Standardisation of HIV-1 Neutralisation:
Provision of Reagents to NeutNet

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Programme EVA
Centre for AIDS Reagents (CFAR)

NIBSC
UK
Provision of Reagents to NeutNet: Programme EVA Centre for AIDS Reagents

- Programme EVA CFAR established in 1989 by MRC and EC

- Currently supported by:
  - EC FP6
    - Europrise – New FP6 Network of Excellence – member of consortium
    - AIDS Vaccine Integrated Project (AVIP) – member of consortium
    - NeutNet Specific Support Action
  - Bill and Melinda Gates Foundation – Collaboration for AIDS Vaccine Discovery
    - Member of Global HIV Vaccine Cryorepository consortium

- Primary repository for WHO-UNAIDS HIV Characterisation Network

- We collaborate closely with NIH AIDS Research and Reagent Project

- Focus on supply of reagents for AIDS vaccine development
  - Some 2000 different reagents available from CFAR on our web-site
  - Over 80,000 reagents distributed worldwide since CFAR started operating

- Provides standards and reference reagents developed at NIBSC
Biological Reference Materials

• Biological standards and reference materials important for standardisation, quality control and potency estimation of medicinal products such as vaccines

• NIBSC plays leading role in producing International Biological Standards – produces over 95% of WHO standards

• Various classes of biological standard/reference reagent:
  – WHO International Standard – assigned value in International Units (IU)
  – WHO International Reference Reagent – may be interim standards
  – WHO Reference Panel – Number of samples eg genotypes, serotypes etc
  – National Standards – primarily for regional use eg BWS for anti-HIV-1
  – Working Standards / reagents
    • Calibrated in IUs against International Standard
    • May be used as run control or ‘go/no-go’ control
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• Wide range of neutralisation assays in use - No agreement as to which assay(s) should be used

• Many variables including cell type used, virus used, number of virus cycles, endpoint etc

• In phase 1 NeutNet attempted to reduce variables by providing:
  – Panel of 12 viruses representing different subtypes, neutralisation sensitivities and co-receptor usage
  – Corresponding panel of env clones
  – Panel of monoclonal antibodies and sCD4
    • TriMab: consists of 2F5, 2G12, b12 (1mg/ml)
    • 447-52D (1mg/ml)
    • 4E10 (1mg/ml)
    • sCD4

• Facilitate the comparison and evaluation of assays

• Help to select suitable assays and reagents for next phase
<table>
<thead>
<tr>
<th>Subtype</th>
<th>Virus code</th>
<th>Virus phenotype</th>
<th>Neutralisation sensitivity</th>
<th>Name of clone*</th>
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<tbody>
<tr>
<td>A</td>
<td>92RW009 ARP178.2</td>
<td>R5</td>
<td>Sensitive</td>
<td>p92RW009.6 ARP2068</td>
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<tr>
<td>A</td>
<td>VI 191 ARP1042</td>
<td>R5</td>
<td>Sensitive</td>
<td>pVI191 cl68 ARP2055</td>
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<tr>
<td>B</td>
<td>SF162 ARP113</td>
<td>R5</td>
<td>Sensitive</td>
<td>pCAGGS SF162 gp160 ARP2125 (NIH10463)</td>
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<tr>
<td>B</td>
<td>MN(P) ARP1048</td>
<td>X4</td>
<td>Sensitive</td>
<td>MNP.ec3 ARP2054</td>
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<tr>
<td>C</td>
<td>DU174 ARP1049</td>
<td>R5</td>
<td>Moderately Sensitive</td>
<td>DU174.15 ARP2052</td>
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<tr>
<td>C</td>
<td>92BR025 ARP179.11</td>
<td>R5</td>
<td>Moderately Sensitive</td>
<td>p92BR025.9 ARP2067 (NIH3083)</td>
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<tr>
<td>D</td>
<td>92UG024 ARP177.4</td>
<td>X4</td>
<td>Moderately Resistant</td>
<td>p92UG024.2 ARP2008 (NIH3107)</td>
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<tr>
<td>A/E CRF_01</td>
<td>CM244 ARP1050</td>
<td>R5</td>
<td>Resistant</td>
<td>CM244.ec1 ARP2053</td>
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<tr>
<td>A/E CRF_01</td>
<td>NP1525 ARP1051</td>
<td>X4</td>
<td>Moderately Sensitive</td>
<td>-</td>
</tr>
<tr>
<td>B (US)</td>
<td>QH0692 ARP1052</td>
<td>R5</td>
<td>Sensitive</td>
<td>QH0692.42 ARP2043 (NIH11018)</td>
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<tr>
<td>B (US)</td>
<td>-</td>
<td>R5</td>
<td>Resistant</td>
<td>CAAN5342.A2 ARP2045 (NIH11038)</td>
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<tr>
<td>B (US)</td>
<td>AC10 ARP1053</td>
<td>R5</td>
<td>Moderately Sensitive</td>
<td>AC10.0.29 ARP2044 (NIH11024)</td>
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<tr>
<td>Total</td>
<td>11 viruses</td>
<td></td>
<td></td>
<td>11 clones</td>
</tr>
</tbody>
</table>
Comparison of TriMab from NIBSC CFAR with TriMab from Duke

Results courtesy of David Montefiori, Duke University

**TriMab: Mixture of three purified MoAbs:**

2F5, 2G12, b12 diluted in PBS to 1mg/ml
(333_g/ml of each antibody)

<table>
<thead>
<tr>
<th>Reagent Name</th>
<th>Reagent Name</th>
<th>SF162. LS</th>
<th>QH0692. 42</th>
<th>AC10.0. 29</th>
<th>CAAN5342. A2</th>
<th>Du174. 15</th>
<th>CM244. ecl</th>
<th>MNP. ec3</th>
<th>V1 191</th>
<th>92BR025 .9</th>
<th>92UG024 .2</th>
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<tbody>
<tr>
<td>TriMab</td>
<td>ARP3240</td>
<td>0.06</td>
<td>0.80</td>
<td>1.75</td>
<td>15.1</td>
<td>1.96</td>
<td>5.30</td>
<td>0.11</td>
<td>4.17</td>
<td>4.37</td>
<td>0.20</td>
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<tr>
<td>TriMab</td>
<td>Duke</td>
<td>0.08</td>
<td>0.69</td>
<td>1.32</td>
<td>13.4</td>
<td>1.07</td>
<td>6.54</td>
<td>0.17</td>
<td>4.66</td>
<td>4.59</td>
<td>0.06</td>
</tr>
</tbody>
</table>

ID50 in TZM-bl cells (mean of Dataset #1 & #2)
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- Viruses, clones and sCD4 stored frozen and shipped on dry ice
- MoAbs stored unfrozen at 4°C and shipped at ambient temperature
- Concern expressed that 447-52D may be less stable if stored or shipped unfrozen
- Checked MoAbs by SDS-PAGE electrophoresis and HPLC
SDS-PAGE analysis of MoAbs

4-12% SDS-PAGE – non-reduced

Reference = Octagam/Vigam (human polyclonal)

MoAbs contain mainly intact (~160Kd) immunoglobulin, with small amounts of fragments
HPLC Analysis of TriMab and Components

- CFAR 2G12 Mab
- CFAR 2F5 Mab
- CFAR b12 Mab
- ARP3240 TriMab (2F5/2G12/b12)
HPLC Analysis of MoAbs 447D-52D and 4E10
Reagents for Next Phase of NeutNet

• Virus/clone panel
  – Reduced number of panel members proposed
  – 7 selected, A, 3xB, C, D, CRF_01
  – Selected clones that have good growth characteristics
  – Growth in PBMCs to be confirmed by all groups

• Proficiency panel to include both monoclonal and polyclonal reagents

• Monoclonal antibodies
  – Trimab-FD (300 ampoules freeze-dried at NIBSC and shown to be comparable with Duke version)
  – Other Mabs being considered eg M9, broad V3
  – Negative Mab control

• Polyclonal human antibody preparations
Reagents for Next Phase of NeutNet

• Human polyclonal antibody preparations available to NeutNet:
  – HIV+ human plasma from Zeptometrix, USA
    • 10 batches screened for use by NeutNet by T Wrin, V Polonis and D Montefiori
    • Three being acquired for NeutNet use (one donated by HJF)
  – 10 HIV+ plasma samples (~200ml) recently acquired by NIBSC – from UK transfusion centres collected in 2005-2006
    • To be screened for HIV-neutralising activity
  – Selected plasma from WHO/UNAIDS virus network to be screened for neutralising activity
  – Negative plasma as control available from NIBSC
  – Selected plasma samples to be included in proficiency panel
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  - S Goriup / M Hurley, Programme EVA CFAR
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  - Dr Carl Dolman, NIBSC for HPLC analysis
  - Dr P Matjeschuk, NIBSC, for freeze-drying TriMab
  - Dr D Katinger, Polymun, Austria for 2F5, 2G12 and 4E10 MoAbs
  - Dr D Burton, Scripps Research Institute, USA for b12 MoAb
  - Dr S Zolla-Pazner, NYUMC, USA for 447-52D MoAb
  - Dr Franti, Progenics, USA for sCD4