CBER update and International Collaboration for development of HIV variant panels

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DETTD/CBER/FDA
XXII SoGAT meeting
HIV genetic diversity: subtypes and homology

HIV-1:
- M
- A
- B
- C
- D
- E
- F
- G
- H
- I
- J
- O
- N

HIV-2:
- A
- B

SIV:
- cpz
- mac/SM

Homology percentages:
- HIV-1 M to HIV-1 A: 70%
- HIV-1 M to HIV-1 B: 50%
- HIV-1 M to HIV-1 C: 60%
- HIV-1 O to HIV-1 N: 60%
- HIV-2 A to HIV-2 B: 70%
- SIV cpz to SIV mac/SM: 75%
Genetic diversity of HIV

- Two major types:
  - HIV-1 and HIV-2;
  - 4 HIV-1 groups: M (major), O (outlier), N (non-M, non-O), P
- Group M has multiple subtypes (A-H) and up to 50 Circulating Recombinant Forms (CRFs)
- HIV-2 has 5 major subtypes – A and B are most prevalent
- HIV diversity is due to:
  - High error rate of HIV RT and absence of proof reading mechanisms
  - Rapid viral turnover ($10^{10}$ viral particles/day) in infected individuals
  - Zoonotic transmission characteristic of lentiviruses
Evolution of HIV-1 Genetic Variants

Group O

1983

1988

Variation – HIV-2

1992

Subtypes

1993

URF

1995

Recombination

1998

Nomenclature

2001

CRF

2003

Co-infection, dual- & super-infections

2004

SGRs

2nd gen. recombinant

Up to 50 CRFs have been identified
Worldwide distribution of predominant HIV-1 group M subtypes and CRFs

North and Central America: B

South America: B, F1, CRF12_BF

Western Europe: B, A, C, G, CRF03_AB

Eastern Europe: A, CRF07_BC, CRF55_BC

China: B, CRF07_BC, CRF55_BC

Southeast Asia: B, CRF01_AB

East Africa: A, D, C

Western Africa: CRF03_AB, A, G

Central Africa: Most CRFs: A, C, D, G, H, J, K, O, N

South Africa: C

Australia: B
Impact of HIV genetic diversity on diagnosis


- Zouhair, S., et al. JCM, (2006), Group O HIV-1 infection that escaped detection in two immunoassays


CBER HIV panel development

- Project initiated in Cameroon, region of high HIV diversity to study virus evolution and have access to new strains for panel development.

Study Goals:
- Collect specimens, viruses; genotyping and virus tropism
- Evaluate sensitivity of blood screening and diagnostic tests for ability to detect diverse strains
- Identify new strains for future use as reference reagents
CBER HIV-1 RNA genotype panel

- HIV-1 RNA genotype panel currently in use for testing.
- Panel consists of one primary isolate each of group M subtypes A-G and groups O and N cultured in PBMC and characterized by sequencing.
- Virus isolates are inactivated by heat treatment at 60°C for 60 mins.
- Panel composed of 3 members of each subtype at $10^3$, $10^4$ and $10^5$ copies/ml spiked in negative plasma.
Seven isolates of HIV-2 subtype A were cultured in PBMC cultures and characterized by partial sequencing.

Viruses were inactivated by heat treatment (60 C/60 minutes and spiked into negative plasma.

Testing was performed in 3 laboratories.

Results from labs were in good agreement.

HIV-2 panel was formulated at 5, 10, 50, 100 copies/ml and is currently in use for lot release testing of HIV-2 NAT assays.
<table>
<thead>
<tr>
<th>Isolate ID</th>
<th>Manufacturer A</th>
<th>Manufacturer B</th>
<th>Manufacturer C</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
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<tbody>
<tr>
<td>B2</td>
<td>8.7782</td>
<td>8.4116</td>
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Emerging new HIV strains are circulating recombinant forms (CRFs)

CRF02_AG and CRF01_AE are currently the most prevalent strains; need for standards

Five primary virus isolates each of CRF02_AG and CRF01_AE cultured in PBMCs were characterized by full genome sequencing

Heat inactivated virus isolates spiked into negative plasma were tested in five laboratories

Panel consists of 3 members for each isolate spiked in negative plasma at $10^3$, $10^4$ and $10^5$ copies/ml (log)
# CRF01_AE and CRF02_AG Isolate Testing Summary

<table>
<thead>
<tr>
<th>Isolate ID</th>
<th>Lab A</th>
<th>Lab B</th>
<th>Lab C</th>
<th>Lab D</th>
<th>Lab E</th>
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<th>SD</th>
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<tr>
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<td>AE-2</td>
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<td>8.84</td>
<td>8.51</td>
<td>8.31</td>
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<td>8.64</td>
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<td>AE-3</td>
<td>8.70</td>
<td>8.69</td>
<td>8.34</td>
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<td>AE-9</td>
<td>8.92</td>
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<td>AE-10</td>
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<td>8.61</td>
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</tr>
<tr>
<td>NYU 360</td>
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<td>9.59</td>
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<td>8.73</td>
<td>9.21</td>
<td>9.0</td>
<td>0.36</td>
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</table>
CBER obtained viruses from NIBSC, UK and Carlos de Salud institute, Spain and in-house project in Cameroon.

Viruses were cultured in PBMC to high titers.

Viral stocks were heat-treated to inactivate the virus, no loss of RNA copy number.

Aliquots were sent to multiple collaborating labs for copy number determination.

Data analysis ongoing; final formulation expected in the near future.
### B/C – B/F Isolate Titer Testing Summary \((\log^{10})\)

<table>
<thead>
<tr>
<th>Isolate ID</th>
<th>Lab A</th>
<th>Lab B</th>
<th>Lab C</th>
<th>Mean</th>
<th>Standard Deviation</th>
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<tr>
<td>P1942</td>
<td>9.11</td>
<td>9.09</td>
<td>8.81</td>
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<td>92023</td>
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<td>8.93</td>
<td>0.22</td>
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<tr>
<td>X531-2</td>
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<td>8.29</td>
<td>7.92</td>
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<td>0.17</td>
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<td>8.78</td>
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<td>2457-2</td>
<td>9.16</td>
<td>9.54</td>
<td>8.93</td>
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<tr>
<td>475-2/754-2</td>
<td>8.93</td>
<td>9.21</td>
<td>8.66</td>
<td>8.93</td>
<td>0.22</td>
</tr>
</tbody>
</table>
NIAID Global HIV Viral Panels Project: Purpose

To establish a set of fully characterized viruses from early acute HIV infections that are consistent with the degree of viral evolution present globally, for

- Developing new assays
- Validating assay platforms
- Assisting regulators to evaluate test kits
- Monitoring HIV drug resistance
- Informing vaccine development
HIV Viral Panels Project Requirements

- Well characterized HIV reference panels encompassing epidemic
- Full length single genome sequencing
- Verified RNA concentration
- Fiebig staging and serological profiles for current assays/platforms including rapid POC assays
- Comparisons of VL from different FDA approved commercially available platforms
- Panels with larger volumes for use on newer diagnostic platforms
HIV Viral Panels: Pilot study

Phase I (Feasibility)
- 20 pre or very early post-SC plasma units
  - South Africa – M. Vermeulen (SANBS)
  - USA – S. Stramer (ARC)
  - Brazil – E. Sabino (REDS II)
  - Cameroon – I. Hewlett (FDA)
- Standardize assays and validated protocols

Phase II (Expansion)
- DAIDS EQAPOL awarded to Duke and started FY11 (7 year contract)
- Dynamic 60 member panels: updated and rebalanced as epidemic evolves
- Focus on window phase and recent seroconversions
- HIV reference stocks housed within four panels:
  1. Major subtypes/CRF (Group M)
  2. Emerging strains (rare subtypes/CRF_cpx, Group N, O, HIV-2)
  3. Challenging strains (VL assay variation)
  4. Subtype representation from diverse geographical locations
## Tier 1 Isolates

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Region of Interest</th>
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<tbody>
<tr>
<td>A1</td>
<td>Uganda, Rwanda, former Soviet Republics (IDU)</td>
</tr>
<tr>
<td>B</td>
<td>North America, Western Europe, Australia, Western South America</td>
</tr>
<tr>
<td>C</td>
<td>South Africa, Botswana, Zambia, Malawi, Tanzania, Ethiopia, India, Southern Brazil</td>
</tr>
<tr>
<td>D</td>
<td>Uganda</td>
</tr>
<tr>
<td>G</td>
<td>Nigeria, Spain (IDU), Portugal (IDU)</td>
</tr>
<tr>
<td>CRF01_AE</td>
<td>Thailand, Vietnam, Cambodia</td>
</tr>
<tr>
<td>CRF02_AG</td>
<td>Senegal, Nigeria, Ghana, Cote d’Ivoire, Cameroon</td>
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## Tier 2 Isolates

<table>
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<th>Subtype</th>
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<td>Brazil, Romania, Spain</td>
</tr>
<tr>
<td>F2</td>
<td>Cameroon</td>
</tr>
<tr>
<td>H, J, K</td>
<td>DRC, Cameroon, Congo</td>
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<tr>
<td>CRF04_cpx (A,G,H,K,U)</td>
<td>Cyprus, Greece</td>
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<tr>
<td>CRF05_DF</td>
<td>DRC, Belgium</td>
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<td>CRF06_cpx (A,G,J,K)</td>
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<tr>
<td>CRF07_BC</td>
<td>China</td>
</tr>
<tr>
<td>CRF08_BC</td>
<td>China</td>
</tr>
<tr>
<td>CRF09_cpx (02,A,U)</td>
<td>Cote d’Ivoire, Mali</td>
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<tr>
<td>CRF11_cpx (A,G,01,J)</td>
<td>Cameroon, DRC, CAR</td>
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<tr>
<td>CRF12_BF</td>
<td>Argentina</td>
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<tr>
<td>CRF13_cpx (A,01,11,G,J,U)</td>
<td>Cameroon, CAR</td>
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<tr>
<td>CRF14_BG</td>
<td>Spain, Portugal</td>
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<tr>
<td>CRF18_cpx (A,F,G,H,K,U)</td>
<td>Cuba</td>
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<td>CRF20_BG, CRF23_BG, CRF24_BG</td>
<td>Cuba</td>
</tr>
<tr>
<td>CRF31</td>
<td>Brazil</td>
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</table>
1. Goal to complete a pilot study before end of FY2010
   - 20 pre or very early post-SC plasma units from 4 countries (US, SA, Brazil, Cameroon)
   - Additional European countries added mid year
   - Obtain country support and resolve IRB issues and logistical challenges; standardize procedures

2. Accomplishments
   - Identified needed strains and geographic locations
   - Partnering with different groups to collaborate and pool resources
   - Procured, isolated, propagated and characterized over 30 isolates
   - Continued support from EQAPOL (additional 7 years)
Viral Isolation Summary

- Samples (L. Hewlett, M. Ramaswamy, E. Brojer, K. Roth, S. Laperche)
- Lab analysis by M. Manak, B. Coombs, T. Denny
- Repository at SeraCare (M. Manak and P. Garrett)

Success rate

<table>
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<th>Fleibig Stage</th>
<th>Pos</th>
<th>Attempt</th>
<th>% Positive</th>
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<td>33</td>
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</tr>
<tr>
<td>II</td>
<td>8</td>
<td>12</td>
<td>67%</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>4</td>
<td>75%</td>
</tr>
<tr>
<td>IV</td>
<td>2</td>
<td>7</td>
<td>29%</td>
</tr>
<tr>
<td>V</td>
<td>9</td>
<td>15</td>
<td>60%</td>
</tr>
<tr>
<td>VI</td>
<td>4</td>
<td>12</td>
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</tr>
<tr>
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<td>5</td>
<td>15</td>
<td>33%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>33</td>
<td>98</td>
<td>34%</td>
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Viral Load

<table>
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<td>27</td>
<td>0%</td>
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<tr>
<td>1,000-10,000</td>
<td>1</td>
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<td>7%</td>
</tr>
<tr>
<td>10,000-100,000</td>
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<td>41%</td>
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<tr>
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<td>23</td>
<td>70%</td>
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<tr>
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</tr>
<tr>
<td>NT</td>
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<td>43%</td>
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<tr>
<td><strong>Total</strong></td>
<td>33</td>
<td>98</td>
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HIV Viral Panels: Plasma summary

Obtained Plasma from early stage HIV infection

- Plasma centers in US, S Africa, and Brazil
  - Large vol (~100 ml) back up available
- Collaborations for rare subtypes
  - Greece (NIBSC), Cameroon (FDA)
- Collaborations with other countries:
  - Poland, Germany, France
  - More (Spain, Thailand, India, Egypt, Japan) coming on board

Plasma Characterization

- Fiebig Stage assignment
  - Viral Load
  - p24 Antigen
  - EIA and Western blot
- Full Length Genome Sequencing
- Virus Infectivity/Virus Isolation
HIV Viral Panels: Cultured Virus Summary

Optimized virus Isolation conditions from plasma

- Clarification by ultracentrifugation
- Infection of CD8-depleted PHA stimulated donor PBMC
- Success rate correlates with VL (65% success for VL >30,000 cp/ml)

Standardized Virus Expansion

- Maximize viral yield at low passage number
- Expand in PBMC to 100 ml; Most at >10^8 copies/ml

Characterization of Expanded Virus

- Subtype Designation based on Full Length sequencing
- Confirm identify to original plasma
- Co-receptor usage (CCR5/CXCR4)
- Viral Load, p24 antigen, Infectivity

Consistent source of High Titer HIV isolates

- Comparison of quantitative NAT assays
- Infectivity studies for Drug and Vaccine design/evaluation
Acknowledgments

CBER
Owen Wood
Sherwin Lee
Ragupathy Viswanath
Jiangqin Zhao
Shixing Tang
Siva Gaddam
Stephen Kerby

Supported by
NHLBI IAG- Y1-HB-5026-01
NIAID IAA -Y1-A1-9447-02
CBER Critical Path Funds

NYU/Cameroon
Phillipe Nyambi
Denis Barengolts

Panel Testing Participants

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Mark Manak
Sodsai Stovanabutra
CHAVI group