.5c

GCS (Glasgow Coma Scale) 15, cooperative and fully orientated

BP 150/90     P 88/min

RS
CVS   NAD
GIT
Fundi – NAD     CN II-XII intact

Mild pronator drift LUL

<table>
<thead>
<tr>
<th></th>
<th>RLL</th>
<th>LLL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power</td>
<td>5/5</td>
<td>4/5 hip and knee flexion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4/5 ankle dorsiflexion</td>
</tr>
<tr>
<td>Tone</td>
<td>normal</td>
<td>↑ (sustained ankle clonus)</td>
</tr>
<tr>
<td>Reflexes</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Plantar</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Coordination</td>
<td>normal</td>
<td>normal</td>
</tr>
</tbody>
</table>

sensation – no abnormality found
no fasiculation
no cerebellar signs

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<table>
<thead>
<tr>
<th>Investigation</th>
<th>Value</th>
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<tbody>
<tr>
<td>Hb</td>
<td>14.6</td>
</tr>
<tr>
<td>Cr</td>
<td>80</td>
</tr>
<tr>
<td>Plts</td>
<td>57</td>
</tr>
<tr>
<td>glu</td>
<td>6.2</td>
</tr>
<tr>
<td>WCC</td>
<td>2.7</td>
</tr>
<tr>
<td>ALT</td>
<td>61</td>
</tr>
<tr>
<td>neuts</td>
<td>1.9</td>
</tr>
<tr>
<td>lymph</td>
<td>0.6</td>
</tr>
<tr>
<td>ESR</td>
<td>29</td>
</tr>
<tr>
<td>ECG</td>
<td>88/min SR</td>
</tr>
<tr>
<td>CRP</td>
<td>&lt;5</td>
</tr>
<tr>
<td>CXR</td>
<td>normal</td>
</tr>
<tr>
<td>UA</td>
<td>NAD</td>
</tr>
</tbody>
</table>
Lumbar Puncture

- Opening pressure 12.5 cm H₂O
- 13 WCC (100% mononuclear)
- pro 0.4 g/dL
- India Ink negative
- glu 3.6 (serum 5.2)
- No bacterial / fungal growth
- Viral PCRs (JCV, EBV, HSV, VZV, enterovirus, CMV)
- TB culture
- Syphilis
- Cryptococcal Ag
- Cytology
- Oligoclonal band synthesis (serum weakly +ve)
Other tests

- MR spine – normal signal
- Echocardiogram – normal appearances
- Carotid doppler USS
- Auto-immune / vasculitic screen
  - ANA, ANCA, TFT, B12
Further investigations

Confirmed HIV Ab +ve

HIV VL 361,837 copies/mL
CD4+ 190 cells/μL

HIV genotype – Clade B virus, no significant resistance mutations

Recent HIV seroconversion with neurological manifestations and marked immunosuppression

No evidence of other CNS opportunistic infection
Primary HIV infection and the CNS

Neurological involvement

aseptic meningitis
meningo-encephalitis
encephalitis
radiculopathy
transverse myelitis
mononeuritis multiplex
Primary HIV infection

Outcome:

• self limiting
• ‘glandular fever’
• presents to family doctor/general ‘on-call’ physicians
• HIV rarely thought about
• recent negative HIV-1ab test doesn’t exclude diagnosis
### When to start treatment - British Guidelines 2008

<table>
<thead>
<tr>
<th>Primary HIV infection</th>
<th>AIDS-defining illness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neurological involvement</td>
</tr>
<tr>
<td></td>
<td>Prolonged CD4 &lt; 200 for &gt;3/12</td>
</tr>
<tr>
<td>Chronic HIV infection</td>
<td>CD4 &lt; 350 treat ASAP</td>
</tr>
<tr>
<td></td>
<td>CD4 350-500 consider treatment in specific situations</td>
</tr>
<tr>
<td>AIDS diagnosis</td>
<td>Treat (unless TB and CD4 &gt;350)</td>
</tr>
</tbody>
</table>
What do you commence?

- 2 NRTIs plus an NNRTI
- 2 NRTIs plus a boosted PI
- Other regimen
- Do not commence ART
Progress

Plasma HIV VL

CD4+ cell count

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Clinical Progress

Plasma HIV VL <50 copies/mL  CD4 360
Clinical improvement, returns to work
Residual leg stiffness, worse when tired

Convalescence MR at 3/12
  no progression of pre-central gyrus lesion
Clinical Progress

Plasma HIV VL <50 copies/mL  CD4 450
Neurology stable

Convalescence MR at 6/12
  no progression of pre-central gyrus lesion
  additional diffuse tiny lesions
Seq: IR
Slice: 5 mm
Pos: -27.1973
TR: 9002
TE: 146.816
AC: 1

HFS
FoV: 260 mm
Image no: 5
Image 5 of 27

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Clinical Progress

CSF

WCC 4, protein 0.47g/L
CSF HIV VL 718 copies/mL (plasma HIV VL<50)
No sequence performed as low sample volume
All other tests repeated

Clinical decision to continue ARVs and observe neurology as stable

Would you routinely measure CSF viral load in this situation?

Is it common to find detectable CSF viraemia if pVL < 50 copies/ml?
1221 plasma and CSF pairs
60% previous AIDS / median CD4 nadir 172

<table>
<thead>
<tr>
<th></th>
<th>ON ART (842)</th>
<th>OFF ART (379)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF VL &gt;50</td>
<td>16%</td>
<td>76%</td>
</tr>
<tr>
<td>CSF VL &lt;50</td>
<td>84%</td>
<td>24%</td>
</tr>
</tbody>
</table>

If plasma VL <50, 4% have detectable CSF VL

Level of CSF viral load as such was not predictive
But CSF viral load above or equal to plasma VL had worse NP performance

Letendre S et al. Abstract 172. CROI 2010

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4 months later

CD4 510    plasma HIV VL<50

3/7 new constant temporal headache
feverish
‘vibration’ sensation down shin, subjective altered sensation

worsening left leg weakness
WCC 44 (100% mononuclear)  
pro 0.53 g/dL

Rx ceftriaxone and aciclovir  
Headaches resolved  
TB culture negative

CSF HIV VL  676 copies/mL  (plasma HIV VL<50)  
Resistance test CSF  
M184V and D67E in reverse transcriptase

Tropism on CSF  
Tropism using proviral DNA  
both predict CCR5-using virus
1. On balance, genotypic testing offers a more easily accessible, rapid, & inexpensive method for tropism diagnostics than phenotypic testing, and is therefore the preferred option (IV).

2. Recommended sample for genotypic tropism testing: plasma with VL >500 cps/ml (IIb), proviral DNA with low-level or undetectable VL (IV).

3. Naive patients: Testing may be considered prior to starting first-line HAART, especially if at recognised risk of ARV toxicity (IV).

4. Treated patients with virological failure: tropism and resistance test results should become available at the same time (IV).

5. Treated patients with suppressed VL: Testing can be performed using proviral DNA (III) or the last sample with VL >500 cps/ml (III).


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Management

ARVs changed - according to genotype and theoretical CNS penetration

Switched to abacavir zidovudine lamivudine (TrizivirTM) nevirapine

4 months later – AZT switched to maraviroc because of concerns around lipoatrophy

Plasma HIV VL remains <50 copies/mL
Is it important to use drugs with good CNS penetration?
## CNS penetration-effectiveness (CPE) rank

<table>
<thead>
<tr>
<th></th>
<th>Low (0)</th>
<th>Intermediate (0.5)</th>
<th>High (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical properties</strong></td>
<td>Suggestive of poor penetration</td>
<td>Do not clearly support penetration</td>
<td>Support high penetration</td>
</tr>
<tr>
<td><strong>CSF concentration</strong></td>
<td>Below quantifiable level or &lt; wild-type IC₅₀</td>
<td>Not consistently detectable. Measurable concentrations not consistently exceeding wild-type IC₅₀</td>
<td>Measurable and consistently exceeds wild-type IC₅₀</td>
</tr>
<tr>
<td><strong>Alterations in viral load (VL) or cognition</strong></td>
<td>No reduction in CSF VL and no improvements in cognition</td>
<td>—</td>
<td>Reduction in CSF RNA or improved cognition</td>
</tr>
</tbody>
</table>


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## Summary of CPE 2010 ranking approach

<table>
<thead>
<tr>
<th>NRTIS</th>
<th>NNRTIs</th>
<th>PIs</th>
<th>Fusion/Entry Inhibitors</th>
<th>Integrase inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>AZT</td>
<td>NVP</td>
<td>IDV/r</td>
<td>MVC</td>
<td>RAL</td>
</tr>
<tr>
<td>ABC</td>
<td>DLV</td>
<td>DRV/r</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTC</td>
<td>EVF</td>
<td>FPV/r</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d4T</td>
<td></td>
<td>IDV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ddl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ddC</td>
<td></td>
<td></td>
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CNS penetration and CSF HIV RNA

Reduction in HIV RNA in CSF at 12 weeks ($\log_{10}$ c/mL)

Number of CNS-penetrating drugs

$P = 0.04$


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Survival PML and CNS penetrating cART

Gasnault et al. CROI 2008 abstract 385

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N=12 (CCR5+ HIV-1 adult ARV-experienced patients receiving MVC-containing regimens for at least 1 month)

CSF, semen plus a blood sample were taken around 12 hours after last MVC dose

12 plasma, 12 CSF, and 9 semen samples were collected

Median CD4 count 281 cells/μL (120 to 759); median HIV-1 VL at screening <40 c/mL

Median time on MVC was 13.5 (4 to 60) weeks

Background regimen in 92% RAL, 62% DRV and 42% ETV. Nucleoside analogues were given in only one case

<table>
<thead>
<tr>
<th>N</th>
<th>Plasma (ng/mL)</th>
<th>Plasma VL copies/mL</th>
<th>Semen (ng/mL)</th>
<th>Semen VL copies/mL</th>
<th>CSF (ng/mL)</th>
<th>CSF VL copies/mL</th>
<th>Semen: Plasma</th>
<th>CSF: Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>99.5</td>
<td>&lt;40</td>
<td>150</td>
<td>&lt;40</td>
<td>3.42</td>
<td>&lt;40</td>
<td>1.508</td>
<td>0.034</td>
</tr>
<tr>
<td>2</td>
<td>159</td>
<td>&lt;40</td>
<td>38.8</td>
<td>&lt;40</td>
<td>2.63</td>
<td>&lt;40</td>
<td>0.244</td>
<td>0.017</td>
</tr>
<tr>
<td>3</td>
<td>60.2</td>
<td>&lt;40</td>
<td>–</td>
<td>–</td>
<td>5.72</td>
<td>&lt;40</td>
<td>–</td>
<td>0.095</td>
</tr>
<tr>
<td>4</td>
<td>263</td>
<td>1777</td>
<td>1170</td>
<td>&lt;40</td>
<td>1.08</td>
<td>111</td>
<td>4.449</td>
<td>0.004</td>
</tr>
<tr>
<td>5</td>
<td>31.7</td>
<td>&lt;40</td>
<td>15.8</td>
<td>1926**</td>
<td>0.5</td>
<td>&lt;40</td>
<td>0.498</td>
<td>0.016</td>
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<tr>
<td>6</td>
<td>220</td>
<td>&lt;40</td>
<td>197</td>
<td>–</td>
<td>2.28</td>
<td>&lt;40</td>
<td>0.895</td>
<td>0.010</td>
</tr>
<tr>
<td>7</td>
<td>7.34</td>
<td>&lt;40</td>
<td>–</td>
<td>–</td>
<td>1.27</td>
<td>&lt;40</td>
<td>–</td>
<td>0.173</td>
</tr>
<tr>
<td>8</td>
<td>517</td>
<td>&lt;40</td>
<td>288</td>
<td>–</td>
<td>3.39</td>
<td>&lt;40</td>
<td>0.557</td>
<td>0.007</td>
</tr>
<tr>
<td>8*</td>
<td>–</td>
<td>213001</td>
<td>–</td>
<td>26276</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>184</td>
<td>202</td>
<td>133</td>
<td>&lt;40</td>
<td>3.48</td>
<td>45</td>
<td>0.723</td>
<td>0.019</td>
</tr>
<tr>
<td>10</td>
<td>93.5</td>
<td>&lt;40</td>
<td>66.5</td>
<td>360**</td>
<td>2.29</td>
<td>&lt;40</td>
<td>0.711</td>
<td>0.024</td>
</tr>
<tr>
<td>11</td>
<td>72.9</td>
<td>99</td>
<td>213</td>
<td>&lt;40</td>
<td>2.54</td>
<td>111</td>
<td>2.922</td>
<td>0.035</td>
</tr>
<tr>
<td>12</td>
<td>150</td>
<td>&lt;40</td>
<td>–</td>
<td>–</td>
<td>7.22</td>
<td>&lt;40</td>
<td>–</td>
<td>0.048</td>
</tr>
<tr>
<td>Median (range)</td>
<td>124.75 (7.3 to 517)</td>
<td>&lt;40</td>
<td>150 (15.8 to 1170)</td>
<td>&lt;40</td>
<td>2.585 (0.5 to 7.22)</td>
<td>&lt;40</td>
<td>0.723 (0.244 to 4.449)</td>
<td>0.022 (0.004 to 0.173)</td>
</tr>
</tbody>
</table>

Patient 8 brought the semen sample several weeks later; he had stopped ARV and a new blood sample was obtained.

** Patient 5 had been on MRV-regimen for 4 weeks, and patient 10, for 58 weeks

Tiraboschi et al, Abstract 612 CROI 2010
Progress

Plasma HIV VL

CSF VL 718

CSF VL 676

CSF VL <50

CD4+ cell count

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Currently

Neurology clinically stable

Repeat CSF

WCC 1
Pro 0.43g/dL
CSF HIV VL <50

MR appearances unchanged to date
33-year old British MSM

Recent HIV seroconversion presenting with neurological symptoms
No CNS opportunistic infection found

Demonstrable ongoing CNS HIV replication despite suppression of plasma compartment

Consider CSF HIV RNA quantification and genotype in an individual with neurological symptoms, irrespective of plasma HIV RNA level
Acknowledgements:
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Dr Steve Kaye

Thank you