<table>
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<tr>
<th><strong>Title</strong></th>
<th>A Novel Methodology for Isolating Broadly Neutralizing HIV-1 Human Monoclonal Antibodies</th>
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<tr>
<td><strong>Author(s)</strong></td>
<td>Sun, Z; Li, J; Shao, Y; Zhang, M</td>
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<tr>
<td><strong>Citation</strong></td>
<td>The 2013 AIDS Vaccine Conference, Barcelona, Spain, 7-10 October 2013. In Abstracts book, 2013, p. 126, abstract no. P03.34</td>
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<td><strong>Issued Date</strong></td>
<td>2013</td>
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AIDS Vaccine 2013
Progress, Partnership and Perseverance
7-10 October | www.vaccineenterprise.org/conference/2013
### AIDS Vaccine 2013 Program at a Glance

**Monday, 7 October**

<table>
<thead>
<tr>
<th>Satellite Sessions</th>
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<tbody>
<tr>
<td>Catalyzing a Health Innovation System that Works for All</td>
<td>08:30 - 10:30</td>
</tr>
<tr>
<td>Supporting HIV Vaccine R&amp;D Using the CHVI R&amp;D Alliance</td>
<td>08:30 - 10:30</td>
</tr>
<tr>
<td>Virtual Community</td>
<td>08:30 - 10:30</td>
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<tr>
<td>Antibody and NK Recognition of HIV-1 Infected Cells</td>
<td>08:30 - 11:30</td>
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<tr>
<td>Speaking Science to the Public</td>
<td>09:00 - 10:30</td>
</tr>
<tr>
<td>Major Battles in HIV Vaccine Development: The Light at the End of the Tunnel</td>
<td>10:30 - 13:30</td>
</tr>
<tr>
<td>Capturing Participant Information for Mucosal Sampling: An Investigator’s Guide</td>
<td>11:00 - 12:15</td>
</tr>
<tr>
<td>Structure and Antigenicity of Soluble, Cleaved (SDSIP) HIV-1 gp140 Trimmers</td>
<td>12:00 - 15:00</td>
</tr>
</tbody>
</table>

**Tuesday, 8 October**

| Plenary 01 | 08:30 - 10:00 |
| Plenary 02 | 08:30 - 10:00 |
| Plenary 03 | 08:30 - 10:00 |
| Plenary 04 | 10:00 - 10:30 |
| Poster Session 01 and Reception | 17:30 to 19:00 |

| HIV Vaccine Strategies | |
|------------------------||

**Wednesday, 9 October**

<table>
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<tr>
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<th>Plenary 02</th>
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</thead>
<tbody>
<tr>
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<tr>
<td>Plenary 04</td>
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<tr>
<td>Poster Session 02 and Reception</td>
<td>17:30 to 19:00</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Innovative HIV Vaccine Discovery</th>
<th>Plenary 03</th>
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</thead>
</table>

**Thursday, 10 October**

<table>
<thead>
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<th>Emerging Clinical Trial Data</th>
<th>Plenary 04</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poster Session 03 and Reception</td>
<td>17:30 to 19:00</td>
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<table>
<thead>
<tr>
<th>Satellite Sessions</th>
<th>15:00 - 17:00</th>
</tr>
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<tbody>
<tr>
<td>Meta-Analysis of HVTN Efficacy Studies with Adenovirus Vectors</td>
<td>15:00 - 17:00</td>
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</tbody>
</table>

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### Plenaries

- **Plenary 01: Satellite Sessions**
  - 08:30 - 10:00
  - Location: LL Room 113 - 116
  - Chairs: Brigitte Autran, George Pavlakis
  - Speakers: Michael Penson, Andrew McMichael, Barton Haynes

- **Plenary 02: HIV Vaccine Strategies**
  - 08:30 - 10:00
  - Location: LL Room 113 - 116
  - Chair: Nicole Bernard
  - Speakers: Robert Kauf, Filip Goodf, Penelope MacK

- **Plenary 03: Catalyzing a Health Innovation System that Works for All**
  - 08:30 - 10:00
  - Location: LL Room 113 - 116
  - Chairs: José Esparrza, Peggy Johnston
  - Speakers: Michael Penson, Andrew McMichael, Barton Haynes

- **Plenary 04: Protecting from Infection & Disease Progression**
  - 08:30 - 10:00
  - Location: LL Room 113 - 116
  - Chairs: Nicole Bernard, Christian Brander
  - Speakers: Robert Kauf, Filip Goodf, Penelope MacK

---

### Satellite Sessions

- **Animal Models**
  - 13:00 - 13:30
  - Location: LL Room 113 - 116
  - Chair: Anna Angle

- **Clinical Trial**
  - 13:00 - 13:30
  - Location: LL Room 113 - 116
  - Chair: José Esparrza, 2013 Chairs

- **HIV Transmission**
  - 10:00 - 10:30
  - Location: LL Room 113 - 114
  - Chair: José Esparrza, 2013 Chairs

- **Innovations in HIV Vaccine Discovery**
  - 08:30 - 10:00
  - Location: LL Room 113 - 116
  - Chair: Brigitte Autran, George Pavlakis
  - Speakers: María José Alonso, Behzade Combadier, Adrian Hill

- **Strategic Directions for Therapeutic HIV Vaccine Research**
  - 14:00 - 15:00
  - Location: LL Room 113 - 116
  - Chair: José Esparrza, 2013 Chairs

- **Protecting from Infection & Disease Progression**
  - 08:30 - 10:00
  - Location: LL Room 113 - 116
  - Chair: Nicole Bernard
  - Speakers: Robert Kauf, Filip Goodf, Penelope MacK

- **Enabling Technologies and Methods for HIV Vaccine Research and Clinical Trials**
  - 13:00 - 15:30
  - Location: LL Room 114
  - Chair: José Esparrza, 2013 Chairs

- **AIDS Vaccine Research at the Crossroads: How to Adapt to a New Prevention Agenda**
  - 13:00 - 15:30
  - Location: LL Room 127 - 128

- **From Bench to Clinical: A Researcher’s Guide to Move Vaccine Candidates into Trials**
  - 12:00 - 13:30
  - Location: LL Room 130

- **New Targets from Acute Infection**
  - 10:00 - 11:00
  - Location: LL Room 113 - 114
  - Chair: José Esparrza, 2013 Chairs

- **Humoral Immunity**
  - 10:00 - 11:00
  - Location: LL Room 113 - 114
  - Chair: José Esparrza, 2013 Chairs

- **Clinical Trial Follow-up and Retention**
  - 10:00 - 11:00
  - Location: LL Room 113 - 114
  - Chair: José Esparrza, 2013 Chairs

- **Strategic Directions for Therapeutic HIV Vaccine Research**
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- **Clinical Trial Follow-up and Retention**
  - 10:00 - 11:00
  - Location: LL Room 113 - 114
  - Chair: José Esparrza, 2013 Chairs

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### Poster Sessions

- **Poster Session 01 and Reception**
  - 17:30 to 19:00
  - Location: LL Room 113 - 116
  - Odd-numbered posters will be presented.

- **Poster Session 02 and Reception**
  - 17:30 to 19:00
  - Location: LL Room 113 - 116
  - Even-numbered posters will be presented.
Abstracts will be published as an online supplement in AIDS Research and Human Retroviruses. Abstracts will be open access at www.liebertpub.com/aid at the conclusion of the conference on Thursday, 10 October.
Please Note: The views expressed in written conference materials or publications and by speakers and moderators at HHS-sponsored conferences do not necessarily reflect the official policies of the Department of Health and Human Services (HHS), nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

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AIDS Vaccine 2013 is proud to introduce this year’s New Investigator Awardees.

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**Jeanette Reece**  
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**Yanqin Ren**  
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**Justen Hoffman Russell**  
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**Wiriya Rutvisutitunnut**  
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**Natasha Samsunder**  
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**Louise Scharf**  
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**Lucas Schifanella**  
National Institutes of Health, National Cancer Institute, USA

**Torben Schiffler**  
University of Oxford, UK

**Veronika Schmid**  
University of Regensburg, Germany

**Sayuri Seki**  
National Institute of Infectious Diseases, Tokyo, Japan

**Liang Shang**  
University of Minnesota, USA

**Xiaoying Shen**  
Duke University, USA

**Aida Sivro**  
University of Manitoba, Canada

**Kate Snyder**  
Desmond Tutu HIV Foundation/UCT, South Africa

**Devin Sok**  
The Scripps Research Institute, USA

**Aloysious Ssemaganda**  
UVRI-IAVI HIV Vaccine Program, Uganda

**Deogratius Ssemwang**  
MRC/UVRI Uganda Research Unit on AIDS, Uganda

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University of Washington, USA

**Yada Tansiri**  
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**Nancy Tumba**  
HIV/STI Centre - NICD, South Africa

**Humberto Valenzuela Ponce**  
Instituto Nacional de Enfermedades Respiratorias, Mexico

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**Constantinos Wibmer**  
National Institute for Communicable Diseases of the National Health Laboratory Services and the University of the Witwatersrand, South Africa

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The University of Hong Kong, Hong Kong

Local Scholars

**Vanessa Bach**  
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**Judith Dalmau**  
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**Amanda Fabra**  
IDIBAPS, Barcelona, Spain

**María Florencia Etcheverry**  
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**Patricia García de Olalla**  
Agència de Salut Pública de Barcelona, Spain

**Nuria Gonzalez**  
Instituto de Salud Carlos III, Spain

**Nuria Izquierdo-Useros**  
IrsiCaixa Institute for AIDS Research, Spain

**Esther Jimenez**  
IrsiCaixa Institute for AIDS Research, Spain

**Joan Joseph**  
Hospital Clinic, IDIBAPS, HIVACAT, Spain


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Agència de Salut Pública de Catalunya, Spain

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IrsiCaixa Institute for AIDS Research, Spain

Michael Meulbroek  
Projecte dels NOMS-Hispanosida, Spain

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Jorge Palacio  
Fedelatina, Spain

Susana Perez-Alvarez  
IrsiCaixa Institute for AIDS Research, HIVACAT, Spain

Maria Pino  
IrsiCaixa Institute for AIDS Research, HIVACAT, Spain

Julia G. Prado  
IrsiCaixa Institute for AIDS Research, HIVACAT, Spain

Maria Puertas  
IrsiCaixa Institute for AIDS Research, HIVACAT, Spain

Joan Romeu  
HIV Unit, Hospital Germans Trias i Pujol, Spain

Sonsoles Sánchez-Palomino  
IDIBAPS, Spain

Narcís Saubi  
Hospital Clinic, IDIBAPS, HIVACAT, Spain
### Monday, 7 October

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<thead>
<tr>
<th>Time</th>
<th>Event</th>
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| 08:30 – 15:30 | **Satellite Sessions**  
See pages 11–15 of Program Book for detailed information. |                |
| 08:00 – 20:00 | **Registration / Coat Check** | L0, Entrance Hall |
| 13:00 – 16:00 | **Speaker Check-in** | L1, Room 118 |
| 16:00 – 18:00 | **Opening Session**  
Chairs: Christian Brander, Bonaventura Clotet, and José M. Gatell | L1, Room 113 – 116 |
| 16:00 – 16:10 | **Welcome Remarks**  
2013 Conference Chairs  
Christian Brander¹, Bonaventura Clotet², José M. Gatell³  
¹IrsiCaixa Inst for AIDS Res, HIVACAT, ICREA, Barcelona, Spain; ²IrsiCaixa Inst for AIDS Res, HIVACAT, Barcelona, Spain; ³Hospital Clinic, IDIBAPS, HIVACAT, University of Barcelona, Spain |                |
| 16:10 – 16:20 | Enric Banda¹  
¹Director of the Area of Science, Research and the Environment, “la Caixa” Foundation |                |
| 16:20 – 16:30 | Boi Ruiz i Garcia¹  
¹Minister of Health, Government of Catalonia |                |
| 16:30 – 16:40 | **Opening Remarks from the Vaccine Enterprise**  
William Snow¹  
¹Global HIV Vaccine Enterprise, New York, NY, USA |                |
| 16:40 – 16:50 | **Partnership Towards a Tough Target: Communities Are an Essential Ingredient, Not a Cherry on Top**  
Ntando Yola¹  
¹NACOSA, Cape Town, South Africa | OS.01 |
| 16:50 – 17:13 | **HIV Vaccines in the Context of Vaccine Development in General**  
Stanley A. Plotkin¹  
¹Univ of Pennsylvania, Doylestown, PA, USA | OS.02 |
| 17:13 – 17:36 | **Insights from HIV Vaccine Efficacy Trials: What We Are Learning from HVTN 505**  
Magdalena Sobieszczyl¹  
¹Columbia Univ, New York, NY, USA | OS.03 |
| 17:36 – 18:00 | **Toward Ending the HIV/AIDS Pandemic: Synergy Between Vaccine and Non-vaccine Interventions**  
Anthony Fauci¹  
¹NIAID, NIH, Bethesda, MD, USA | OS.04 |
| 18:00 – 20:00 | **Welcome Reception** | L2, Banquet Hall |
Tuesday, 8 October

A full complimentary breakfast buffet is available in the conference hotels: The AC Hotel Barcelona Forum by Marriott and the Hotel Barcelona Princess. Breakfast will not be available at the CCIB.

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<th>Time</th>
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<td>07:30 – 18:00</td>
<td>Registration / Coat Check</td>
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<tr>
<td>07:30 – 16:30</td>
<td>Speaker Check-in</td>
<td>L1, Room 118</td>
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<tr>
<td>08:30 – 10:00</td>
<td>HIV Vaccine Strategies Plenary Session 01</td>
<td>L1, Room 113 – 116</td>
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<td>Chairs: José Esparza and Peggy Johnston</td>
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<tr>
<td>08:30 – 09:00</td>
<td>Manufacturing HIV Envelope Proteins for HIV Vaccines: An NIAID Perspective of Where We Are and What Is Still Needed</td>
<td>PL01.01</td>
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<tr>
<td></td>
<td>Michael Pensiero1</td>
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<td>1NIAID, NIH, Bethesda, MD, USA</td>
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<td>09:00 – 09:30</td>
<td>Vaccine Induction of T Cell Immunity to HIV-1</td>
<td>PL01.02</td>
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<td></td>
<td>Andrew McMichael1</td>
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<td>1Univ of Oxford, Oxford, UK</td>
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<td>09:30 – 10:00</td>
<td>Pathways of Affinity Maturation of HIV-1 Broadly Neutralizing Antibodies</td>
<td>PL01.03</td>
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<td>Barton Haynes1</td>
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<td>1Duke Human Vaccine Inst, Durham, NC, USA</td>
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<tr>
<td>10:00 – 10:30</td>
<td>Refreshment Break</td>
<td>L1, Room 111 – 112</td>
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<td>L2, Room 211 – 212</td>
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<tr>
<td>10:30 – 12:00</td>
<td>T Cell Immunology</td>
<td>L1, Room 113 – 114</td>
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<tr>
<td></td>
<td>Oral Abstract Session 01</td>
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<td>Chairs: Zabrina Brumme and Paul Goepfert</td>
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<tr>
<td>10:30 – 10:45</td>
<td>Effective CD8+ T Cell Responses Restrict SIV Replication to Follicular Helper T Cells</td>
<td>OA01.01</td>
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<td>Yoshinori Fukazawa1, R. Lum1, J. Bae1, A. Okoye1, S.I. Hagen1, H. Park1, A.W. Legasse1, M.K. Axthelm1, J.D. Estes2, M. Piatak Jr.2, J.D. Lifson2, L.J. Picker1</td>
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<td>1VGTI, Oregon Hlth &amp; Science Univ, Beaverton, OR, USA; 2SAIC-Frederick, Inc., Frederick Nat’l Laboratory, Frederick, MD, USA</td>
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<td>10:45 – 11:00</td>
<td>The Breadth of Expandable Central Memory CD8 T Cells Inversely Correlates with Residual Viral Loads in HIV Elite Controllers</td>
<td>OA01.02</td>
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<td>Zaza Ndhllovu1, E. Stampouloglou2, K. Cesa2, D. Alvino2, A. Piechocka-Trocha2, F. Pereyra2, B. Walker2</td>
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<td>1Univ of KwaZulu-Natal, Durban, South Africa; 2Ragon Inst of MGH, MIT and Harvard, Cambridge, MA, USA</td>
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<td>11:00 – 11:15</td>
<td>PD-1 Expression on HIV-1 Specific CD8+ T Cells Is Shaped by Epitope Specificity, TcR Clonotype Usage and Driven by Antigen Load</td>
<td>OA01.03</td>
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<td>Henrik N. Kloverpris1, A. Stryhn2, S. Buus2, D. Price3, P. Goulder4</td>
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<td>1Univ of KwaZulu-Natal, Durban, South Africa; 2Univ of Copenhagen, Copenhagen, Denmark, 3Cardiff Univ School of Medicine, Cardiff, Wales, UK; 4Univ of Oxford, Oxford, UK</td>
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| 11:15 – 11:30 | Immunotherapeutic Interventions to Restore HIV-1-Specific CD4 T Cell Help to NK Cells  
Filippos Porichis¹, M. Hart¹, L. Barblu², T.E. Brennan¹, D. Kavanagh¹, D. Kaufmann²  
¹Ragon Inst of MGH, MIT and Harvard, Cambridge, MA, USA; ²Centre de Recherche du Centre Hospitalier de l’Université de Montréal, Montreal, Canada | OA01.04     |
| 11:30 – 11:45 | Integrative Analysis of Responses to Dendritic-Cell Vaccination Identifies Signatures Correlated with Control of HIV Replication: The DALIA Trial  
Rodolphe Thiebaut¹, B. Hejblum¹, J. Skinner², M. Montes², G. Chêne¹, K. Palucka², J. Banchereau¹, Y. Levy³  
¹INSERM U897, Bordeaux, France; ²Baylor Inst for Immunology Res, Dallas, TX, USA; ³INSERM U955, Créteil, France | OA01.05     |
| 11:45 – 12:00 | Effective Antiviral CD8+ T Cells Responses Are Rare in HIV-Positive Step & Phambili Study Participants but Are Targeted to Low Entropy Viral Epitopes  
Gemma Hancock¹, H. Yang¹, A.J. McMichael¹, N. Frahm², J.M. McElrath², L. Dorrell¹  
¹Univ of Oxford, Oxford, UK; ²FHCRC, Seattle, WA, USA | OA01.06 LB   |
| 10:30 – 12:00 | Adjuvants  
Oral Abstract Session 02  
Chairs: Gunilla Karlsson Hedestam and Rick King | L1, Room 115 – 116 |
| 10:30 – 10:45 | Engineering an HIV Envelope Protein to Activate Germline B Cell Receptors of Broadly Neutralizing VRC01-Class Antibodies  
Andrew McGuire¹, A.M. Dreyer¹, S. Hoot², A. Lippy¹, A. Stuart¹, K.W. Cohen¹, J. Jardine¹, S. Menis¹, J.F. Scheid³, A.P. West³, W.R. Schief³, L. Stamatatos¹  
¹Seattle Biomed, Seattle, WA, USA; ²Altravax Inc., Sunnyvale, CA, USA; ³IAVI Neutralizing Antibody CTR at The Scripps Res Inst, La Jolla, CA, USA; ⁴The Rockefeller Univ, New York, NY, USA; ⁵California Inst of Technology, Pasadena, CA, USA | OA02.01     |
| 10:45 – 11:00 | Focusing Antibody Responses to Specific Protein Surfaces Through Site-Selective Glycan Addition  
Torben Schiffner¹, K. Leonavicius¹, H. Schuster¹, L. Kong¹, R. Saliba¹, F. Wegmann¹, P. Huang¹, G.B. Stewart-Jones¹, W.R. Schief³, B.C. Davis¹, Q.J. Sattentau¹  
¹Univ of Oxford, Oxford, UK; ²Univ of Washington, Seattle, WA, USA; ³IAVI Neutralizing Antibody CTR at The Scripps Res Inst, La Jolla, CA, USA | OA02.02     |
| 11:00 – 11:15 | Comparative Antigenicity and Immunogenicity of Indian and South African HIV-1 Subtype C Native and CD4 Liganded Envelope Glycoproteins  
M.L. Grant¹, M.A. Killick¹, N. Cerutti¹, A. Capovilla¹, Maria A. Papathanasopoulos¹  
¹Univ of the Witwatersrand Med School, Johannesburg, South Africa | OA02.03     |
| 11:15 – 11:30 | Immunogenicity of a CD207-Targeted Anti-Gag Vaccine and TLR Ligand-Dependent Enhancement  
O. Epaulard¹, L. Adam³, C. Poux¹, G. Zurawski¹, P. Rosenbaum³, N. Dereuddre-Bosquet³, S. Zurawski¹, S. Oh¹, G. Romain³, J. Banchereau¹, Y. Lévy³, R. Le Grand³, Frederic Martinon³  
¹Baylor Inst for Immunology Res, Dallas, TX, USA; ³Vaccine Res Inst, Créteil, France; ⁴CEA, Fontenay-aux-Roses, France | OA02.04     |
| 11:30 – 11:45 | Impact of TLR9 Stimulation on HIV-1 Env-Specific Immune Responses Elicited in Non-human Primates  
Paola Andrea Martinez Murillo¹, C. Sundling¹, Y. Feng², S. O’Dell¹, K. Reimann², J. Mascola³, R. Wyatt¹, G.B. Karlsson Hedestam¹  
¹Karolinska Inst, Stockholm, Sweden; ²IAVI Neutralizing Antibody CTR at The Scripps Res Inst, La Jolla, CA, USA; ³VRC, NIAID, NIH, Bethesda, MD, USA; ⁴MassBiologics, Univ of Massachusetts Med School, Boston, MA, USA | OA02.05     |
11:45 – 12:00  
**Lipid Components in MPER-Based Immunization Regimens Are Critical for Inducing Broadly Neutralizing Antibody Responses in 2F5 and 4E10 Knockin Mice**  
Y. Chen\(^1\), J. Zhang\(^1\), H. Bouton-Verville\(^1\), A. Newman\(^1\), S. Xia\(^1\), H. Liao\(^1\), D.C. Montefiori\(^1\), M.A. Moody\(^1\), L. Armand\(^1\), K. Hwang\(^1\), B. Lockwood\(^1\), S.M. Dennison\(^1\), S.M. Alam\(^1\), B.F. Haynes\(^1\), Laurent Verkoczy\(^1\)  
\(^1\)Duke Human Vaccine Inst, Durham, NC, USA

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10:30 – 12:00  
**Clinical Trials**  
Oral Abstract Session 03  
**Chairs:** Lucy Dorrell and Beatriz Mothe

10:30 – 10:45  
The HIV consv Vaccines Induce Polyfunctional and Highly Proliferative T Cells that Control In Vitro HIV Replication: HIV-CORE002 Phase-I Clinical Trial  
Beatrice O. Ondondo\(^1\), T. Ahmed\(^1\), B. Nicola\(^1\), E. Hayton\(^1\), E. Vasilyeva\(^2\), N. Frahm\(^2\), S. Colloca\(^3\), A. Nicosa\(^3\), A. McMichael\(^1\), L. Dorrell\(^1\), T. Hanke\(^1\)  
\(^1\)Univ of Oxford, Oxford, UK; \(^2\)FHCRC, Seattle, WA, USA; \(^3\)Okaïros, Rome, Italy

10:45 – 11:00  
Phase I Clinical Trials of DNA-Protein Vaccine (CombiHIVvac) Containing Artificial Multi-Clade Immunogens  
Larisa Karpenko\(^1\), N. Ryndyuk\(^2\), Z. Gin’ko\(^2\), V. Kuzubov\(^2\), S. Bazhan\(^1\), L. Lebedev\(^1\), O. Kapлина\(^1\), A. Reguzova\(^1\), A. Ryzhikov\(^1\), M. Bogryantseva\(^1\), S. Usova\(^1\), V. Masycheva\(^1\), E. Nechaeva\(^1\), and A. Ilyichev\(^1\)  
\(^1\)State Res CTR of Virology and Biotechnology VECTOR, Koltsovo, Novosibirsk, Russian Federation; \(^2\)Hospital Number 163 of Russian Federal Med and Biological Agency, Koltsovo, Novosibirsk, Russian Federation

11:00 – 11:15  
Comparative Analysis of Binding Antibody Responses Elicited by a Cross-Section of Human HIV-1 Vaccine Clinical Trials  
Shelly J. Krebs\(^1\), S. Narpal\(^2\), A. Wheatley\(^2\), B.M. Slike\(^2\), M. Eller\(^2\), S. Ratto-Kim\(^1\), V.R. Polonis\(^1\), J.H. Kim\(^1\), M.L. Robb\(^1\), M.A. Marovich\(^1\), R.A. Koup\(^2\), B.S. Graham\(^1\), N.L. Michael\(^1\), A. McDermott\(^2\)  
\(^1\)U.S. MHRP, Silver Spring, MD, USA; \(^2\)VRC, NIAID, NIH, Bethesda, MD, USA

11:15 – 11:30  
Antibody Repertoire Induced by the Multiclade (Env A,B,C) HIV-1 DNA Prime, rAd5 Boost VRC Vaccine  
Wilton B. Williams\(^1\), K. Jones\(^1\), P. Liu\(^1\), A.M. Trama\(^1\), K. Seaton\(^1\), M.A. Moody\(^1\), N. Vandergrift\(^1\), K. Wlie\(^1\), H. Liao\(^1\), D.C. Montefiori\(^1\), C. Ochsenbauer\(^1\), J. Kappes\(^2\), S.M. Hammer\(^1\), J. Mascloa\(^1\), R. Koup\(^1\), L. Corey\(^1\), G. Nabel\(^1\), P. Gilbert\(^1\), C.A. Morgan\(^1\), G. Churchyard\(^1\), J. Maenza\(^1\), L.R. Baden\(^1\), M. Keefe\(^1\), B.S. Graham\(^1\), G.D. Tomaras\(^1\), B.F. Haynes\(^1\)  
\(^1\)Duke Univ Med CTR, Durham, NC, USA; \(^2\)Univ of Alabama at Birmingham, Birmingham, AL USA

11:30 – 11:45  
A Phase I Clinical Trial of an HIV-1 (CN54), Clade C, Trimeric Envelope Vaccine Delivered by Parenteral, Nasal and Vaginal Routes of Immunisation  
Catherine A. Cosgrove\(^1\), C. Lacey\(^2\), A.V. Cope\(^3\), A. Bartolf\(^1\), G. Morris\(^3\), C. Yan\(^1\), S. Baden\(^1\), T. Cole\(^1\), D. Carter\(^1\), E. Brodnicki\(^5\), W. Stoehr\(^5\), S. McCormack\(^6\), R.J. Shattock\(^3\)  
\(^1\)St George’s, Univ of London, London, UK; \(^2\)Hull York Med School, Univ of York, York, UK; \(^3\)Imperial College, London, UK; \(^4\)Infectious Disease Res Inst, Seattle, WA, USA; \(^5\)MRC Clinical Trials Unit, London, UK; \(^6\)MRC Clinical Trials Unit and Imperial College, London, UK

11:45 – 12:00  
HIV-1 Fusion Protein (F4/AS01) and Adenovirus-35 Gag-RT-Int-Nef Induce Potent and Durable T-Cell and Antibody Responses in Healthy African Adults  
Juliet Mpendo\(^1\), G. Omosa-Manyonyi\(^1\), E. Ruzagirwa\(^1\), W. Kilembe\(^1\), F. Roman\(^1\), M. Koutsoukos\(^1\), A. Lombardo\(^6\), A. McDermott\(^2\), J. Gilmour\(^1\), J. Cox\(^1\), F. Priddy\(^1\)  
\(^1\)Uganda Virus Res Inst, Entebbe, Uganda; \(^2\)Kenya AIDS Vaccine Initiative, Nairobi, Kenya; \(^3\)MRC-Uganda, Entebbe, Uganda; \(^4\)Zambia Emory HIV Res Program, Lusaka, Zambia; \(^5\)GSK Vaccines, Rixensart, Belgium; \(^6\)IAVI, New York, NY, USA; \(^7\)Emmes Corp., Rockville, MD, USA
Lunch tables have been reserved for young and early-career investigators to network with senior researchers. The list of participating researchers is available at the conference registration desk along with a sign-up sheet. Space is limited and will be reserved on a first-come, first-served basis.

**13:00 – 14:30 Animal Models**  
**L1, Room 113 – 114**  
**Chairs:** Amitinder Kaur and María Ángeles Muñoz Fernández

13:00 – 13:15  
**PET/CT Imaging of Simian Immunodeficiency Virus Reveals the Dynamics of Viral Replication In Vivo**  
*P. Santangelo,* K. Rogers, C. Zurita, E. Alonza, P. Armancha, J. Hong, P. Sharma, D.K. Machiah, K. Strait, A.A. Ansari, François Villinger

*Georgia Inst of Technology and Emory Univ, Atlanta, GA, USA; Emory Univ, Atlanta, GA, USA*

13:15 – 13:30  
**Non-human Primate Model to Study the Role of Serum and Vaginal IgA in Acquisition of Infection**  

*Drexel Univ, Philadelphia, PA, USA; Univ of Alabama at Birmingham, Birmingham, AL, USA;
Tulane Univ, New Orleans, LA, USA; Inovio Pharmaceuticals, Blue Bell, PA, USA; Duke Univ, Durham, NC, USA; Univ of Pennsylvania, Philadelphia, PA, USA*

13:30 – 13:45  
**Vaccine Induced Epitope Specific Antibodies to the SIV Envelope Are Distinct from Those Induced to the HIV-1 Envelope**  

*Duke Univ, Durham, NC, USA; Oregon Hlth & Science Univ, Beaverton, OR, USA; Emory Univ, Atlanta, GA, USA; GeoVax Inc., Smyrna, GA, USA; Imperial College, London, UK; EuroVac Foundation, Amsterdam, Netherlands; U.S. MHRP, Silver Spring, MD, USA; VRC, NIAID, NIH, Bethesda, MD, USA; Univ of Washington, Seattle, WA, USA; Novartis Vaccines and Diagnostics, Inc., Cambridge, MA, USA; Nat’l Cancer Inst, NIH, Frederick, MD, USA; Univ of Lausanne, Lausanne, Switzerland*

13:45 – 14:00  
**Co-administration of Rapamycin with a DNA/MVA SIV Vaccine Improves Memory CD8 T Cell Response and Enhances Control of SIV251 Infection**  
*Shanmugalakshmi Sadagopal,* S. Kwa, R. Basu, S. Gangadharan, K. Araki, R. Ahmed, R. Amara

*Emory Univ, Atlanta, GA, USA*

14:00 – 14:15  
**Protection of Cynomolgus Macaques from Pathogenic SIV Following Vaccination with Varicella-Zoster Virus Based Vaccines**  

*Canadian Food Inspection Agency, Lethbridge, Canada; Univ of Toronto, Toronto, Canada; Publ Hlth Agency of Canada, Ottawa, Canada; Health Canada, Ottawa, Canada; Mt. Sinai Hospital, Univ of Toronto, Toronto, Canada*

14:15 – 14:30  
**Therapeutic Efficacy of Potent Neutralizing HIV-1-Specific Monoclonal Antibodies in SHIV-Infected Rhesus Monkeys**  
*Dan Barouch,* J. Whitney, B. Moldt, F. Klein, K. Shekhar, A. Chakraborty, M. Nussenzweig, D. Burton

*Beth Israel Deaconess Med CTR, Boston, MA, USA; The Scripps Res Inst, USA; The Rockefeller Univ, USA; MIT, Cambridge, MA, USA*
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<tr>
<td>13:00 – 13:15</td>
<td>Oral Abstract Session 05</td>
<td>Structural Basis for HIV-1 gp120 Recognition by a Germ-line Version of a Broadly Neutralizing Antibody</td>
<td>Louise Scharf¹, A.P. West¹, J.F. Scheid², M.C. Nussenzweig³, P.J. Bjorkman¹, R. Diskin³</td>
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<td>¹California Inst of Technology, Pasadena, CA, USA; ²The Rockefeller Univ, New York, NY, USA; ³Weizmann Inst, Rehovot, Israel</td>
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<td>13:15 – 13:30</td>
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<td>Structural Characterization of Potent, Longitudinally-Defined HIV-1 V1/V2-Directed Antibodies</td>
<td>Jason Gorman¹, N. Doria-Rose³, C.A. Schramm², P.L. Moore³, M. Pancera¹, Y. Yang¹, R.P. Staupe¹, Z. Zhang², I.S. Georgiev¹, J. Bhiman¹, N.L. Longo¹, L. Morris¹, L. Shapiro², J.R. Mascola¹, P.D. Kwong¹</td>
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<td>¹VRC, NIAID, NIH, Bethesda, MD, USA; ²Columbia Univ, New York, NY, USA; ³Nat'l Inst for Communicable Diseases of the Nat'l Hlth Labs Service, Johannesburg, South Africa</td>
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<td>13:30 – 13:45</td>
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<td>Interplay Between Broadly Cross-Neutralizing V2 Monoclonal Antibodies and Autologous Viral Evolution</td>
<td>Jinal N. Bhiman¹, N. Doria-Rose³, P.L. Moore¹, M. Nonyane³, S.S. Abdooll Karim⁴, P.D. Kwong⁵, J.R. Mascola¹, L. Morris¹</td>
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<td>¹Nat'l Inst for Communicable Diseases of the Nat'l Hlth Laboratory Service and Univ of the Witwatersrand, Johannesburg, South Africa; ²VRC, NIAID, NIH, Bethesda, MD, USA; ³Nat'l Inst for Communicable Diseases of the Nat'l Hlth Labs Service, Johannesburg, South Africa; ⁴CAPRISA, Univ of KwaZulu-Natal, Durban, South Africa</td>
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<td>13:45 – 14:00</td>
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<td>A Diverse Antibody Response to the gp120 N332 Glycan Epitope in an HIV-Infected Donor</td>
<td>Katie J. Doore², K. Le², D. Sok², U. Laserson¹, F. Vigneault¹, J. Laserson⁴, D. Koller⁴, G. Church², P. Poignard², D. Burton²</td>
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<td>²King's College London and The Scripps Res Inst, London, UK; ³The Scripps Res Inst, La Jolla, CA, USA; ⁴Harvard Med School, Boston, MA, USA; ⁵Stanford Univ, Stanford, CA, USA</td>
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<td>14:00 – 14:15</td>
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<td>MF59 and ALUM, in Combination with an ALVAC-SIV/gp120 Vaccine, Induce Plasmablasts that Differ in the Expression of Homing Markers</td>
<td>Luca Schifanella¹, S. Gordon¹, M. Vaccari¹, N. Binello¹, F. Caccuri¹, M. Blackburn¹, C. Fenizia¹, M. Doster¹, P. Pegu¹, N. Liynage¹, X. Shen², G. Tomaras², M. Rao³, G. Ferrati¹, D. Venzon¹, D. Stabliens¹, S. Barnett⁵, J. Tartaglia¹, G. Franchini¹</td>
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<td>¹Nat'l Cancer Inst, NIH, Bethesda, MD, USA; ²Duke Human Vaccine Inst, Durham, NC, USA; ³U.S. MHRP, Silver Spring, MD, USA; ⁴Duke Univ Med CTR, Durham, NC, USA; ⁵The EMMES Corporation, Rockville, MD, USA; ⁶Novartis Vaccines and Diagnostics Inc., Cambridge, MA, USA; ⁷Sanofi Pasteur, Swiftwater, PA, USA</td>
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<td>14:15 – 14:30</td>
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<td>HIV-1 gp120 Vaccination Elicits a Robust and Durable Anti-V1/V2 IgG Response and Yet No HIV-1 Env-Specific IgA Response in HIV-Exposed Infants</td>
<td>Genevieve G. Fouda¹, C.K. Cunningham¹, E.J. McFarland², W. Borkowsky³, P. Muresan⁴, B.E. Liebl¹, H. Liao¹, B.F. Haynes¹, R. Overman¹, N.L. Yates¹, G.D. Tomaras¹, S.R. Permar¹</td>
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<td>¹Duke Univ Med CTR, Durham, NC, USA; ²Univ of Colorado, Aurora, CO, USA; ³New York Univ, New York, NY, USA; ⁴SDAC-FSTRF/Harvard School of Publ Hlth, Boston, MA, USA</td>
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<tr>
<td>13:00 – 14:30</td>
<td>Vaccine Concepts</td>
<td>Chairs: Nicole Frahm and Ralf Wagner</td>
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<td>13:00 – 13:15</td>
<td>Identifying the Minimal Mutations in VRC01 Required for Neutralization Potency and Breadth to Inform Vaccine Design</td>
<td>Joseph G. Jardine¹, D. Sok², O. Kalyuzhniy¹, M. Kubitz³, Y. Adachi⁴, D.R. Burton², W.R. Schief¹</td>
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<td>¹The Scripps Res Inst, IAVI, CHAVI-ID, Univ of Washington, La Jolla, CA, USA; ²The Scripps Res Inst, IAVI, CHAVI-ID, La Jolla, CA, USA; ³The Scripps Res Inst, IAVI, CHAVI-ID, La Jolla, CA, USA; ⁴The Scripps Res Inst, IAVI, La Jolla, CA, USA</td>
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<td>13:15 – 13:30</td>
<td>Structural Organization and CD4-Induced Reorganization in Soluble HIV-1 Trimers</td>
<td>M. Guttman², A. Cupo¹, R.W. Sanders¹, J.P. Moore¹, Kelly K. Lee²</td>
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<td>¹Weill Cornell Med College, New York, NY, USA; ²Univ of Washington, Seattle, WA, USA</td>
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<td>13:30 – 13:45</td>
<td>Cell-Surface Display and Panning of HIV-1 Derived Envelope Proteins</td>
<td>Veronika Schmid³, T.H. Bruun³</td>
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<td>³Univ of Regensburg, Regensburg, Germany</td>
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<td>13:45 – 14:00</td>
<td>DNA-MVA Prime-Boost Vaccine Eliciting T-Cell Specificities Associated with HIV-1 Control Is Highly Immunogenic in Mice and Breaks CTL Immunodominance</td>
<td>A. Olvera¹, A. Llano², M. Rosati³, S. Pérez-Álvarez³, V. Kulkarni³, B. Chowdhury³, C. Alicia³, R.K. Beach³, N.Y. Sardesai³, T. Hanke³, B. Clotet⁴, G.N. Pavlakis³, B.K. Felber³, C. Brander³, Beatriz Mothe³</td>
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<td>¹IrsiCaixa Inst for AIDS Res, HIVACAT; ‘Lluita contra la Sida’ Hospital Universitari “Germans Trias i Pujol”, Barcelona, Spain; ²IrsiCaixa Inst for AIDS Res, HIVACAT, Barcelona, Spain; ³Nat’l Cancer Inst-Frederick, NIH, Frederick, MD, USA; ⁴Inovio Pharmaceuticals Biomedical Corp., Blue Bell, PA, USA; ⁵Univ of Oxford, Oxford, UK; ⁶IrsiCaixa Inst for AIDS Res, HIVACAT; ‘Lluita contra la Sida’ Hospital Universitari “Germans Trias i Pujol”; Universitat Autònoma de Barcelona, Barcelona, Spain</td>
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<td>14:00 – 14:15</td>
<td>Detection of Early CTL Escape in Gag and Nef Using Population-Level Approaches</td>
<td>Eric Martin¹, J. Carlson², A. Le¹, R. McGovern³, C. Ng³, M. Rahman¹, D. Chopera¹, M. Wainberg¹, R. Harrigan¹, Z. Brumme¹</td>
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<td>¹Simon Fraser Univ, Burnaby, Canada; ²Microsoft Research, Los Angeles, CA, USA; ³BC Centre for Excellence in HIV/AIDS, Vancouver, Canada; ⁴McGill Univ, Montreal, Canada</td>
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<td>14:15 – 14:30</td>
<td>Neutralizing and Non-neutralizing Antibody Binding to Cleaved, SOSIP-Stabilized and Uncleaved Soluble HIV-1 Env Trimers, Protomers, and gp120</td>
<td>A. Yasmeen³, R.W. Sanders¹, R. Ringe³, R. Derking³, A. Cupo³, J. Julien³, A.B. Ward³, I.A. Wilson³, J.P. Moore³, Per Johan Klasse³</td>
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<td>³Academic Med CTR, Amsterdam, Netherlands; ²The Scripps Res Inst, La Jolla, CA, USA; ³Cornell Univ, New York, NY, USA</td>
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<td>14:30 – 15:00</td>
<td>Refreshment Break</td>
<td>L1, Room 111 – 112, L2, Room 211 – 212</td>
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<td>Time</td>
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<tr>
<td>15:00 – 16:20</td>
<td>Innate Immunity Symposium 01</td>
<td>L1, Room 113 – 114</td>
<td>Gallit Alter and Karin Lore</td>
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<tr>
<td>15:00 – 15:20</td>
<td>Antiviral Activity of NK Cells in HIV-1 Infection</td>
<td>S01.01</td>
<td>Marcus Altfeld1</td>
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<td>1Heinrich-Pette-Inst, Hamburg, Germany</td>
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<td>15:20 – 15:40</td>
<td>Adjuvants Modify Antibody Effect or Function</td>
<td>S01.02</td>
<td>Alison Mahan1</td>
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<td>1Ragon Inst of MGH, MIT and Harvard, Cambridge, MA, USA</td>
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<td>15:40 – 16:00</td>
<td>Systems Analysis of Innate Immune Responses Triggered by Viral Vector-</td>
<td>S01.03</td>
<td>Daniel Zak1</td>
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<td>Based HIV Vaccine Candidates</td>
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<td>1Seattle Biomed, Seattle, WA, USA</td>
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<td>16:00 – 16:20</td>
<td>Systems Vaccinology: Enabling Rational Vaccine Design with Systems Biology</td>
<td>S01.04</td>
<td>Bali Pulendran1</td>
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<td>1Emory Univ, Atlanta, GA, USA</td>
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<td>15:00 – 16:20</td>
<td>Linking T and B Cell Responses</td>
<td>L1, Room 115 – 116</td>
<td>Andreas Meyerhans and Hendrik Streeck</td>
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<td>15:00 – 15:20</td>
<td>Memory CD8 T Cells: Issues of Quantity, Quality, and Location</td>
<td>S02.01</td>
<td>David Masopust1</td>
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<td>1Univ of Minnesota, Minneapolis, MN, USA</td>
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<td>15:20 – 15:40</td>
<td>Impaired B Cell Help During HIV Infection</td>
<td>S02.02</td>
<td>Elias Haddad1</td>
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<td>1Vaccine and Gene Therapy Inst of Florida, Port St. Lucie, FL, USA</td>
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<td>15:40 – 16:00</td>
<td>Role of Tfh in HIV Infection</td>
<td>S02.03</td>
<td>M. Perreau1*, A.L. Savoye1*, E. De Crignis1*, J.M. Corpataux2*, R. Cubas3*, E. Haddad3*,</td>
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<td>L. De Leval4, C. Graziosi1, Giuseppe Pantaleo1,3,5</td>
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<td>1Divisions of Immunology and Allergy, 2Thoracic Surgery, 3Vaccine and Gene Therapy Institute of Florida,</td>
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<td>Port St.Lucie, FL, USA, 4Institute of Pathology and 5Swiss Vaccine Research Institute, Lausanne University Hospital, University of Lausanne, Lausanne, Switzerland</td>
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<td>*The first two authors are listed in alphabetic order and have equally contributed to this study.</td>
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<td>16:00 – 16:20</td>
<td>SIAE Inhibition and Enhanced Somatic Hypermutation Against gp140</td>
<td>S02.04</td>
<td>H. Mattoo1, Shiv Pillai1, R. Wyatt2</td>
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<td>1Massachusetts General Hospital, Boston, MA, USA; 2The Scripps Res Inst, La Jolla, CA, USA</td>
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<td>15:00 – 16:40</td>
<td>NHP Vaccines and Correlates of Immune Protection</td>
<td>L1, Room 117</td>
<td>Roger LeGrand and Jeffery Lifson</td>
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<td>15:00 – 15:20</td>
<td>Can AIDS Virus-Specific Classical CD8+ T Cells Control Virus Replication?</td>
<td>S03.01</td>
<td>David Watkins1</td>
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<td>1Univ of Miami, Miami, FL, USA</td>
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<td>15:20 – 15:40</td>
<td>Strengths and Limitations of Macaque Models as Predictors of HIV Vaccine Efficacy in Humans</td>
<td>S03.02</td>
<td>Genoveffa Franchini1</td>
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<td>1Nat’l Cancer Inst, NIH, Bethesda, MD, USA</td>
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15:40 – 16:00 Pre-clinical Development of Cytomegalovirus Vectors  
Louis Picker1
1Oregon Hlth & Science Univ, Beaverton, OR, USA
S03.03

16:00 – 16:20 Vaccine Responses to Four Different gp140 Trimmers  
Robin Shattock2
2Imperial College, London, UK
S03.04

16:20 – 16:40 Impressive Vaccine Protection Against SIVmac239 by Recombinant Gamma-2 Herpesvirus  
Y. Shin1, W. Laufer1, G. Bischof3, M. Platack1, J. Lifson1, D. Watkins2, Ronald C. Desrosiers3
1SAIC Frederick, Inc., Frederick Nat’l Labs, Frederick, MD, USA; 2University of Miami, Miami, FL, USA; 3New England Primate Research Center, Harvard Medical School, Southborough, MA, USA
S03.05

16:45 – 16:52 Heterogeneity of Anti-V2 ADCC Ab Responses and Implications for Vaccine Development  
Justin Pollara1, M. Bonsignori1, M. Moody1, S. Alam1, K. Hwang1, T.C. Gurley1, D.M. Kozink1, D.J. Marshall1, J.F. Whitesides2, C. Tsao1, J. Kaewkungwal2, S. Nitayaphan3, P. Pilisutthitum2, S. Rerk-Ngam1, G.D. Tomaras1, J.H. Kim2, N.L. Michael2, D.C. Montefiori1, H. Liao1, B.F. Haynes1, G. Ferrari1, and the CTR for HIV/AIDS Vaccine Immunology1
1Duke Univ, Durham, NC, USA; 2Mahidol Univ, Bangkok, Thailand; 3Armed Forces Res Inst of Med Sciences, Bangkok, Thailand; 4Ministry of Publ Hlth, Thailand; 5U.S. MHRP, Silver Spring, MD, USA
P03.01 D

16:53 – 17:00 Modalities of Broad and Potent Antibody Neutralization at the CD4-Binding Site on HIV-1 gp120  
Tongqing Zhou1, L. Chen1, R. Lynch1, X. Wu1, S. Srivatsan1, S. Moquin1, Y. Yang1, B. Zhang1, J.R. Mascola1, P.D. Kwong1
1NIAID, NIH, Bethesda, MD, USA
P03.02 D

17:01 – 17:08 Vaccine-Elicited CD4 Binding Site-Directed Antibodies Utilizing the Macaque Equivalent of the Human VH1-2 Heavy Chain Variable Gene Segment  
Marjon Navis1, K. Tran2, Y. Li1, G. Phad1, C. Sundling1, R. Wilson1, M. Soldemo1, K. McKe1, J. Mascola3, R.T. Wyatt1, G.B. Karlsson Hedestam1
1Karolinska Inst, Stockholm, Sweden; 2IAVI Neutralizing Antibody Center at The Scripps Res Inst, La Jolla, CA, USA; 3VRC, NIAID, NIH, Bethesda, MD, USA
P03.03 D

17:09 – 17:16 Analysis of Vaccine-Elicited Antibodies to the HIV-1 gp120 CD4 Binding Site Reveals Distinct Modes of Env Recognition to Guide Immunogen Re-design  
K. Tran1, C. Poulsen1, J. Guenaga1, N. de Val Alda1, C. Sundling2, R. Wilson1, Y. Li1, R. Stanfield1, I. Wilson1, A.B. Ward1, G.B. Hedestam Karlsson2, Richard T. Wyatt1
1The Scripps Res Inst, La Jolla, CA, USA; 2Karolinska Inst, Stockholm, Sweden
P03.62 LB D

16:45 – 17:24 T Cell Immunology  
Poster Discussion Session 02  
Chair: Montserrat Plana  
L1, Room 115 – 116

16:45 – 16:52 Host Immune Environment Impact the Level of CD4 Central Memory Reconstitution in HIV Subjects Receiving ZFN CCR5 Modified CD4 T-Cells (SB-728-T)  
1Vaccine and Gene Therapy Inst - Florida, Port St Lucie, FL, USA; 2Sangamo Biosciences, Richmond, CA, USA; 3Quest Clinical Res, San Francisco, CA, USA; 4UCLA, Los Angeles, CA, USA
P12.01 D
16:53 – 17:00 | HIV-1 Protease Inhibitor Alter Cellular Protease Activity, Epitope Processing and Presentation to CD8 T Cells
Georgio Kourjian1, Y. Xu1, J. Boucau1, M. Shimada1, P. Gourdain1, S. Le Gall1
1Ragon Inst of MGH, MIT and Harvard, Cambridge, MA, USA

17:01 – 17:08 | Targeting HIV-1 Where It Hurts
Tina Ahmed1, P. Hayes2, N. Borthwick3, B. Ondondo1, E. Hayton1, S. Colloca3, A. Nicosia3, A. McMichael1, J. Gilmour2, L. Dorrell1, T. Hanke1
1Univ of Oxford, Oxford, UK; 2IAVI Human Immunology Laboratory, Chelsea and Westminster Hospital and Imperial College, London, UK; 3Okaïros, Rome, Italy

17:09 – 17:16 | Local Cellular Immune Responses Precede Systemic Responses Following Intravaginal SIV Infection in Rhesus Monkeys
Jinyan Liu1, H. Li1, K. Smith1, E. Boudoucri1, K. Stanley1, W. Wagner1, M. Lewis1, D. Barouch1
1Beth Israel Deaconess Med CTR, Harvard Med School, Boston, MA, USA; 2Bioqual, Inc., Rockville, MD, USA

17:17 – 17:24 | Immune-Correlates Analysis of the Step HIV Vaccine Efficacy Trial–A Post-Hoc Analysis of HIV-Specific and Non-specific Cellular Immune Responses
Yunda Huang1, A. Duerr1, Z. Moodie1, N. Frahm1, S. DeRosa1, J. McElrath1, P. Gilbert1
1FHCRC, Seattle, WA, USA

16:45 – 17:24 | Clinical Trials
Poster Discussion Session 03
Chair: Eric Sandström

16:45 – 16:52 | Phase I Safety and Immunogenicity of Electroporated HIV DNA with or Without Interleukin 12 in Healthy HIV-Seronegative African Volunteers
1Projet San Francisco, Kigali, Rwanda; 2UVRI-IAVI HIV Vaccine Programme, Entebbe, Uganda; 3KAVI-Kangemi, Univ of Nairobi, Nairobi, Kenya; 4IAVI, New York, NY, USA; 5IAVI Human Immunology Laboratory, London, UK; 6Profectus BioSciences, Inc., Terrrytown, NY, USA; 7ICHOR Med Systems, Inc., San Diego, CA, USA; 8The EMMES Corporation, Rockville, MD, USA; 9Emory Univ, School of Medicine, Atlanta, GA, USA

16:53 – 17:00 | Enhanced HIV-Specific CD8+ T Cell Responses Following Polytopic Administration of VRC rAd5 gag-pol/env A/B/C in HIV-Uninfected Healthy Adults
Ian Frank1, N. Grunenberg2, J. Hural2, P. Edlesfsen2, A. DeCamp2, M. Allen3, P. Spearman3, K. Bar1, S. Kalams2, L. Baden6, M. Keefer7, J. Fuchs6, R. Novak8, B. Graham10, M. Enama10, N. Frahm2
1Univ of Pennsylvania, Philadelphia, PA, USA; 2Univ of Washington, Seattle, WA, USA; 3NIAID, NIH, Bethesda, MD, USA; 4Emory Univ, Atlanta, GA, USA; 5Vanderbilt Univ, Nashville, TN, USA; 6Brigham and Women’s Hospital, Boston, MA, USA; 7Univ of Rochester, Rochester, NY, USA; 8San Francisco Dept of Publ Hlth, San Francisco, CA, USA; 9Univ of Illinois, Chicago, IL, USA; 10VRC, NIAID, NIH, Bethesda, MD, USA

17:01 – 17:08 | Distinct HIV-Specific Antibody Fc-Profiles in RV144 and VAX003 Vaccinees
Amy Chung1, M. Ghebremichael1, H. Robinson1, E. Brown2, I. Choi2, M. Rolland1, A. Dugast1, T.J. Suscovich1, L. Liao4, A.E. Mahan1, H. Streeck3, S. Reks-Ngarm1, S. Nitayaphan1, M.S. de Souza1, P. Pitsutthithum1, D. Francis1, N.L. Michael1, J.H. Kim1, C. Bailey-Kellog2, M.E. Ackerman2, G. Alter1
1Ragon Inst of MGH, MIT and Harvard, Cambridge, MA, USA; 2Dartmouth College, Hanover, NH, USA; 3U.S. MHRRP, Silver Spring, MD, USA; 4Duke Univ Human Vaccine Inst and the CTR for HIV/AIDS Vaccine Immunology, Durham, NC, USA; 5Ministry of Publ Hlth, Nonthaburi, Thailand; 6Armed Forces Res Inst of Med Sciences, Bangkok, Thailand; 7Mahidol Univ, Bangkok, Thailand; 8Global Solutions for Infectious Diseases, South San Francisco, CA, USA
Feasibility of Enrolling Female Commercial Sex Workers at High Risk of HIV Infection for HIV Vaccine Trials in the Caribbean

M. Deschamps¹, Cecilia Morgan², C. Zorrilla³, B. Metch², Y. Donastorg⁴, P. Joseph¹, E. Swann⁵, T. Madenwald², G. Escamilla², J.W. Pape¹, HVTN 907 Protocol Team²

¹Groupe Haitien d’Etude du Sarcome de Kaposi et des Infections Opportunistes (GESKIO), Port au Prince, Haiti; ²FHCRC, Seattle, WA, USA; ³Maternal and Infant Studies CTR (CEMI), Univ of Puerto Rico School of Medicine, San Juan, Puerto Rico; ⁴Unidad de Vacunas IDCP-COIN-DIGECITSS, Santo Domingo, Dominican Republic; ⁵NIAID, NIH, Bethesda, MD, USA

Phase I Clinical Trial HIV-CORE002 of a Universal T-Cell Vaccine: One Year Follow On and Epitope Mapping

Nicola J. Borthwick¹, A. Nicosia², A. McMichael¹, L. Dorrell¹

¹Univ of Oxford, Oxford, UK; ²CEINGE, Naples, Italy

Poster Session 01 and Reception

Poster presentations are scheduled by topic and the last two digits of the abstract presentation number. For example, 1.01 is an odd-numbered abstract; whereas 1.02 is an even-numbered abstract.

Posters are available for viewing throughout the conference, however, Poster Session 01 will feature odd-numbered posters only.

**Poster Topics Presented in L1, Room 111 – 112**
- P01: Adjuvants, Immunogens and Inserts
- P02: Animal Models and Preclinical Trials
- P03: B Cell Immunology and Antibody Functions
- P04: Clinical Vaccine Trials and Trial Site Challenges
- P05: HIV Transmission and Viral Diversity

**Poster Topics Presented in L2, Room 211 – 212**
- P06: Immunogenetic Factors
- P07: Innate Immunity
- P08: Mucosal Immunity
- P09: Non-Vaccine Prevention
- P10: Pediatric and Adolescent Infections and Trials
- P11: Social/Ethical/Access/Regulatory Issues
- P12: T Cell Immunity
- P13: Vaccine Concepts and Design

AIDS Vaccine 2013 11
## Wednesday, 9 October

A full complimentary breakfast buffet is available in the conference hotels: The AC Hotel Barcelona Forum by Marriott and the Hotel Barcelona Princess. Breakfast will not be available at the CCIB.

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tr>
<td>07:30 – 18:00</td>
<td>Registration / Coat Check</td>
<td>L0, Entrance Hall</td>
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<td>07:30 – 16:30</td>
<td>Speaker Check-in</td>
<td>L1, Room 118</td>
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<td>08:30 – 10:00</td>
<td>Protection from Infection and Disease Progression</td>
<td>L1, Room 113 – 116</td>
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<td>Plenary Session 02</td>
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<td>Chairs: Nicole Bernard and Christian Brander</td>
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<tr>
<td>08:30 – 09:00</td>
<td>Genital Immunology and HIV Susceptibility</td>
<td>PL02.01</td>
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<td>Rupert Kaul1</td>
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<td>1Univ of Toronto, Ontario, Canada</td>
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<td>09:00 – 09:30</td>
<td>Immunodeficiency Virus and the MHC: Past, Present and Future</td>
<td>PL02.02</td>
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<td>Philip Goulder1</td>
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<td>1Univ of Oxford, Oxford, UK</td>
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<td>09:30 – 10:00</td>
<td>The Role of HIV-1 Viral Evolution in Shaping Broadly Neutralizing Antibodies</td>
<td>PL02.03</td>
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<td>Penelope Moore1</td>
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<td>1Nat’l Inst for Communicable Diseases, Johannesburg, South Africa</td>
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<td>10:00 – 10:30</td>
<td>Refreshment Break</td>
<td>L1, Room 111 – 112</td>
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<td>L2, Room 211 – 212</td>
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<tr>
<td>10:30 – 12:00</td>
<td>HIV Transmission</td>
<td>L1, Room 113 – 114</td>
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<td>Oral Abstract Session 07</td>
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<td>Chairs: Jacob Estes and Lynn Morris</td>
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<td>10:30 – 10:45</td>
<td>A Role for Glycosylation in HIV Transmission</td>
<td>OA07.01</td>
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<td>F. Nawaz1, C. Cicala2, C. Schwing2, D. Wei2, J. Hiatt2, K. Jelcic2, D. Van Ryk2, A. Fauci2, James Arthos2</td>
<td>1New York Univ, New York, NY, USA; 2NIAID, NIH, Bethesda, MD, USA</td>
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<td>10:45 – 11:00</td>
<td>Role of Integrin α4β7 in HIV Transmission and Pathogenesis</td>
<td>OA07.02</td>
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<td>Simone Irene Richardson1, N. Mkhize1, S. Abdool Karim2, E. Gray1, L. Morris1</td>
<td>1Nat’l Inst for Communicable Diseases, Johannesburg, South Africa; 2CAPRISA, Univ of KwaZulu-Natal, Durban, South Africa</td>
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<tr>
<td>11:00 – 11:15</td>
<td>Profound Alterations in Cholesterol Metabolism Restrict HIV-1 Transfection of CD4 T Cells in Viremic Controllers</td>
<td>OA07.03</td>
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<td>Giovanna Rappoccio1, M. Jais1, P. Piazza1, P. Gupta1, C.R. Rinaldo1</td>
<td>1Univ of Pittsburgh, Pittsburgh, PA, USA</td>
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<td>11:15 – 11:30</td>
<td>Highly Potent Broadly Neutralizing Antibodies Lack Potential to Inhibit HIV-1 Cell-to-Cell Transmission</td>
<td>OA07.04</td>
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<td>Lucia Reh1, I.A. Abela1, P. Rusert1, A. Trkola1</td>
<td>1Univ of Zurich, Zurich, Switzerland</td>
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</table>
11:30 – 11:45

**Highly Pathogenic Adapted HIV-1 Strains Limit Immune Responses and Dictate Rapid Disease Progression in Early Infection**

J. Dalmau¹, M. Rotger¹, I. Erkizia³, A. Rauch², P. Reche³, M. Pino³, Y. Lie⁴, R. Bellido⁵, A. Esteve⁵, E. Coackley⁴, E. Palou⁶, C. Brander⁷, R. Paredes⁷, T. Wrin⁴, B. Clotet⁵, A. Telenti¹, J. Martinez-Picado⁷, Julia G. Prado⁷

¹Univ Hospital CTR and Univ of Lausanne, Switzerland; ²Univ Hospital Bern and Univ of Bern, Switzerland; ³Universidad Complutense de Madrid, Spain; ⁴Monogram Biosciences, USA; ⁵Centre d’Estudis Epidemiològics sobre les Infeccions de Transmissió Sexual i Sida de Catalunya, Spain; ⁶Banc de Sang i Teixits de Barcelona, Spain; ⁷IrsiCaixa Inst for AIDS Res. HIVACAT, Barcelona, Spain

11:45 – 12:00

**Genetic Selection Bias at the Heterosexual HIV-1 Transmission Bottleneck**

Jonathan Carlson¹, D. Monaco², M. Schaefer², D. Claiborne², J. Prince², D. Dilemnia², K. Denis³, W. Kilembe³, J. Tang³, P. Farmer³, R. Kaslow⁴, P. Goulder⁵, S. Allen⁶, P. Goepfert⁶, D. Heckerman¹, E. Hunter²

¹Microsoft Research, Los Angeles, CA, USA; ²Emory Univ, Atlanta, GA, USA; ³Zambia Emory HIV Res Project, Lusaka, Zambia; ⁴Univ of Alabama-Birmingham, Birmingham, AL, USA; ⁵Univ of Oxford, Oxford, UK

10:30 – 12:00

**Innate and Mucosal Immunity**

Oral Abstract Session 08

**Gut-Homing of Plasmacytoid Dendritic Cells Persists in the Absence of HIV/SIV Replication and Contributes to Residual Chronic Immune Activation**

R. Keith Reeves¹, H. Li¹, T. Evans¹

¹Harvard Med School, Southborough, MA, USA

**HIV-1 Infection Induces Potent Type I IFN Signatures in Conventional Dendritic Cells from HIV-1 Elite Controllers**

Enrique Martin-Gayo¹, T. Hickman¹, Z. Ouyang¹, D. Pimenova¹, F. Pereyra¹, B.D. Walker¹, M. Lichterfeld¹, X.G. Yu¹

¹Ragon Inst of MGH, MIT and Harvard, Cambridge, MA, USA; ²Howard Hughes Med Inst, Chevy Chase, MD, Boston, MA, USA; ³MGH, Boston, MA, USA

**Acute-Phase Response Proteins Are Overexpressed in Vaginal Mucosa and Plasma of HIV-Resistant Women upon Viral Challenge**

Adam Burgener¹, Y. Keynan², A. Meyers², K. Birse², M. Abou³, G. Westmacott³, J. Kimani³, F. Plummer³, K. Fowke³, T. Ball³

¹Publ Hlth Agency of Canada, Winnipeg, Canada; ²Univ of Manitoba, Canada; ³Nat’l Laboratory for HIV Immunology, Canada; ⁴Nat’l Microbiology Laboratory, Canada; ⁵Univ of Nairobi, Nairobi, Kenya

**Optimization of Systemic and Mucosal Immune Responses to SIV and HIV-1 Antigens Using Different Protocols of DNA and Protein Immunization in Macaques**

George N. Pavlakis¹, R. Jalali¹, J. Li¹, A. Valentini¹, M. Rosati¹, V. Patel¹, V. Kulkarni¹, C. Alicea¹, R. Kelly Beach¹, Y. Guan¹, J.D. Litson³, V.M. Hirsch⁴, D. Venzon¹, K.E. Broderick¹, N.Y. Sardesai¹, T.R. Fouts¹, A. Pinter⁷, C. LaBrancie⁸, D.C. Montefiori³, S. Sherr¹, G.D. Tomaras⁸, B.K. Felber¹

¹Nat’l Cancer Inst, NIH, Frederick, MD, USA; ²Inst of Human Virology, Baltimore, MD, USA; ³SAIC-Frederick, Inc., Frederick, MD, USA; ⁴NIAID, NIH, Bethesda, MD, USA; ⁵Inovio Pharmaceuticals, Inc., Blue Bell, PA, USA; ⁶Projectus BioSciences, Inc., Baltimore, MD, USA; ⁷Univ of Medicine and Dentistry of New Jersey, Newark, NJ, USA; ⁸Duke Univ Med CTR, Durham, NC, USA
11:45 – 12:00  Immune Correlates of a Functional Cure Following Therapeutic Vaccination of SIV-Infected Rhesus Macaques  
1Univ of Washington, Seattle, WA, USA; 2Albany Med College, Albany, NY, USA; 3Univ of Pittsburgh, Pittsburgh, PA, USA; 4GSK, Stevenage, UK

10:30 – 11:50  Viral Vaccine Vectors  
Symposium 04  
Chairs: Dan Barouch and Tomas Hanke

10:30 – 10:50  Development of HIV Vaccines— A Vaccine Manufacturer’s Perspective  
Gerald Voss1  
1GSK Vaccines, Rixensart, Belgium

10:50 – 11:10  Sendai Virus Vector Vaccine  
Tetsuro Matano1  
1Nat’l Inst of Infectious Diseases, Tokyo, Japan

11:10 – 11:30  Reinventing the Nucleic Acid Vaccine  
Andrew Geall1  
1Novartis Vaccine and Diagnostics, Cambridge, MA, USA

11:30 – 11:50  Durable Antibody Responses Induced by DNA and Recombinant Modified Vaccinia Ankara Vaccines Expressing HIV-1 Virus-like Particles  
Harriet Robinson1, G. Tomaras2, K. Seaton2, A. Sato3, J. Hural3, S. DeRosa3, N. Frahm3, B. Moss4, P. Goepfert5  
1GeoVax, Inc., Smyrna, GA, USA; 2Duke University Med CTR, Durham, NC, USA; 3FHCRC, Seattle, WA, USA; 4NIAID, NIH, Bethesda, MD, USA; 5Univ of Alabama at Birmingham, Birmingham, AL, USA

12:00 – 13:00  Lunch  
L2, Banquet Hall

12:00 – 13:00  Delegate Networking Lunch  
L2, Banquet Hall

Lunch tables have been reserved for young and early-career investigators to network with senior researchers. The list of participating researchers is available at the conference registration desk along with a sign-up sheet. Space is limited and will be reserved on a first-come, first-served basis.

13:00 – 14:20  New Targets from Acute Infection  
Symposium 05  
Chairs: Bruce Walker and Carolyn Williamson

13:00 – 13:20  Targeting the Female Reproductive Tract Epithelium to Prevent Transmission and Acute Systemic Infection: Lessons Learned in the SIV-NHP Model  
Ashley Haase1  
1Univ of Minnesota, Minneapolis, MN, USA

13:20 – 13:40  Host-Virus Interactions in HIV-1 Rapid Disease Progression  
Javier Martinez-Picado1  
1IrsiCaixa Inst for AIDS Res, HIVACAT, Autonomous Univ of Barcelona, Barcelona, Spain
13:40 – 14:00  Phenotypic Properties of Acute HIV-1 Associated with Transmission Fitness and Virulence
Eric Arts¹
¹Case Western Reserve Univ School of Med, Cleveland, OH, USA

14:00 – 14:20  Modeling the Effects of Vaccination on Acute Phase CD8+ T Cell Immunodominance Patterns to HIV in Humanized Mice
Todd Allen¹
¹Ragon Inst of MGH, MIT and Harvard, Cambridge, MA, USA

13:00 – 13:20  A Pure Native Env Trimer Vaccine Elicits Potent Tier 2 Neutralization to a Quaternary, Glycan-Sensitive Epitope
James Binley¹, B. Chakrabarti², E. Crooks³, S. Du³, J. Muranaka³, K. Narayan³, K. Osawa¹, T. Tong¹, R. Whalen¹, R. Wyatt⁴
¹Torry Pines Inst for Molecular Studies, San Diego, CA, USA; ²IAVI Neutralizing Antibody CTR at The Scripps Research Institute, La Jolla, CA, USA; ³Altravax, Sunnyvale, CA, USA; ⁴The Scripps Res Inst, La Jolla, CA, USA

13:20 – 13:40  Genetic Development of HIV-1 Neutralizing Antibody Lineages
John Mascola¹
¹VRC, NIAID, NIH, Bethesda, MD, USA

13:40 – 14:00  HIV Vaccine Design to Induce Broadly Neutralizing Antibodies
William Schief¹
¹The Scripps Res Inst, La Jolla, CA, USA

14:00 – 14:20  Recombinant Env Interactions with the BCRs of Broadly Neutralizing Anti-HIV Antibodies
Leonidas Stamatatos¹
¹Seattle Biomedical Res Inst, Seattle, WA, USA

13:00 – 14:30  Clinical Trial Follow-up and Retention
Roundtable Discussion
Chairs: Sheena McCormack and Jose Maria Miró

The efforts of researchers and institutions and the support and participation of communities impacted by HIV has been and continues to be essential to advancing efforts to develop an HIV vaccine. This roundtable will discuss the importance of and strategies around recruiting, retaining and following clinical trial participants throughout the entirety of the clinical trial process; lessons learned from strata analysis and sub-studies; as well as challenges and strategies to encourage adherence to additional preventive measures during and after the trial. The session will include brief presentations followed by an open discussion around the issues raised with attendees.

Susan Buchbinder
San Francisco Department of Public Health, San Francisco, CA, USA

Glenda Gray
Perinatal HIV Research Unit, Johannesburg, South Africa

Scott Hammer
Columbia University Medical Center, NY, USA

Punnee Pitisuttithum
Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

14:30 – 15:00  Refreshment Break

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<table>
<thead>
<tr>
<th>Time</th>
<th>Session Title</th>
<th>Location</th>
<th>Chairs</th>
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<tbody>
<tr>
<td>15:00 – 15:20</td>
<td>Therapeutic Vaccines and Viral Latency</td>
<td>L1, Room 113 – 114</td>
<td>Felipe Garcia and Rafick-Pierre Sekaly</td>
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<td>1Mcgill Univ, Montreal, Canada; 2Argos Therapeutics, Durham, NC, USA; 3VGTI Florida, Port St. Lucie, FL, USA</td>
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<td>15:20 – 15:40</td>
<td>Viral Persistence Under ART and the Challenges of Eradicating Myeloid Reservoirs</td>
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<td>Mario Stevenson1</td>
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<td>1Univ of Miami Miller School of Med, Miami, FL, USA</td>
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<td>15:20 – 15:40</td>
<td>Therapeutic Concepts for HIV Eradication</td>
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<td>Romas Geleziunas1</td>
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<td>1Gilead Sciences, Inc., Foster City, CA, USA</td>
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<td>16:00 – 16:20</td>
<td>Immune Mechanisms and HIV Eradication</td>
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<td>Michael Lederman1</td>
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<td>1Case Western Reserve Univ, Univ Hospitals Case Med CTR, Cleveland, OH, USA</td>
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<td>15:00 – 16:20</td>
<td>New Vaccine Concepts</td>
<td>L1, Room 115 – 116</td>
<td>Julia Blanco and Yves Levy</td>
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<td>15:00 – 15:20</td>
<td>Refocusing CTL and Antibody Responses with p24gag Conserved Elements Vaccines</td>
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<td>James Mullins1</td>
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<td>1Univ of Washington, Seattle, WA, USA</td>
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<td>15:20 – 15:40</td>
<td>HIVACAT T- and B-Cell Immunogen Design and Preclinical Testing</td>
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<td>Christian Brander1</td>
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<td>1IrsiCaixa Inst for AIDS Res, HIVACAT, Barcelona, Spain</td>
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<td>15:40 – 16:00</td>
<td>Protection Against HIV: Vaccination to Block Inflammation</td>
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<td>D. Hartigan-O’Connor1, A. Tarantal1, Joseph M. McCune2</td>
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<td>1Univ of California-Davis, Davis, CA, USA; 2Univ of California-San Francisco, San Francisco, CA, USA</td>
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<td>16:00 – 16:20</td>
<td>Immunological and Virological Mechanisms of Vaccine-Mediated Protection Against SIV</td>
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<td>A. Buzby1, L. Mach1, L. Shen1, M. Alam2, T. Denny2, G. Ferrari2, R. Gottardo3, W. Fischer4, B. Korber4, W. Gu5, M. Nason5, Mario Roederer6</td>
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<td>1Beth Israel Deaconess Med CTR, Boston, MA, USA; 2Duke Univ, Durham, NC, USA; 3FHCRC, Seattle, WA, USA; 4LANL, Los Alamos, NM, USA; 5NIAID, NIH, Bethesda, MD, USA; 6VRC, NIAID, NIH, Bethesda, MD, USA</td>
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<td>15:00 – 16:20</td>
<td>Mucosal Immunity</td>
<td>L1, Room 117</td>
<td>Jason Brenchley and Ronald Desrosiers</td>
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<td>15:00 – 15:20</td>
<td>Visualizing Cellular Dynamics of HIV Infection In Vivo</td>
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<td>Thomas Murooka2, M. Deruaz2, F. Marangoni2, V. Vrbac2, E. Seung2, U. von Andrian1, A. Tag2, A. Luster2, T. Mempe2</td>
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<td>1Harvard Med School, Boston, MA, USA; 2Massachusetts General Hospital, Boston, MA, USA</td>
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<td>15:20 – 15:40</td>
<td>Dynamic Interplay Between Virus and Host Occurring at the Female Reproductive Tract During Acute HIV Infection and Treatment</td>
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<td>J. Victor Garcia-Martinez1</td>
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<td>1Univ of North Carolina at Chapel Hill, Chapel Hill, NC, USA</td>
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15:40 – 16:00  Identification and Characterization of Specific IgG-mucin Interactions to Enhance Vaccine Function
Thomas Hope1, G. Alter2, B. Gunn2, M. Shansab2
1Northwestern Univ, Chicago, IL, USA; 2Ragon Inst of MGH, MIT and Harvard, Cambridge, MA, USA

16:00 – 16:20  Prime and Pull: A Vaccine Strategy for Viral Sexually Transmitted Infections
Haina Shin1, A. Iwasaki1
1Yale Univ School of Med, New Haven, CT, USA

16:20 – 16:45  Break

16:45 – 17:32  HIV Transmission
Poster Discussion Session 04
Chairs: Eric Hunter and Alexandra Trkola

16:45 – 16:52  Rapid Detection of Early HIV-1 Infections by Direct Sequencing of an Hypervariable Segment of the Viral Env Gene
Gabriel Damilano1, E. Socías2, C. Magneres2, G. Turk1, O. Sued1, J. Ruiz1, M. Gomez-Carrillo1, H. Salomon1, D. Dilemía1
1Inst for Biomedical Res on Retroviruses and AIDS, Buenos Aires, Argentina; 2HUESPED Fundation, Buenos Aires, Argentina; 3Emory Vaccine CTR, Atlanta, GA, USA

16:53 – 17:00  Mechanism of HIV Entry into the Columnar Epithelium of the Female Genital Tract
Ann Carias1, M. Anderson1, T. Hope1
1Northwestern Univ, Chicago, IL, USA

17:01 – 17:08  Impact of Host Cell-Specific Glycosylation Differences on SIV Infectivity and Mucosal Transmission
Christina Karsten1, F.F. Buettner2, I. Nehlmeier1, U. Sauermann1, B. Neumann1, C. Stahl-Hennig1, R. Gerardy-Schahn2, S. Pöhlmann1
1German Primate CTR, Göttingen, Germany; 2Inst for Cellular Chemistry, Hannover Med School, Hannover, Germany

17:09 – 17:16  The V1V2 Domain Preserves Envelope Functionality During Free Virus Transmission but is Largely Dispensable for Cell-Cell Transmission
Oliver Brandenberg1, P. Rusert1, C. Magnus1, J. Weber1, R.R. Regoes2, A. Trkola1
1Univ of Zurich, Zurich, Switzerland; 2ETH Zurich, Zurich, Switzerland

17:17 – 17:24  Relative Contribution of Gag, Nef, and Env to Minority Variant Transmission Revealed by Deep Sequencing of Transmission Pairs
Rebecca Batorsky1, M. Schaefer1, K. Power1, D. Tully1, E. Hunter2, T. Allen1
1Ragon Inst of MGH, MIT and Harvard, Cambridge, MA, USA; 2Emory Univ, Atlanta, GA, USA

17:25 – 17:32  The Nervous System Can Restrict ‘At a Distance’ Langerhans Cell-Mediated HIV-1 Transmission via the Neuropeptide Calcitonin Gene-Related Peptide
Morgane Bomsel1, A. Drillet1, L. Lopalco2, G. Tambussi1, D. Tudor1, M. Zerbib3, N. Bary de Lonchamps1, Y. Canor1
1Institut Cochin- CNRS UMR 8104- INSERM U1016- Paris Descartes Univ, Paris, France; 2San Raffaele Scientific Inst, Italy; 3GH Cochin-St Vincent de Paul, Paris, France
### Poster Discussion Session 05

**Chair:** Barbara Felber and Julie McElrath

#### 16:45 – 16:52

**Cross-Neutralizing Antibodies Are Elicited in Macaques Immunized with DNA and Protein Env Vaccines Derived from Subjects During Development of Breadth**


1. VGTI, Oregon Health & Science Univ, Beaverton, OR, USA; 2. Seattle Biomed, Seattle, WA, USA; 3. FHCRC, Seattle, WA, USA

#### 16:53 – 17:00

**Synthetic Enhanced EP Delivered Ig DNA Vector Drives Biologically Relevant Anti-HIV-1 Envelope Responses In Vivo**


1. Univ of Pennsylvania School of Medicine, Philadelphia, PA, USA; 2. Inovio Pharmaceuticals, Blue Bell, PA, USA; 3. Univ of South Florida Morsani College of Medicine, Tampa, FL, USA

#### 17:01 – 17:08

**Increased Neutralizing Antibody Responses Elicited by a Bivalent HIV-1 Env Protein Vaccine Comprising Stable Mosaic and Clade C HIV-1 gp140 Trimers**

Joseph Nkolola, C.A. Bricault, A. Cheung, J. Perry, M. Seaman, D. Barouch

1. Beth Israel Deaconess Med CTR, Boston, MA, USA

#### 17:09 – 17:16

**Association of CD8+ T Cell Responses to Conserved HIV Epitopes with Viral Control: Implications for HIV Vaccine Design**


1. Seattle Biomedical Res Inst, Seattle, WA, USA; 2. FHCRC, Seattle, WA, USA; 3. Polyclinic Infectious Disease, Seattle, WA, USA; 4. Univ of Washington, Seattle, WA, USA

#### 17:17 – 17:24

**High Affinity Recognition of Synthetic Glycopeptides by HIV-1 gp120 V1V2 Loop Broadly Neutralizing Antibodies and Their Unmutated Common Ancestors**


1. Duke University Medical Center, Durham, NC, USA; 2. Sloan-Kettering Institute for Cancer Research, New York, NY, USA; 3. Boston University School of Medicine, Boston, MA, USA; 4. Dana-Farber Cancer Institute, Boston, MA, USA

#### 17:25 – 17:32

**Novel R5 Tropic Founder Env SHIV for Nonhuman Primate Immunoprophylactic Studies**


1. SAIC Frederick, Inc., Frederick Nat’l Labs, Frederick, MD, USA; 2. The Scripps Res Inst, La Jolla, CA, USA; 3. Nat’l Cancer Inst, Frederick, MD, USA; 4. The Rockefeller Univ, New York, NY, USA
16:45 – 17:30  Non-vaccine Prevention and Social-ethical Aspects  
Poster Discussion Session 06  
Chair: Michael Meulbroek

16:45 – 16:52  Anti-HIV Activity of 5-Hydroxytyrosol, a Microbicidal Candidate  
P.09.01 D  
L. Bedoya1, M. Belfrán2, D. Auñón3, E. Gómez-Acebo1, Jose Alcami3  
1Seprox Biotech, Spain; 2Centro Nacional de Microbiología Instituto de Salud Carlos III, Madrid, Spain

16:53 – 17:00  Neutralization of Diverse HIV Strains by V3 Specific DARPins with Different Structural Preferences in Epitope Recognition  
P.09.02 D  
Nikolas Friedrich1, A. Mann1, E. Stiegeler1, Y. Wu1, A. Batyuk1, P. Rusert1, J. Weber1, B. Dreier1, M. Robbiani2, J.A. Robinson1, A. Plückthun1, A. Trkola1  
1Univ of Zurich, Zurich, Switzerland; 2Population Council, New York, NY, USA

17:01 – 17:08  Getting to Zero with an HIV Vaccine: Modeling the Impact of Introduction of the Current HIV Vaccine Pipeline Under the Investment Framework  
P.11.01 D  
J. Aylward2, E.D. Donaldson1, K. Fisher1, T. Harmon4, M. McGlynn4, Arne Naeveke2, J. Stover4, M. Warren4  
1AVAC, New York, NY, USA; 2IAVI, New York, NY, USA; 4Futures Inst, Glastonbury, CT, USA

17:09 – 17:16  A Survey of Community Opinions and Preferences on PrEP, Microbicides and Vaccines in 5 Regions and with Key Populations in Kenya  
P.11.02 D  
Judie Mbogua1, B.P. Ngongo1, J. Ndegwa2, B. Bender1, F. Manguyu1  
1IAVI, Nairobi, Kenya; 2Kenya AIDS NGO Consortium, Nairobi, Kenya

17:17 – 17:24  The Role of Stakeholders Involvement Towards the Success of Vaccine/Clinical Trials—The Partners PrEP Study  
P.11.03 D  
Charles Brown1  
1Infectious Diseases Inst - PrEP Study, Kampala, Uganda

17:30 – 19:00  Poster Session 02 and Reception  
L1, Room 111 – 112  
L2, Room 211 – 212

Poster presentations are scheduled by topic and the last two digits of the abstract presentation number. For example, 1.01 is an odd-numbered abstract; whereas 1.02 is an even-numbered abstract.

Posters are available for viewing throughout the conference, however, Poster Session 02 will feature even-numbered posters only.

**Poster Topics Presented in L1, Room 111 – 112**  
- P01: Adjuvants, Immunogens and Inserts  
- P02: Animal Models and Preclinical Trials  
- P03: B Cell Immunology and Antibody Functions  
- P04: Clinical Vaccine Trials and Trial Site Challenges  
- P05: HIV Transmission and Viral Diversity

**Poster Topics Presented in L2, Room 211 – 212**  
- P06: Immunogenetic Factors  
- P07: Innate Immunity  
- P08: Mucosal Immunity  
- P09: Non-Vaccine Prevention  
- P10: Pediatric and Adolescent Infections and Trials  
- P11: Social/Ethical/Access/Regulatory Issues  
- P12: T Cell Immunity  
- P13: Vaccine Concepts and Design
Thursday, 10 October

A full complimentary breakfast buffet is available in the conference hotels: The AC Hotel Barcelona Forum by Marriott and the Hotel Barcelona Princess. Breakfast will not be available at the CCIB.

07:30 – 14:30 **Registration / Coat Check**
L0, Entrance Hall

08:30 – 10:00 **Innovations in HIV Vaccine Discovery**
Plenary Session 03
Chairs: Brigitte Autran and George Pavlakis
L1, Room 113 – 116

08:30 – 09:00 **Nanotechnology Approaches for Designing Novel HIV Vaccines**
M. Luo¹, María José Alonso²
¹Univ of Manitoba, Manitoba, Canada; ²Univ of Santiago de Compostela, Santiago de Compostela, Spain
PL03.01

09:00 – 09:30 **Cutaneous Routes for HIV Vaccination Strategies**
Behazine Combadiere³
³INSERM, UPMC, Paris, France
PL03.02

09:30 – 10:00 **HIV Vaccine Development: A View from the Outside**
Adrian Hill⁴
⁴Univ of Oxford, Oxford, UK
PL03.03

10:00 – 10:30 **Refreshment Break**
L1, Foyer

10:30 – 13:00 **Emerging Clinical Trial Data**
Plenary Session 04
Chairs: Mariano Esteban and Britta Wahren
L1, Room 113 – 116

10:30 – 10:55 **The First Clinical Evaluation of Conserve-Region Vaccines in Humans**
P. Hayes⁵, J. Vasilyeva⁶, N. Frahm⁷, J. Cox², S. Colloca³, A. Nicosia⁴, J. Gilmour⁵, Tomas Hanke⁶
⁵IAVI, Imperial College, London, UK; ⁶FHCRC, Seattle, WA, USA; ⁷Okairos, Rome, Italy; ⁸Okairos, CEINGE, Univ of Naples, Naples, Italy; ⁹Univ of Oxford, Oxford, UK
PL04.01

10:55 – 11:20 **The HIVIS and TaMoVac Studies, a North–South Collaboration**
Muhammad Bakari⁸
⁸Muhimbili Univ of Hlth and Allied Sciences, Dar es Salaam, United Republic of Tanzania
PL04.02

11:20 – 11:45 **Looking Back to Move Forward: Understanding ALVAC/AIDSVAX Immune Responses**
Robert O’Connell¹
¹AFRIMS, Bangkok, Thailand
PL04.03

11:45 – 12:10 **Vaccine Induced Antibody Responses in HVTN 505, a Phase IIb HIV-1 Efficacy Trial**
Georgia D Tomaras¹, X. Shen¹, K. Seaton¹, H. Janes², D. Grove³, A. deCamp³, Y. Fong³, H. Liao⁴, Z. Yang⁵, T. Xu⁴, J. H. Kim⁶, N. L. Michael⁷, R. T. Bailer³, G. Ferrari¹, J. Mascola¹, R. A. Koup³, G. Nabel⁴, L. Corey³, S. Karuna², D. C. Montefiori¹, M. J. McElrath², B. F. Haynes³, P. Gilbert⁴, B. S. Graham³, M. Sobieszczyk⁵, S. M. Hammer³
¹Duke University Medical Center, Durham, NC; ²Fred Hutchinson Cancer Research Center, Seattle, WA; ³Vaccine Research Center, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.; ⁴Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD; ⁵Division of Infectious Diseases, Columbia University Medical Center, New York, NY; ⁶Sanofi, USA
PL04.04
12:10 – 12:35 An Update on the Phambili/HVTN 503 Study, a Phase IIB HIV Vaccine Efficacy Study Investigating MRK Ad5 HIV-1 Subtype B gag/pol/nef Vaccine
Glenda Gray1
1Perinatal HIV Res Unit, Johannesburg, South Africa

12:35 – 13:00 Meta-Analysis of Ad5-vector HIV Vaccine Trials to Assess the Vaccine Effect on HIV Acquisition
1FHCRC, Seattle, WA, USA; 2NIAID, Bethesda, MD, USA; 3Merck, USA; 4Univ of Rochester Med Center, Rochester, NY, USA; 5International AIDS Vaccine Initiative (IAVI); 6Aurum Institute, Johannesburg, South Africa; 7Columbia Univ, New York, NY, USA; 8Perinatal HIV Research Unit, South Africa; 9San Francisco Dept of Publ Hlth, San Francisco, CA, USA; 10U.S. Military HIV Research Program, Bethesda, MD, USA

13:00 – 13:50 Closing Session
L1, Room 113 – 116
Chairs: Christian Brander, Bonaventura Clotet, and José M. Gatell

13:00 – 13:20 What Has 30 Years of HIV Vaccine Research Taught Us? Lessons from The Past To Inform The Future
José Esparza1
1Bill & Melinda Gates Foundation, Seattle, WA, USA

2013 Conference Chairs
Christian Brander1, Bonaventura Clotet2, José M. Gatell3
1IrsiCaixa Inst for AIDS Res, HIVACAT, ICREA, Barcelona, Spain; 2IrsiCaixa Inst for AIDS Res, HIVACAT, Barcelona, Spain; 3Hospital Clinic, IDIBAPS, HIVACAT, University of Barcelona, Spain

13:25 – 13:35 AIDS Vaccine Research, Where We Are and Where We Are Going
Nina Russell1,2
1Bill & Melinda Gates Foundation, Seattle, WA, USA; 2Global HIV Vaccine Enterprise, New York, NY, USA

13:35 – 13:50 HIV Research for Prevention
2014 Conference Co-Chairs
Eric Hunter1, Robin Shattock2
1Emory Univ, Atlanta, GA, USA; 2Imperial College London, UK

15:00 – 17:00 Satellite Session
See pages 16 of Program Book for detailed information.
OS.01

Partnership Towards a Tough Target: Communities Are an Essential Ingredient, Not a Cherry on Top

N. Yola1
1NACOSA, Cape Town, South Africa

History has taught us that the way to eradicate a global epidemic is to design, produce massively and then systematically vaccinate the population with an effective prophylactic vaccine. We have also learned from the past that this route to a "silver bullet" may be a long and complicated one. Today almost 60 million men, women and children having been infected and nearly 25 million attributable deaths, twenty five years of this epidemic has taken a horrific toll. In the small country of South Africa, more people live with HIV than anywhere else in the world. Too many are being infected in that country and around the world as we speak. The urgency we felt 3 decades ago to find an effective vaccine has in no way diminished. We saw in 2009, the first hope that a prophylactic HIV vaccine may be possible, but it is likely that this endeavor will, in order to realize real Progress require in addition meaningful Partnership and diligent Perseverance. It is a time, therefore, to take stock of the role stakeholders have played and to ensure strategic engagement so they not only continue to support the field, but make it better.

The global race against HIV/AIDS presents realities for all concerned. The scientific agenda is not only that of discovering antibodies or demystifying the immune response, but of addressing the realities of affected communities. Scientific research is less likely to achieve what it sets to if these realities are overlooked. The social, economic, political and personal realities of people from around the world vary and are significant. The real conditions of people in developing countries have potential to present both challenging and supportive environment for the successes of the trials and ultimately in the implementation of an effective vaccine. The theme of this meeting highlights the power of drawing scientific Progress together with the ideals of Partnership and Perseverance. As the vaccine field advances, we can celebrate strong partnerships with trial communities and wider stakeholders. There is willingness to work towards a supportive enabling environment for clinical trials which can only be achieved through expressive and practical partnership. To persevere, we must not desert this investment, but look at opportunities to sustain it for the long term, until an end to AIDS is realized.

OS.02

HIV Vaccines in the Context of Vaccine Development in General

S.A. Plotkin1
1University of Pennsylvania, Doylestown, PA, USA

The HIV vaccine picture continues to be chiaroscuro, with bright spots and shadows. However, real progress has been made, with evidence for efficacy, identification of possible correlates of protection, definition of immune priming in prime-boost regimens, structural analysis of gp160, and the possible utility of effector CD8+ cells, just to name a few. On the other hand, it appears that non-specific immune activation may be undesirable, so the properties of vectors are critical. I will point out that other vaccines in development have had similar problems and that vaccine development is not always straightforward.
OS.03

**Insights from HIV Vaccine Efficacy Trials: What We Are Learning from HVTN 505?**

*M. Sobieszczyn*  
1Columbia University, New York, NY, USA

Thirty years following the discovery of HIV-1, a safe and effective vaccine remains elusive. The modest protective effect seen in RV144 contrasts with the negative results of VAX 003, VAX 004, Step, and Phambili trials. To this latter list must now be added HVTN 505, a phase 2b trial of a multigene DNA prime/rAd5 boost vaccine regimen. This study enrolled 2504 men and transgender women who have sex with men. Vaccinations were discontinued following a DSMB review in April 2013 as efficacy futility was declared for both the HIV acquisition and post-acquisition setpoint viral load endpoints. Although disappointing, data generated in HVTN 505 about the immunogenicity profile of the vaccine will add new knowledge to the vaccine discovery process and provide guidance for prioritizing immunogenicity endpoints for subsequent Phase I/II vaccine trials.

OS.04

**Toward Ending the HIV/AIDS Pandemic: Synergy Between Vaccine and Non-Vaccine Interventions**

*A. Fauci*  
1NIAID, NIH, Bethesda, MD, USA

Significant decreases in rates of HIV acquisition likely can be achieved with the growing armamentarium of non-vaccine prevention and treatment modalities. However, accomplishing this goal without an HIV vaccine will take much longer and, importantly, may not be sustainable. Recent advances in understanding the complex B-cell pathways to potentially protective immune responses, together with greater insight into the role of T cells in this process has heightened the feasibility of developing next-generation HIV vaccines. A synergistic process involving non-vaccine and vaccine modalities could lead to effective and sustained prevention of HIV acquisition, and perhaps, for some infected individuals, a functional cure.
PL01.01

Manufacturing HIV Envelope Proteins for HIV Vaccines: An NIAID Perspective of Where We Are and What Is Still Needed

M. Pensiero

1NIAID, NIH, Bethesda, MD, USA

The recent success of RV144 has refocused the HIV vaccine field on the importance of including an Env immunogen as part of an efficacious vaccine regimen. Over the past two years, there has been considerable effort focused on attempting to manufacture HIV Env proteins towards that purpose. However, despite the best efforts, there still is a dearth of HIV Env available for clinical testing, let alone for use in clinical research, to answer basic fundamental questions of how best to improve neutralizing breadth and durability. This abstract will provide a perspective from an extramural NIAID scientist responsible for managing a portfolio of large grants and contracts targeted to support HIV Env manufacturing. Not only will common manufacturing hurdles be discussed, but also a potential path forward to accelerate advancing HIV Env protein manufacturing will be presented, such as using transient transfection, management of common product development pitfalls, preferred CMO/Analytics outsourcing, etc.

Understanding the manufacturing issues will provide a clearer framework to develop possible solutions and a more realistic expectation of the timelines necessary to manufacture an HIV Env of sufficient quality attributes (e.g., potency, purity, and identity) for use in human clinical trials.

PL01.02

Vaccine Induction of T Cell Immunity to HIV-1

A. McMichael

1University of Oxford, Oxford, UK

Because a vaccine that stimulates highly protective antibody immunity to HIV-1 is still distant, development of a ‘T cell vaccine’ remains an option. In macaques, such a vaccine can clear virus early in infection or reduce virus load after infection. Combining a T cell vaccine with an antibody inducing vaccine could offer significant benefits if the latter was not 100% efficient. However, the STEP and HVTN505 trials of T cell stimulating vaccines showed no efficacy in humans. Possibly these vaccines generated T cell responses that were too weak, too narrow and too focused on variable HIV-1 epitopes. In macaques, protective CD8 T cell responses were of much greater magnitude and breadth. Indeed, very strong broad activated CD8 T cells can clear early SIV infection. What T cell response features determine virus control? Elite HIV-1 controllers have: polyfunctional Gag specific T cell responses, often restricted by HLA allotypes B57, B58, B27 or B81 which present conserved epitopes, cross reacting T cell clonotypes and T cells that suppress virus replication in vitro. However, while antigen sensitivity and virus inhibition correlate strongly they are not specific for clonotype; state of T cell activation rather than polyfunctionality may determine antigen sensitivity. Focus on conserved and vulnerable epitopes, not only those presented by protective HLA types, must be important. How these relate the fitness landscape is also critical. Vaccines should target these epitopes rather than following normal immunodominance patterns. There are at least four immunogen designs aimed at doing this. Our phase one trial of the HIVconsv construct shows that such constructs are immunogenic in humans and stimulate T cells that can recognize HIV infected cells and suppress virus replication. There is now a need to agree on how to optimize such vaccines and to progress them to efficacy testing.
The induction of broad neutralizing antibodies (BnAbs) is hindered by a myriad of roadblocks, not the least of which includes host immune regulatory controls. In HIV-1 infected individuals who do develop plasma BnAb activity, the evolved virus variants are responsible for BnAb induction. The Duke CHAVI-ID team has begun to map both BnAb and virus evolution pathways in 17 individuals who were followed from the time of HIV-1 infection through induction of plasma BnAbs. The status of this work will be presented and new immunization strategies that derive from this work will be discussed.
Genital Immunology and HIV Susceptibility

R. Kaul

1University of Toronto, Ontario, Canada

Although HIV transmission is relatively rare after sexual exposure, it has resulted in a devastating global pandemic. Susceptibility to HIV is heterogeneous, but understanding the mucosal immune determinants of sexual transmission is complicated by the low frequency of transmission.

We have carried out ex vivo studies of cervical and foreskin mucosal immunology in cohorts of low risk, HIV-infected and HIV-exposed, persistently seronegative (HEPS) participants from Canada and East Africa. Studies use endocervical cytobrush samples and ectocervical biopsies from female participants, and foreskin tissues obtained during elective male circumcision. Our focus to date has been on mucosal T cell parameters and soluble genital immune factors associated with HIV susceptibility. Mucosal T cell immune activation and increased expression of the HIV co-receptor CCR5 and/or α4β7 characterize HIV-enhancing conditions such as asymptomatic herpes infection, bacterial vaginosis and candidate microbicides that cause genital irritation. Studies performed by blinded investigators demonstrate striking similarities between the genital immune environment of HEPS women and men. However, only some of these HEPS parameters translate into reduced HIV acquisition in prospective cohort studies, while others are associated with increased HIV susceptibility and may be markers for high-risk sexual activity. Soluble immune factors present in genital secretions, including pro-inflammatory cytokines/chemokines and alpha defensins, correlate with increased mucosal T cell “targets” and with HIV susceptibility, while IgA with virus-neutralizing capacity is associated with protection. Mucosal immunology is an important determinant of HIV transmission and susceptibility, but is difficult to study in human cohorts and even more difficult to manipulate therapeutically. Better tools to rapidly define the impact of candidate microbicides, vaccines and other agents on mucosal HIV susceptibility are urgently needed.

Immunodeficiency Virus and the MHC: Past, Present and Future

P. Goulder

1University of Oxford, Oxford, UK

The role of the MHC in control of immunodeficiency virus infection will be addressed, focusing on HIV infection in humans and its SIV model in macaques. The studies that have brought us to the current state of the art will be reviewed and the potential future impact of HLA/HIV coevolution discussed.
The Role of HIV-1 Viral Evolution in Shaping Broadly Neutralizing Antibodies

P. Moore

1National institute for Communicable Diseases, Johannesburg, South Africa

Broadly neutralizing antibodies develop in about a fifth of HIV infected people, but usually only do so after years of infection, in the presence of high levels of antigenic stimulation. Other factors that lead to the development of these kinds of antibodies remain unclear. However, the association between viral diversity, superinfection, and the development of breadth supports a role for viral evolution in driving such responses. We have used detailed longitudinal analyses from the CAPRISA cohorts to describe the role of HIV envelope evolution in shaping broad antibody responses. This ranges from the creation and/or exposure of conserved epitopes through viral escape, to the formation of multiple co-circulating immunotypes, all of which contribute to the development of broadly neutralizing responses. Understanding these interactions between the virus and the host may inform novel immunization strategies to elicit protective antibodies.
Our group has designed novel nanostructures intended to transport antigens across biological barriers and to target them to the immune system. These nanocarriers have been made of safe materials, i.e. poly(lactic acid) and polysaccharides. Within this frame, we have identified some critical parameters that confer special properties to these nanocarriers, i.e. overcoming mucosal barriers and being attracted by macrophages and dendritic cells. Using antigen models such as tetanus toxoid (TT) and recombinant Hepatitis B surface Antigen (rHBsAg) we have shown the ability of the resulting nanovaccines to elicit significant and long-lasting responses following either nasal or i.m. administration.

More recently, in collaboration with the University of Manitoba, we have applied the nanodelivery technology to a novel HIV vaccine targeting sequences around the 12 protease cleavage sites (PCS). We have packaged the 12 20-amino acid peptides into a polymer nanomatrice and tested the resulting nanovaccine as a nasal boost in a preclinical study in Cynomolgus macaques. The results showed that nanopackaged antigens were able to boost T cell and antibody responses to the peptides in Cynomolgus macaques. Moreover, we observed that the macaques could withstand higher dose of intrarectal SIVmac239 challenge. The vaccinated macaques could also maintain higher CD4+ T cell counts than the controls. These promising results led us to plan further preclinical studies to test whether nanodelivery of this PCS peptide-based vaccine can be used to prime and boost T cell and antibody response and protect macaques from multiple SIVmac251 intravaginal challenge. The conclusion of this preclinical study is that polymer nanostructures are able to ensemble peptide antigens and help them overcoming the nasal mucosal barrier and reaching the underlying immunocompetent cells. Overall, this nanovaccine approach may represent a new paradigm in the development of novel peptide-based vaccines and, in particular, HIV vaccines.
HIV vaccine development faces an extremely difficult scientific challenge and innovative new technologies are likely required for an efficacious product. The fundamental issues in HIV vaccine design and development largely overlap with problems being addressed in other areas of both human and veterinary vaccinology. But HIV vaccine development probably benefits less from this extensive resource of experience and know-how than other vaccine fields. Why this rather inward-looking approach persists is unclear but may relate to the extensive size of the HIV vaccine field and its exceptional level of funding support. I will highlight some of the striking parallels between HIV and other vaccine development programmes and illustrate how HIV vaccine developers might benefit from lessons learned in other areas. These areas include adjuvant choice, optimisation of T cell induction with vectored vaccine regimes, mucosal immunisation, small scale efficacy trial design, and evaluation of clinical trial results of marginal statistical significance. A remarkably large number of new vaccine technologies have been pioneered in the malaria field, because of the availability of established challenge models in both human and mice, and some recent exciting developments will be outlined. Some current widespread views in the HIV field appear to outsiders to have very little scientific and statistical support. These include the belief that true efficacy, and even a correlate of vaccine efficacy, was demonstrated in the RV144 Thai trial, that there is likely to be a safety issue with the use of Ad5 viral vectors, and that antibody-based vaccines are more likely to show true efficacy in the short to medium term than T cell-based HIV vaccines.
**PL04.01**

The First Clinical Evaluation of Conserve-Region Vaccines in Humans

P. Hayes¹, J. Vasilyeva², N. Frahm³, J. Cox¹, S. Colloca³, A. Nicosia⁴, J. Gilmour¹, T. Hanke⁵
¹Imperial College, London, UK; ²Fred Hutchinson Cancer Research Center, Seattle, WA, USA; ³Okairos, Rome, Italy; ⁴Okairos, CEINGE, University of Naples, Italy; ⁵University of Oxford, Oxford, UK

Vaccines focusing T cells on the conserved regions of the HIV-1 proteome as a way of addressing HIV-1 diversity and escape were tested for the first time in humans. The vaccines were safe and showed efficacy against HIV-1 in vitro.

**PL04.02**

The HIVIS and TaMoVac Studies, a North-South Collaboration

M. Bakari³
³Muhimbili University of Health and Allied Sciences, Dar es Salaam, United Republic of Tanzania

HIV remains a significant health problem globally, but more so in developing countries. Efforts towards a safe, affordable and efficacious vaccine have seen the development of partnerships between the North and the South in the search for such a vaccine. The HIVIS and TaMoVac programmes have built and consolidated capacity in Tanzania and Mozambique to conduct HIV vaccine trials, following initial investments by European partners. The specific activities conducted have included studies aimed at optimizing HIV vaccine delivery; expansion of capacity to perform future phase IIB and III trials; extension of current adult target cohorts to youths; and developing capacity to investigate preventive HIV vaccines in neonates. The presentation will focus on clinical trial data so far accrued.
Looking Back to Move Forward: Understanding ALVAC/AIDSVAX Immune Responses

R. O’Connell
1USAMC-AFRIMS, Bangkok, Thailand

Improving upon the 31.2% vaccine efficacy seen following ALVAC-HIV/AIDS V/C prime-boost administration in the RV144 trial is one potential avenue for development of a licensed preventive HIV vaccine. Building upon earlier identification of correlates of risk and sieve analyses suggesting the importance of anti-V2 antibody responses, recent analyses have identified cross-clade reactivity to scaffolded V1V2 constructs as correlates of risk and suggested an important role in IgG3 anti-envelope responses. Work with new monoclonal antibodies has provided insight into the evolution of antibody sequences and potential structural interactions with the V2 domain of gp120 protein. Recent animal studies have also provided important corroborative information. Host genetic analyses suggest an association between HLA and acquisition. Additionally, correlates analysis in combination with molecular sieve analysis have highlighted the potential importance of anti-V3 responses.

Gene expression analysis has defined potential mechanisms underlying cellular responses associated with vaccine protection. Finally, late boosting of RV144 vaccine recipients, 6-8 years following their last RV144 vaccination, provides preliminary data regarding antibody and cellular responses that may assist in assessing schedule adjustment. Finally, the availability of potential correlates of risk in RV144 permit review of previous studies to look at the impact of immunogens, primes, and adjuvants on the development of anti-V1V2 antibody. This talk will address these results, as well as plans for the next efficacy trial.

Vaccine Induced Antibody Responses in HVTN 505, a Phase Ib HIV-1 Efficacy Trial

1Duke University Medical Center, Durham, NC; 2Fred Hutchinson Cancer Research Center, Seattle, WA; 3Vaccine Research Center, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.; 4Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD; 5Division of Infectious Diseases, Columbia University Medical Center, New York, NY; 6Sanofi, USA

The phase Ib multiclade (A,B,C) HIV-1 DNA prime, multiclade (A,B,C) rAd5 boost vaccine (HVTN 505) given to U.S. Ad5 seronegative volunteers was halted due to lack of efficacy. A previous vaccine trial, the RV144 ALVAC prime, A/E gp120 protein boost trial, with 31.2% efficacy, demonstrated that gp70 V1/V2 IgG antibodies significantly correlated with decreased risk of infection and plasma HIV-1 Env IgA antibodies correlated with decreased vaccine efficacy. We examined humoral responses in HVTN 505 for vaccine immunogenicity and then compared these responses to the RV144 vaccine induced antibody response. Analyses of humoral responses between the two trials with different efficacy outcomes will be presented. Comparative immunogenicity analyses can provide insights into assessment of potentially protective antibody responses.
**PL04.05**

**An Update on the Phambili/HVTN 503 Study, a Phase IIB HIV Vaccine Efficacy Study Investigating MRK Ad5 HIV-1 Subtype B gag/pol/nef Vaccine**

G. Gray

1 Perinatal HIV Research Unit, Johannesburg, South Africa

The Phambili study, conducted in South Africa, evaluating the efficacy of MRK Ad5 HIV-1 subtype B gag/pol/nef vaccine in test of concept HIV vaccine trial. Enrolment into Phambili was paused, vaccinations stopped and vaccinations unblinded when the Step study evaluating the same vaccine in the Americas, Caribbean and Australia was shown to be non-efficacious at the first interim DSMB. We present data on the long-term follow-up of Phambili participants. We present the final analysis of the Phambili endpoint data for three time periods: all study follow-up, the first 18 months of follow-up, and 18 months after enrollment to the end of follow-up. Cox proportional hazards model were used to estimate the hazard ratios (HR) for HIV-1 infection due to vaccination (vaccine:placebo), overall and within subgroups, adjusted for baseline HSV-2 status. Of the 801 participants enrolled, 100 acquired HIV-1 infection. More infections occurred in vaccinees (n=63) as compared to placebo (n=37) (adjusted HR (vaccine:placebo) 1.70, 95% CI 1.13-2.55). In unplanned subgroup analyses, the HR was most pronounced after 18 months (adjusted HR 2.32, 95% CI: 1.26-4.25); during this period, it was highest in men (HR=4.26, 95% CI 1.43-12.65) and less evident in women (HR=1.66, 95% CI:0.78-3.55) (interaction p = 0.16). There was no increase in HR with the number of vaccinations received. Although there was no overall significant difference in retention between vaccine and placebo (p=0.40), amongst men there was a trend toward a higher drop-out rate in the placebo group (p=0.18). We saw an increased HR two years after vaccination. This was not associated with the number of vaccinations received. As most study follow up was in the unblinded period, our analyses maybe subject to confounding. Nonetheless, our observations of possible vaccine induced- enhancement warrant further investigation. To this end, Phambili participants are being recalled.

**PL04.06**

**Meta-Analysis of Ad5-vector HIV Vaccine Trials to Assess the Vaccine Effect on HIV Acquisition**

Y. Huang1, D. Follmann2, M. Nason2, L. Zhang1, Y. Huang1, D. Mehrotra3, Z. Moodie1, B. Metcalf1, H. Janes1, M. Keefer4, G. Churchyard6, M. Robb10, P. Fast5, A. Duerr1, L. Corey1, J. Mascola2, B. Graham2, M. Sobieszczyk7, J. Kublin1, M. Robertson3, S. Hammer7, G. Gray8, S. Buchbinder9, P. Gilbert1

1Fred Hutchinson Cancer Research Center, Seattle, WA, USA; 2National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA; 3Merck, USA; 4University of Rochester Medical Center, Rochester, NY, USA; 5IAVI, NY, New York, USA; 6Aurum Institute, Johannesburg, South Africa; 7Columbia University, New York, NY, USA; 8Perinatal HIV Research Unit (PHRU), South Africa; 9San Francisco Department of Public Health, San Francisco, CA, USA; 10U.S. Military HIV Research Program, Bethesda, MD, USA

Three randomized, double-blinded, placebo-controlled proof-of-concept Phase 2b efficacy trials, Step, Phambili and HVTN505, failed to demonstrate a protective effect on HIV acquisition using regimens containing Adenovirus serotype 5 (Ad5) vector HIV vaccines. Vaccinations in all three were halted early and studies subsequently unblinded. Step and Phambili tested a Merck vaccine regimen of HIV-Ad5 immunization at Weeks 0, 4 and 26 in the Americas and South Africa, respectively. HVTN505 tested a VRC vaccine regimen of HIV-DNA immunization at Weeks 0, 4 and 8, and HIV-Ad5 immunization at Week 26 in the US. Extended follow-up of Phambili and HVTN505 are ongoing.

To better estimate the effect of HIV Ad5 vaccines on the risk of HIV infection, both overall and in important subgroups, a meta-analysis was conducted using individual-level data available through August 26, 2013. The mean (range) months of follow-up was 35 (0−51) for Step, 35 (0−43) for Phambili, and 15 (0−28) for HVTN505. Three pre-efficacy trials, HVTN204, RV172 and IAVI001 and their extended follow-up studies of the VRC vaccine regimen also accrued HIV infections and were included.

Cox proportional hazard models adjusting for potential baseline confounding factors including common demographic and risk behavior variables were used to estimate hazard ratios (HRs, vaccine vs. placebo) of HIV infection, allowing separate placebo group hazards for each study. Nonparametric smooth estimates of the HRs over continuous follow-up time were used to assess time-varying HRs. Co-primary analyses measured follow-up based on time since first vaccination and time since first Ad5 vaccination. Interaction tests were used to assess whether HRs differed across studies, time periods, and subgroups defined by gender, Ad5 serostatus, and circumcision status. This talk will present the results of the meta-analysis and discuss the strengths and limitations of such an approach.
**S01.01**

Antiviral Activity of NK Cells in HIV-1 Infection

*M. Altfeld*¹

¹Heinrich-Pette-Institut, Hamburg, Germany

NK cells play a critical role in the control of viral infections, and increasing data also suggest an antiviral activity against HIV. The mechanisms by which NK cell recognize HIV-1 infected cells and can mediate anti-HIV activity will be discussed.

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**S01.02**

Adjuvants Modify Antibody Effector Function

*A. Mahan*¹

¹Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA

Antibody N-glycosylation is important for both determining the inflammatory profile of IgGs and modifying their capacity to induce effector functions, such as antibody-dependent cellular cytotoxicity and phagocytosis. This talk will present research into the innate and adaptive signals that program specific changes in IgG glycosylation in order to determine which signals can be used to elicit highly functional HIV-specific antibodies in the context of a vaccine. We have shown that activation of nucleic acid–sensing TLRs as well as a combination of adaptive signals can induce significant changes in the expression of key glycosylation enzymes and are currently determining how the expression of these enzymes is regulated and programmed. Ultimately, understanding if and how antibody glycosylation is recalled will be important for eliciting antibodies with enhanced function through vaccination.
An efficacious HIV vaccine has the greatest potential to halt the HIV pandemic. Systems-level analysis of clinical and preclinical trials involving HIV vaccine candidates will accelerate the realization of this goal in four ways: (1) identification of correlates of protection (when possible), immunogenicity, and reactogenicity; (2) identification of candidate regulatory networks controlling host immune responses; (3) guiding the re-engineering of vaccine regimens to modulate responses; and (4) reverse engineering failed candidate vaccines to find out what went wrong and to open new avenues of investigation.

In this presentation, I describe our work applying systems vaccinology to clinical and preclinical trials of several viral vector-based HIV vaccine candidates. There are two components of the approach. First, we employ transcriptional profiling to extensively characterize local and/or systemic innate immune responses induced hours or days after vaccination. This analysis reveals the extent to which known and novel innate inflammatory pathways are triggered specifically or generically by the different vectors. Second, we computationally integrate the innate immune responses with vaccine induced functional responses that are measured months or years after vaccination. Functional responses include vaccine efficacy, immunogenicity, and safety. In this manner, we define the relationship between the earliest events after vaccination, which are the most readily manipulated, and the ultimate clinical outcome. I will present results of three collaborative studies: (a) investigating the relationship between adenoviral vector innate response magnitude and CD8+ T cell immunogenicity in mice, (b) investigating the hierarchy of adeno- and pox-viral innate stimulation in nonhuman primates, and (c) comparing innate immune responses induced by different adenoviral vector vaccines in humans.

References
Symposia Sessions

Symposium 02: Linking T and B Cell Responses

S02.01

Memory CD8 T Cells: Issues of Quantity, Quality, and Location

D. Mosopust

1University of Minnesota, Minneapolis, MN, USA

Memory CD8 T cell quantity, quality, and location relate to protective efficacy. This presentation will summarize three series of investigations, 1) the exploitation of heterologous prime boost vaccination to discover parameters regulating effector contraction and memory differentiation in mice, 2) the consequences of CD8 T cell reactivation within the mouse female reproductive tract, and 3) the establishment of robust polyfunctional SIV-specific memory CD8 T cells in rhesus macaques via heterologous prime-boost vaccination. We demonstrate that anamnestic memory CD8 T cell differentiation is flexible, and abundant quantity can be achieved while maximizing protective efficacy and preserving proliferative potential. Moreover, we describe an additional function for nonlymphoid memory CD8 T cells; as local sensors of previously encountered antigens that precipitate innate-like alarm signals and draw circulating memory CD8 T cells into the tissue. These data indicate that robust anamnestic responses in nonlymphoid tissues result from an integrated collaboration between both nonlymphoid and circulating populations of memory CD8 T cells. Ongoing studies will test the hypothesis that establishing preterminally abundant and functional memory CD8 T cells within frontline and circulating compartments may maximize rapid responses at the portal of pathogen exposure in the rhesus macaque model of stringent vaginal SIV challenge.

S02.02

Impaired B Cell Help During HIV Infection

E. Haddad

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Follicular helper T cells (Tfh) constitute a distinct subset of CD4+ T cells that are specialized in providing help to B cells and are required for the formation and maintenance of GCs. We show that these cells are impaired during HIV infection in HIV infected lymph nodes. We show that this impairment is dependent on PD-1/IL-21/ICOS axis. We further show that memory Tfh (mTfh) cells present in the periphery are surrogates for LN resident follicular helper T cells. We provide convincing evidence demonstrating a strong relationship between mTfh cells and LN resident Tfh cells. We show, at the transcriptional level, that mTfh cells express a significant number of Tfh-specific genes. We further show that mTfh cells have the ability to provide B cell help similar to LN resident Tfh cells. We also show direct evidence supporting a physiological association of mTfh cell function with humoral immunity. Interestingly, these memory circulating Tfh cells have lost their ability to elicit anti-HIV humoral immunity during HIV infection. We demonstrate that mTfh cells obtained from long-term non-progressors are better in providing B cell help than those obtained from progressors. Our data indicates that this could be due to weak triggering of the TCR on mTfh that would lead to an aberrant interaction with memory B cells. Overall, understanding the biology of these cells will reveal important information that can be used in the development of HIV vaccines and immunotherapies.
Role of Tfh in HIV Infection

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In the present study, we have investigated the distribution of HIV-specific and HIV infected CD4 T cells within different populations of memory CD4 T cells isolated from lymph nodes of viremic HIV-infected subjects. Four memory CD4 T-cell populations were identified on the basis of the expression of CXCR5, PD-1 and Bcl-6: CXCR5-PD-1-Bcl-6-, CXCR5-PD-1-Bcl-6+, CXCR5-PD-1-Bcl-6+, and CXCR5-PD-1-Bcl-6+. On the basis of Bcl-6 expression and functional properties (IL-21 production and B-cell help), the CXCR5+PD-1+Bcl-6+ cell population was considered to correspond to the Tfh cell population. We show that Tfh and CXCR5-PD-1+ cell populations are enriched in HIV-specific CD4 T cells, and these populations are significantly increased in viremic HIV infected subjects as compared to healthy subjects. The Tfh cell population contained the highest percentage of CD4 T cells harboring HIV DNA and was the most efficient in supporting productive infection in vitro. Replication competent HIV was also readily isolated from Tfh cells in subjects with nonprogressive infection and low viremia (<1000 HIV RNA copies). However, only the percentage of Tfh cells correlated with the levels of plasma viremia. These results demonstrate that Tfh cells serve as the major CD4 T-cell compartment for HIV infection, replication and production.

SIAE Inhibition and Enhanced Somatic Hypermutation Against gp140

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One of the goals in HIV vaccination is the enhancement of somatic hypermutation in order to increase the likelihood of obtaining neutralizing antibodies against conserved epitopes in HIV gp 140. Sialic acid acetyl esterase (SIAE) is a negative regulator of B cell signaling and defective SIAE variants have been linked to human autoimmunity. SIAE functions in concert with CD22, Lyn, and SHP-1 to maintain peripheral tolerance. We examined whether SIAE prevents B cells that recognize low affinity antigens from promiscuous collaboration with T cells, since T-B collaboration and germinal center formation in the context of self-antigens could be deleterious. In the absence of SIAE, B cells respond to a low affinity antigen by inducing CCR7 expression, migrate to the T cell zone, and enhance the generation of follicular helper phenotype CD4+ T cells. The absence of SIAE induces promiscuous B-T collaboration and enhances follicular helper T cell differentiation, somatic hypermutation and CD4+ memory T cell responses. Immunization with gp 140 in mice lacking SIAE resulted in the generation of markedly higher affinity gp140 specific B cells.
Symposia Sessions

Symposium 03: NHP Vaccines and Correlates of Immune Protection

S03.01

Can AIDS Virus-Specific Classical CD8+ T Cells Control Virus Replication?

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The induction of HIV-specific broadly reactive, potent, neutralizing antibodies by vaccination is the ultimate goal of the HIV vaccine field. There have been encouraging recent reports of the isolation of neutralizing antibodies from HIV-infected individuals. However, it is now clear that these antibodies require considerable somatic mutation to evolve into effective neutralizing antibodies. Induction of these neutralizing antibodies by vaccination, therefore, remains one of the key challenges of the field.

In contrast to neutralizing antibodies, we already know how to induce HIV-specific CD8 T cells by vaccination. Furthermore, the T cell receptors expressed by these antigen-specific CD8 T cells do not require somatic mutation to be effective. It is generally thought that these vaccine-induced CD8 T cells might provide some measure of control of acute phase viral replication and then reduce viral replication in the chronic phase. Indeed, there is considerable evidence that this can be achieved in vaccinated rhesus macaques. Accumulating data also suggests that vaccine-induced CD8 T cells can prevent chronic phase replication of the AIDS virus.

In this presentation, we will explore the hypothesis that classical CD8 T cells can prevent chronic phase viral replication after infection with the AIDS virus. We have induced CD8 T cells using a vaccine regimen consisting of a recombinant DNA prime, followed by recombinant Yellow Fever and recombinant Adenovirus 5 boosts. The majority of these vaccine-induced high frequency classical CD8 T cells express effector memory cell markers and are focused on a single, conserved immunodominant Nef epitope restricted by the elite controller MHC class I allele Mamu-B*08. We will present data as to whether these TEM CD8 T cells can control chronic phase viral replication after repeated low dose rectal challenge of Indian rhesus macaques.

S03.02

Strengths and Limitations of Macaque Models as Predictors of HIV Vaccine Efficacy in Humans

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To date, the SIVmac251 macaque model has predicted the efficacy of five HIV Phase III vaccine trials conducted in humans. Gp120/Alum (AIDSVAX), Ad5 Gag-pol-nef (in the STEP and Phambili trials), and DNA-Ad5 gag-pol-env (the HTVN 505 trial, VRC) have not been protective, whereas ALVAC-HIV or SIV/gp120Alum (in the RV144 Trial) have protected, albeit partially, humans against HIV acquisition and Rhesus macaques against SIVmac251 acquisition. Indeed, early data from animal studies have contributed to the design of the RV144 trial, the first human HIV vaccine trial that has demonstrated some degree of protection from HIV acquisition with a 31% efficacy. The potency of this vaccine needs to be improved. However, it remains unclear whether changing the antigens, vector, or adjuvants can improve efficacy. The decreased risk of acquisition in RV144 correlated with antibodies to V2 and perhaps with subclasses of antibodies that mediate ADCC. Since this vaccine regimen elicited negligible CD8 responses, a plausible hypothesis is that antibodies, rather than CD8 cells, contribute to the decreased risk of HIV acquisition. Assuming that these premises are correct, there is a gap in our knowledge of how to induce specific subclasses of antibodies and the appropriate CD4 help to maintain them overtime. Thus, the development of a more effective vaccine for HIV remains largely empirical and requires trial and error studies. Thus, predictive, animal models are vital as they may be instrumental in testing approaches that can improve the level of protection of RV144. Recently, we have reproduced the level of protection of a vaccine regimen similar to RV144 in macaques and attempted to improve it by changing the antigen design, adjuvants or by priming with vaccines other than ALVAC-SIV. Overall, the paradigm that has emerged is that more B or T cell responses do not translate to better protection from virus acquisition, as observed for HIV in human vaccine trials. Improving protection may instead require alterations in the quality of the CD4 helper response, such as T<sub>em</sub> and modulating the generation, homing and differentiation of antibody responses. While macaque studies will facilitate the development of better HIV vaccine candidates, there are several limitations of the animal models that must be considered.
**S03.03**

**Pre-clinical Development of Cytomegalovirus Vectors**

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Cytomegalovirus (CMV) vectors elicit and indefinitely maintain high frequency, pathogen-specific, CD4+ and CD8+ T cells at both lymphoid and extra-lymphoid effector sites. CMV vector-elicited T cells have multiple unique properties, including constitutive effector differentiation, the ability to immediately intercept pathogens without anamnestic expansion, and unconventional epitope recognition, which make these responses well-suited to control and clear persistent pathogens with sophisticated immune evasion strategies. This talk will discuss the latest data on the efficacy of these vectors in non-human primate models and an update on the translation of the CMV vector concept to the clinic.

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**S03.04**

**Vaccine Responses to Four Different gp140 Trimers**

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Immunization with soluble trimeric glycoprotein is considered an improvement upon the use of gp120 monomers. However, soluble recombinant trimeric gp140, while more physiologically relevant than gp120 monomers, differs from native trimers in that the natural cleavage site between gp120 and gp41 is usually mutated and only the ectodomains of gp41 with the trimerization motif are included. Nevertheless, glycosylation patterns are unchanged between the native envelope and the recombinant protein, as is the trimeric configuration. The exposure of the CD4 binding site and its binding behaviour relative to whole virions is preserved in recombinant trimeric proteins. We have performed a series of macaque immunogenicity and challenge studies using different gp140 constructs based on Clade C (1085, TV-1 and CN54) and Clade B (SF-162). Adjuvant choice and route of administration had clear impact on humoral responses providing differing levels of protection against vaginal challenge by homologous and heterologous SHIV strains. Differences in epitope specificity induced by the different trimers was observed, and for one (CN54), responses were compared to those elicited in a human phase I clinical trial.
Impressive Vaccine Protection Against SIVmac239 by Recombinant Gamma-2 Herpesvirus

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Herpesviruses persist for the lifetime of the infected individual and immune responses to them similarly persist at readily detectable levels for life. Distinct advantages may accrue to use of herpesviruses as vaccine vectors because of this persistence. Eight distinct human herpesviruses have been defined in three sub-groupings: alpha, beta, and gamma. The Desrosiers laboratory has been using the gamma-2 herpesvirus of rhesus monkeys called rhesus monkey rhadinovirus (RRV) as a vaccine vector for monkey studies. RRV is a close monkey homolog of human herpesvirus 8 (HHV-8), the Kaposi sarcoma herpesvirus (KSHV). In a previously published study (J Virol 85: 12708, 2011), six rhesus monkeys received recombinant RRV containing SIV gag, env and a rev-tat-nef fusion gene. Cellular responses of high frequency were elicited and these persisted until the time of challenge at 18 weeks. Despite the ability of the RRV-SIV env recombinant containing a codon-optimized version of the gp160 cassette to make gp160 protein in cultured cells infected in vitro, detectable anti-gp160 antibody responses were not elicited. Vaccinated monkeys exhibited a 1.7 log reduction in viral loads both at peak height and at set point following i.v. challenge with 10 i.v. infectious doses of SIVmac239. We have now succeeded in achieving anti-env antibody responses from RRV-SIV env by altering the codon usage in the gp160 cassette. In work not yet published, Y Shin and R Desrosiers have shown that natural induction of gp160 by rev and natural induction of RRV glycoprotein gH by its natural transinducer orf57 are dependent upon the nature of the bad codon usage. While gH and gp160 both have a bad codon usage, the nature of the bad codon usage for these two glycoproteins is very different. Furthermore, orf57 inducibility and rev inducibility can be switched simply by altering the codon usage. By changing 10% of the codons in the gp160 cassette to reflect the bad codon usage of RRV gH, we were able to regulate gp160 expression as a late RRV glycoprotein gene product; this relatively simple change in codon usage resulted in readily-detectable, persistent anti-gp160 antibody responses and the induced antibodies neutralized the SIVmac239 derivative SIV316. Using this new RRV-SIV env recombinant, six rhesus monkeys were immunized with RRV-SIV gag, RRV-SIV env, plus RRV-SIV rev tat nef. Challenge of the six vaccinated monkeys and six unvaccinated controls began at 18 weeks following the single immunization using repeated marginal dose SIVmac239 i.v. challenge spaced three weeks apart. Three of the 6 vaccinated monkeys showed a transient viral blip two weeks after the second exposure (viral loads of 60, 140 and 340 RNA copies per ml of plasma), one has remained uninfected and the other two have become SIV-infected with low viral loads. Five of the six controls have become infected so far, with peak viral loads between 10,000,000 and 120,000,000. These results, and the results of the Picker laboratory, indicate that persisting viral vectors should be a point of emphasis for ongoing AIDS vaccine research.
S04.01

Development of HIV Vaccines—A Vaccine Manufacturer’s Perspective

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The presentation will provide an industry perspective on the global effort to develop an HIV vaccine. First, the principal steps in developing novel vaccines against infectious diseases from discovery to launch will be covered. Second, the specific challenges related to the development and introduction of new vaccines against diseases of the developing world will be described. And third, the additional scientific obstacles for an HIV vaccine and the rationale and need for public-private development partnerships will be explored.

S04.02

Sendai Virus Vector Vaccine

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Viral antigen specific T-cell responses are crucial for the control of HIV replication. Viral vectors are promising vaccine tools for inducing antigen-specific T-cell responses. We have developed an AIDS vaccine using a Sendai virus (SeV) vector and showed the potential of this vector to efficiently induce antigen-specific T-cell responses in non-human primates. A vaccine trial using a Gag-expressing SeV vector showed control of SIVmac239 challenge in rhesus macaques. A clinical trial phase I of SeV vector AIDS vaccine by IAVI started in April, 2013. SeV, whose natural host is mice, is nonpathogenic in humans and replication-competent SeV vectors are available. Although anti-human para-influenza virus type 1 antibodies can crossreact with SeV, most people do not have high anti-SeV neutralizing titers. Our recent study in macaques has indicated the potential of intranasal SeV vector immunization to induce mucosal immune responses. These suggest that SeV vectors can serve as a vaccine tool for inducing effective T-cell responses against HIV infection. Combination of this vector with other viral vectors and antigen optimization may lead to the development of an effective AIDS vaccine.

Symposia Sessions

Symposium 04: Viral Vaccine Vectors

S04.03

Reinventing the Nucleic Acid Vaccine

A. Geall

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• Novartis has developed the SAM® vaccine platform
• The Platform takes advantage of cell-free RNA production from a transcription reaction and delivery with a synthetic delivery system
• The broad utility of this novel vaccine technology has been demonstrated with genes encoding antigens from several pathogens and found to elicit broad and potent protective immune responses
• Responses are comparable to a viral delivery technology, but without the inherent limitations of viral vectors

S04.04

Durable Antibody Responses Induced by DNA and Recombinant Modified Vaccinia Ankara Vaccines Expressing HIV-1 Virus-like Particles

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A challenge for HIV-1 vaccines is to induce long lasting antibody (Ab) responses to the envelope glycoprotein (Env). Here Clade B DNA and recombinant modified vaccinia Ankara (MVA) vaccines producing virus like particles (VLPs) displaying trimeric membrane-bound Env are tested for safety, immunogenicity, and the 6 month durability of elicited immune responses. HVTN trial 205 enrolled 299 individuals who were randomly assigned to receive two DNA followed by two MVA inoculations (DDMM regimen, n=149), three MVA inoculations (MMM regimen, n=75) or placebo (saline, n=75). Humoral and cellular responses induced by vaccination were measured. Both regimens were safe and well tolerated. At 2 weeks post the final vaccination, 93.2% and 98.4% of the DDMM and MMM groups, respectively had binding Ab for gp140 Env. At 6 months post vaccination, binding Ab in both groups had contracted by < 3-fold. Binding Abs were both more frequent and higher titer for gp41 than consensus gp120. The Abs elicited by both regimens had avidity indices similar to those observed in 6 month infections. Both regimens elicited low titer Tier 1 and 2 neutralizing Ab. For both regimens, T cell responses were biased towards Gag, and response rates were higher for CD4+ (66.4% DDMM, 43.1% MMM) than CD8+ (21.8% DDMM, 14.9% MMM) T cells. The DDMM and MMM VLP regimens elicited avid gp41-biased binding Ab that was durable and had low titer Tier 1 and Tier 2 neutralizing activity.
S05.01

Targeting the Female Reproductive Tract Epithelium to Prevent Transmission and Acute Systemic Infection: Lessons Learned in the SIV-NHP Model

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In the SIV-rhesus macaque model of HIV-1 transmission to women, vaginal exposure to high doses of virus infects initially small populations of mainly resident CD4 T cells with an ostensibly resting phenotype. Local expansion of these founder populations then disseminates infection throughout the lymphoid tissues where additional expansion results in a robust systemic acute infection. The initially small size of the infected founder populations at the portal of entry point to the favorable odds for containment by a vaccine-induced immune response and I will discuss targeting the early stages of transmission, but I will also describe for the live attenuated SIV-delta-nef vaccine an important and active role for the mucosal epithelium in concentrating antibodies on the path of virus entry, and in moderating the innate immune response to virus exposure that facilitates local expansion and systemic infection.

S05.02

Host-Virus Interactions in HIV-1 Rapid Disease Progression

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Infectious disease have had a significant impact on history. The unanticipated AIDS pandemic clearly shows the influence that contagious diseases can have in our world. The examination of how we controlled and improved health in previous epidemics should facilitate how to deal with future encounters with emergent diseases. From a clinical perspective, humans show a remarkable variation in the clinical outcome following HIV-1 infection. In absence of highly-active antiretroviral therapy, median time for development of AIDS has been estimated to vary from 8 to 11 years depending on age at seroconversion. However, there are departures from this estimate and meanwhile some individuals are able to control HIV-1 replication for longer periods (elite controllers) others progress quickly to AIDS or meet criteria for antiretroviral treatment within the first 3 years after seroconversion (rapid progressors). Over the last years, many studies have focused in these extreme HIV-1 phenotypes searching for the clues of viral pathogenesis and accumulative evidences suggest that a combination of viral and host factors are involved in the definition of HIV-1 disease outcome. This presentation will focus on the immunological and virological factors that contributed to the rapid development of AIDS-defining pathogenesis.
**S05.03**

**Phenotypic Properties of Acute HIV-1 Associated with Transmission Fitness and Virulence**

*E. Arts*¹

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Only one or a limited number of HIV-1 clones are transmitted from recipient to donor during primary infection. Little is known about the phenotypic properties of the transmitted HIV-1 aside from preference for CCR5 usage and the reduced glycan shield. We performed a series of genotypic and phenotypic assays to determine possible differences between acute/early HIV-1 and chronic virus during transmission and to determine how the infecting HIV-1 clone(s) can influence subsequent disease progression. In contrast to the homogeneity of acute HIV-1 in their blood, HIV-1 genetic diversity in the female genital tract (FGT) was very heterogeneous at acute/early infection, i.e., similar to the HIV-1 diversity observed during chronic disease. These diverse FGT HIV-1 isolates had more N-linked Env glycosylation sites than the acute blood HIV-1. Are these distinct genotypic features during acute HIV infection related to possible selection processes during transmission? Transmitted and chronic HIV-1 have similar entry kinetics, sensitivity to entry inhibitors, and replicative fitness in PBMCs, T cells, and macrophages. However, by exposing human penile and cervical explant tissues to mixtures of chronic and acute HIV-1, we clearly observed that acute HIV-1 was transmitted with greater efficiency than chronic virus. Chronic HIV was trapped and replicated only in the tissue explants; known to contain high levels of extracellular mannose binding lectins (MBLs) and lectins (e.g., Dectin-1) on mucosal epithelial cells that prevent infection of high mannose-containing pathogens. Higher transmission fitness of acute HIV-1 via DC capture in the cervical/penile tissue was correlated with fewer N-linked glycosylation sites and reduced C-type lectin binding affinity. Thus, the HIV-1 clone(s) may be “favored” for transmission based on escape from the lectin “trap” as well as DC capture via enhanced CD4 interactions (based on Affinofile and binding studies).

**S05.04**

**Modeling the Effects of Vaccination on Acute Phase CD8+ T Cell Immunodominance Patterns to HIV in Humanized Mice**

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Vaccination of rhesus macaques with a RhCMV vector demonstrates the ability of CD8+ T cell responses to rapidly contain viral replication following mucosal SIV challenge, supporting the importance of cellular responses in the design of an effective HIV vaccine. Importantly, the immunodominant targeting of conserved regions of the virus where escape mutations impose a fitness cost is associated with the ability of CD8+ T cell responses to control of HIV, although most individuals fail to naturally mount such protective responses. However, additional studies in the macaque model now demonstrate the ability of vaccination to overcome natural immunodominance hierarchies to redirect acute phase CD8+ T cell responses towards more critical regions of SIV resulting in enhanced immune control. A more precise understanding of protective versus easily escaping decoy CD8 epitopes in HIV, and an in vivo model to explore strategies that overcome natural acute phase CD8+ T cell responses towards more critical regions of SIV through vaccination in the humanized mouse model. This approach may aid in the design of vaccine antigens that maximize the potential of vaccine-induced CD8+ T cell responses to contain or control HIV.
**S06.01**

**A Pure Native Env Trimer Vaccine Elicits Potent Tier 2 Neutralization to a Quaternary, Glycan-Sensitive Epitope**

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As the exclusive target of nAbs, the native Env trimer is a logical basis for a nAb vaccine. Given its immunorecessive nature, purity may be essential for its success as a nAb immunogen. In other words, it must be free of contamination by other, more immunogenic forms of Env. 4 groups of rabbits were immunized with: 1) “trimer VLPs”, expressing pure native JR-FL Env trimer, cleared of non-functional Env by a protease digest, 2) undigested control VLPs, 3) a JR-FL gp140F trimer DNA prime-protein boost, and 4) JR-FL gp120 prime-boost. Immune sera were characterized for binding, neutralization and specificity. All 4 immunogens were effective, as judged by monomeric gp120 ELISA, although “trimer VLP”-immune sera bound only weakly. One serum each from groups 1 and 3 exhibited remarkable IC50s >1:1,000 against the JR-FL index virus in the TZM-bl assay. Two group 4 sera exhibited titers of ~1:100. Mapping revealed that both potent sera targeted sites unusually lacking glycans. Specifically, the group 1 serum neutralization was regulated by a glycan at residue 197 in the V1V2 loop stem and the group 3 serum was regulated by a glycan at residue 230 in the C2 of gp120. Interestingly, the N197-regulated activity recognized a quaternary epitope. The two neutralizing sera of group 4 mapped to the C3-V4 region. It is perhaps no coincidence that the two most potent sera targeted sites that unusually lack a glycan. Similar glycan-deprived sites have also accounted for autologous neutralization in natural infection. We are now working on strategies to elicit this activity more consistently, and to broaden its neutralization coverage.

**S06.02**

**Genetic Development of HIV-1 Neutralizing Antibody Lineages**

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Recent advances in antibody isolation and structural characterization have provided insights into the conserved neutralization epitopes on the viral spike and the mode of antibody recognition. Most of the well-characterized broadly neutralizing bNAbs derive from donors with unusually broad and potent serum neutralization. These bNAbs often have a high number of somatic mutations and some have unusually long CDRH3 loops, however relatively little is known about the immunological pathways that produce these antibodies. We are using next generation sequencing of antibody gene transcripts to track the development of neutralizing antibody lineages. Among donors with for whom early time point and longitudinal samples are available, it is possible to trace the origin of a specific antibody lineage and infer the unmutated ancestor ( naïve BCR) of the mature bNAbs. This analysis has been done for donors making bNAbs to the CD4 binding site and to the V1V2-glycan epitope. These analyses provide insights into the naïve BCR that produce broadly neutralizing antibodies, and the maturation pathway required for the antibody to acquire high affinity neutralization.
Symposium 06: Humoral Immunity

S06.03

HIV Vaccine Design to Induce Broadly Neutralizing Antibodies

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Developing a vaccine that induces sustained titers of potent, broadly neutralizing antibodies against multiple epitopes is probably necessary, though possibly not sufficient, for robust protection against HIV infection. At least four different epitopes on the HIV spike are targeted by potent, broadly neutralizing antibodies: the VRC01-class epitope within the CD4 binding site, the 10E8 epitope within the membrane-proximal external region, the N332 glycan-dependent supersite at the base of V3, and the N160-glycan dependent site on V1V2. Recent and newly emerging structural information about these epitopes and their antibody accessibilities provides critical data to inform vaccine design. This talk will review our efforts employing computational design, in vitro screening and nanoparticle engineering to devise immunogens and immunization regimens that attempt to focus immune responses to particular structural epitopes, activate appropriate germline B cells and drive appropriate somatic mutation, with the goal of inducing broadly neutralizing antibodies.

S06.04

Recombinant Env Interactions with the BCRs of Broadly Neutralizing Anti-HIV Antibodies

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Broadly neutralizing antibodies (bNAbs) against HIV are believed to be a critical component of an effective vaccine. Such antibodies are generated during HIV-infection, but have not yet been elicited during immunization with recombinant forms of Env (the sole target of anti-HIV neutralizing antibodies). We and others reported that recombinant Env proteins are very inefficient in engaging the germline BCR forms of a particular type of bNAbs that target the CD4-binding site; one of the most conserved regions of the HIV Env. This lack of engagement is a major contributing factor in our unsuccessful attempts to elicit this type of antibodies by immunization. However, it is currently not well understood whether or not recombinant Envs can engage the germline BCR forms of bNAbs that bind to other conserved Env regions. We will discuss our efforts to design immunogens, based on circulating HIV Envs, that are capable of activating B cells expressing germline BCR forms of diverse bNAbs.
Antiretroviral therapy (ART) has dramatically reduced the morbidity and mortality of HIV-infected patients, whereas HIV-specific immune responses remain unchanged, even after years of successful viral control. Therapeutic immunization strategies have been proposed to enhance immune-specific responses that could allow ART discontinuation over time. Dendritic cell (DC)–based immunotherapy that bridges innate and adaptive immune responses is the only strategy that has shown encouraging results in HIV infection. Type of antigen, immunogen delivery method, optimal interaction of antigentic peptide with T cells and avoidance of tolerogenic responses are key elements for an efficient immunotherapy. DC-based therapy electroporated with RNA encoding both CD40 ligand and patients’ own HIV antigens have the advantage to induce CD8 HIV-specific T cell responses in the absence of requiring CD4T cell help. This approach by not inducing antigen cross-presentation, thus avoiding CD4T cell activation should prevent the potential for increased viral replication. Several groups have reported different levels of HIV-specific immune restoration by promoting the effector functions of T helper (Th)-1 cells and/or cytotoxic T lymphocytes in ART-treated patients receiving autologous monocyte-derived DC. Following ART discontinuation, a decrease of plasma viral load setpoint ≥1 log was observed in several studies. Recently, DC-based immunotherapy is also being considered as effort aimed at decreasing the HIV reservoir, especially when combined with reservoir-mobilizing agents. Clinical study design, patient selection, ethics consideration and optimal condition to safely discontinue ART as a read-out for HIV remission represent unresolved challenges. The induction by DC-based therapy of a robust and long-lasting cellular response that recognize the broad set of viruses that escaped the immune system will be needed to transform this experimental approach into an effective and large scale strategy.
A general pessimism about the plausibility of HIV eradication is being replaced by a cautious optimism. But for persons with established infection, how to get there? Eradication strategies will need to deplete cells harboring virus or somehow coax virus out of them. As the determinants of HIV activation are largely dependent upon signals shared with immune activation, immunologic mechanisms may prove useful for activating virus from latency but may not be without risk as early efforts to activate HIV expression have not been well tolerated. And while newer approaches have generated only modest signals of HIV activation from latency, the effects of this approach on host elements - especially as more potent strategies are implemented - are not yet fully understood. It is also not yet clear whether activation of HIV expression will result in the destruction of latently infected cells without the participation of innate or adaptive immune mechanisms. To this end, strategies that involve administration of cytokines and a renewal of interest in therapeutic vaccines may help to enlist innate and adaptive defenses to clear cells that have been induced to newly express viral antigens or peptides on their surface. And while this approach may not yet be adaptable to most infected persons, the exciting results seen in a few persons who have undergone myeloablative therapies and allogeneic stem cell transplantation may have been mediated at least in part by the immune clearance of graft versus host disease. Will more selective targeting of latently infected cells be aided by the identification of markers that can more selectively target these cells? As chronic exposure to uncontrolled HIV infection appears to result in structural damage to immunologic organs in secondary and gut associated lymphoid tissue, it is also not yet clear whether viral eradication alone will allow full restoration of immunity and attenuation of the inflammatory environment that are linked to HAART-era morbidity and mortality.
Symposium Sessions

S08.01

Refocusing CTL and Antibody Responses with p24gag Conserved Elements Vaccines

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HIV sequence diversity is a major hurdle for AIDS vaccine development. Our Conserved Elements (CE) vaccine design circumvents this problem with immunogens composed of conserved segments of the viral proteome and excluding potentially immunodominant regions capable of mutating without destroying or limiting virus viability (i.e., “decoy” epitopes). The prototype HIV-1 p24gag CE DNA vaccine construct (p24CE) encodes 7 viral peptides (of 12-24 amino acids) that were generally conserved in >98% of all known HIV-1 M Group sequences. While we found a surprisingly weak relationship between sequence conservation and viral fitness, we have identified residues critical for viral infectivity and fitness, primarily at the capsid hexamerization interface. The 7 peptides were linked via short spacer sequences and DNA constructs allowed expression and appropriate processing of 15 of 16 known A-list HIV epitopes in human cells. These peptides were recognized in HIV infection and high avidity responses against them were associated with virologic control. Exposure to CE-expressing autologous dendritic cells in humans induced de novo CD4 and CD8 responses at levels similar to full-length Gag. Immunization of mice and macaques with p24CE DNA elicited strong, cross-clade cellular and humoral responses. In contrast, vaccination with full-length, p55gag DNA primarily elicited responses to epitopes outside of the CE. Boosting of p24CE-primed macaques with p55gag DNA greatly augmented CE-specific multifunctional cellular responses as well as humoral responses. In a therapeutic vaccine experiment in macaques, existing T cell responses induced by infection were found to be overcome by subsequent CE vaccination. These results provide a novel and effective strategy to avoid immunodominant responses against decoy epitopes in both prophylactic and therapeutic settings, while focusing responses to critical features of the virus for which limited viable escape pathways exist.

S08.02

HIVACAT T- and B-Cell Immunogen Design and Preclinical Testing

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The HIVACAT program is dedicated to the development of effective preventive and therapeutic HIV vaccines. Started in 2008, the program includes about 60 investigators at two major HIV research centers in Barcelona with expertise in retrovirology and cellular, humoral and innate immunity, including mucosal immunity. T-cell immunogen development was based on screening more than 1,000 HIV-1 clade-B and -C infected individuals for total virus-specific T cell immune responses and identifying those specificities that dominate the response in subjects with relative viral control. The identified regions emerge to be highly conserved and to induce responses of high functional avidity and superior ability to suppress in vitro viral replication. They are highly enriched for regions that contain sites for which mutations cause significant loss of viral replicative capacity. This approach has led to the design of an immunogen sequence of roughly 500 amino acids encompassing more than 50 optimally defined CTL epitopes and all described regions rich in CD4 T cell targets. Preclinical testing in mice using DNA and MVA-vectors shows broad and evenly distributed responses of high magnitudes. HIVACAT’s B-cell immunogen development includes a MPER and a full-length Gag and Env sequences. Elevated neutralization activity has been obtained in murine studies by in-house developed Env variants when compared to the wild-type sequence. Prime-boost strategies combining DNA/MVA and VLP delivery of HIVACAT’s immunogen, a variant-coveting full-length Gag and MPER and Env sequences are under way.
Symposia Sessions

Symposium 08: New Vaccine Concepts

S08.03 Protection Against HIV: Vaccination to Block Inflammation

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Though candidate vaccines against HIV have generally been designed to induce a robust adaptive immune response against the virus, we wonder whether an effective vaccine might induce tolerance instead. Tolerance to HIV may result in abortive infection because, absent the effects of inflammation that normally drive viral replication and spread, infected cells may be cleared at a faster rate than additional cells can be infected. Although such tolerance can be attributed to the deletion of reactive T cell clones, we have more recently found that there is a surprisingly high degree of maternal microchimerism within the human fetus, inducing a robust and tolerogenic CD4+CD25highFoxP3+CD127low regulatory T cell compartment (Mold et al., Science 322:1562, 2008) emanating from a developmentally-distinct fetal hematopoietic stem cell (Mold et al., Science 331:534, 2010). This observation, in turn, prompts the question: if maternal cells can so readily move into the human fetus and induce such tolerance, why then are not all babies born to HIV-infected mothers infected as well? Since in utero infection appears to occur only infrequently, the fetus might be “protected” against HIV in utero precisely because a tolerogenic response is mounted when HIV spreads transplacentally, limiting further viral replication and spread. Given previous studies demonstrating that fetal-maternal microchimerism also exists in the monkey (Jimenez et al., Transplantation 79:142, 2005), we are now testing the hypothesis that induction of tolerance to SIV in utero and/or at birth results in protection of rhesus macaques against subsequent challenge with SIV. Results will be presented from experiments in which tolerance induction has been attempted by (a) injection of dams with the attenuated strain SIVmac1A11, (b) injection of first trimester fetal macaques with SIVmac1A11 or a lentiviral vector encoding SIV Env, and/or (c) oral administration of AT2-inactivated SIV to newborn macaques.

S08.04 Immunological and Virological Mechanisms of Vaccine-Mediated Protection Against SIV

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Vaccination followed by simian immunodeficiency virus challenge is often used to model potential HIV-1 vaccine protection of humans. We conducted a large (n=80) macaque vaccine-challenge study to define mechanisms of protection, comparing control animals to those vaccinated only with mosaic Gag, only with mosaic Env, or only with native Env. We show that antibodies to the SIV envelope glycoprotein are necessary and sufficient to protect against infection. Both mosaic Env and native Env immunization elicit the same protective response, but the mosaic Env does so inefficiently. These data suggest caution for using mosaic Env for the purpose of eliciting functional antibodies. Sequencing of viruses from breakthrough infections revealed a strong vaccine-mediated selective pressure against neutralization sensitive viruses within the challenge swarm. We identified a two amino acid signature in the envelope that confers a near-global resistance to antibody neutralization, manifested as resistance against a diverse set of monoclonal antibodies as well as to sera from all vaccinated animals. Interconversion of these two amino acids is sufficient to confer resistance on easy-to-neutralize SIV clones (e.g., CP3C), as well as sensitivity on hard-to-neutralize clones (e.g., CR54). Our data are consistent with SIV envelope comprising two serologically-distinct and functional structures, either of which can be adopted to varying extents by any given clone. Analysis of all transmitted/founder (T/F) viruses shows that the native Env vaccination has an efficacy (VE) of 90% against the sensitive viruses, but a VE of only 30% against the resistant viruses. Thus, highly protective antibody responses against lentiviral infection can be elicited by vaccination; broad efficacy will require elicitation of antibodies specific to structurally-distinct forms of the envelope. Our analyses suggest that determining the resistance profile of T/F from clinical trials (RV144, HVTN505) may reveal mechanisms underlying the differential, partial efficacy of these vaccines.
S09.01

Visualizing Cellular Dynamics of HIV Infection In Vivo

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The mechanisms by which HIV spreads among target cells within lymphoid organs are not known. While T cells can be infected by cell-free HIV, cell-to-cell mediated HIV infection has been proposed to play a major role in viral spread, given the high cellular density in lymphoid tissues. Stable contacts between infected and uninfected T cells permit the formation of virological synapses (VS), which facilitate efficient cell-cell transfer of virus in vitro. However, it is unclear whether T cell – T cell contacts are sufficiently stable in vivo to allow for functional VS formation under the conditions of perpetual cell motility in lymphoid tissues. To address this, we have taken a visualization approach using multiphoton intravital microscopy (MP-IVM) to examine the dynamic behavior of HIV-infected T cells in lymph nodes of BLT humanized mice. We have found that the majority of productively infected T cells migrated robustly, resulting in their even distribution throughout the lymph node cortex. Unexpectedly, a subset of infected cells formed multinucleated syncytia through HIV envelope (Env)-dependent cell-cell fusion. Blocking the egress of migratory T cells from LNs into efferent lymph, and thus interrupting T cell recirculation, limited HIV dissemination and strongly reduced plasma viremia. Thus, we found that HIV-infected T cells retain their motility, contributing to local viral spread, as well as to systemic dissemination of HIV through T cell recirculation through distant tissues. We are currently extending these studies to the female reproductive tract. Initial observations from our ongoing work will be discussed.

S09.02

Dynamic Interplay Between Virus and Host Occurring at the Female Reproductive Tract During Acute HIV Infection and Treatment

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We utilized BLT humanized mice to elucidate and study critical events occurring in the FRT and CVS during HIV infection. By performing comprehensive analyses of the T cell subsets present in the FRT and CVS, we have gained insight into the human immune cell populations in this organ. Consistent with observations made in healthy women, the majority of the human lymphocytes present in the FRT and CVS from BLT mice are memory T cells. Consistent with the preferential vaginal transmission of CCR5-tropic viruses, a high percentage of CD4+ T cells present in the FRT and CVS express CCR5. Also consistent with humans a significant number of the memory CD4+ T cells present in the FRT and CVS of BLT mice expressed α4β7. We next investigated the effect of ART on HIV levels in CVS of BLT mice. Consistent with results obtained in humans, ART treatment of infected BLT mice resulted in a significant decrease in the levels of HIV in both PB and CVS. However, the finding showing the absence of cell-free HIV in CVS during ART, but the continued presence of infected cells producing HIV RNA could have potentially important implications for HIV prevention and eradication strategies.

Consistent with the lack of transmission observed in heterosexual couples where the infected partner is undergoing ART our analysis showed that HIV RNA+ cells present in the mice receiving ART couldn’t produce replication competent infectious virus. The availability of a small animal model that accurately recapitulates key aspects of human conditions represents a unique tool for the in vivo study of the intricate cellular dynamics occurring during HIV infection in the FRT. Also, this model could prove helpful in evaluation of novel approaches to prevent HIV transmission and potentially in the evaluation of interventions aimed at HIV eradication from cellular reservoirs.
**S09.03**

Identification and Characterization of Specific IgG-mucin Interactions to Enhance Vaccine Function

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Current HIV vaccines can generate IgG antibodies that bind to, but do not broadly neutralize virions. Such antibodies were generated by the antigens delivered in the RV-144 vaccine trial. How such antibodies could provide the modest protection observed remains to be determined. One potential mechanism is the interaction of vaccine-induced antibodies with mucus. Antibodies tethered to mucus could function to trap viral particles in mucus, effectively neutralizing the virus by preventing it from reaching potential target cells. We have observed the stable interaction of IgG with cervical and cervicovaginal mucus using several different approaches. To begin to identify the specificity of interactions, we set up a series of ELISA based assays to detect IgG interactions with individual mucins. With this approach, we were able to detect a specific interaction of IgG with mucin 16 (MUC16). MUC16 is a cell associated mucin which is shed into mucus. The IgG-MUC16 interaction is mediated by the Fc portion of IgG and sensitive to deglycosylation, suggesting that IgG glycoform plays a role in the specific interaction. Interestingly, serum IgG binding is enhanced in individuals chronically infected with HIV, independent of virus control of HAART treatment. Likewise, individuals with Rheumatoid Arthritis show a similar increase in serum IgG binding to MUC16 indicating that chronic inflammation increases the IgG-MUC16 interaction. This increase in interaction also reveals that the targeting of IgG to interact with MUC16 can be regulated by the immune response. Using biochemical and proteomics approaches, we are gaining insights into the molecular determinants of IgG interaction with MUC16. We are also generating preliminary results revealing that antibodies can interact with other mucins, potentially through different specificities. These data demonstrate that antibodies can be targeted to interact with mucins and possibly enhance vaccine generated antibody function by targeting responses to preferentially interact with mucus.

**S09.04**

Prime and Pull: A Vaccine Strategy for Viral Sexually Transmitted Infections

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Viral sexually transmitted infections (STIs) such as herpes simplex virus-2 (HSV-2) and HIV account for a significant amount of morbidity and mortality around the world. However, efficacious vaccines for viral STIs have been elusive. Strong evidence for the role of T cells in controlling viral STIs has led to the design of prophylactic vaccines that elicit both systemic cellular and humoral immunity, and yet these vaccines have not been successful. In peripheral sites, tissue-resident memory T cells have been shown to provide superior protection compared to circulating memory T cells. However, the female genital tract, which is a portal of entry for viral STIs, is an immunologically restrictive tissue that does not allow entry of activated T cells in the absence of infection or inflammation. To overcome these obstacles, we designed a vaccine strategy called ‘prime and pull’ to establish tissue-resident memory T cell population at the barrier tissue. Prime and pull relies on two steps: 1) systemic parenteral priming and 2) recruitment or ‘pulling’ of activated T cells to the restrictive genital tract by topical application of chemokines. We show that prime and pull effectively recruit virus-specific effector T cells to the genital tract, where the cells then establish a resident memory pool within the tissue. Furthermore, we find that this tissue-resident memory T cell population provides protective immunity and may prevent the spread of virus from the initial infection site. Thus, prime and pull appears to be a promising vaccination strategy for HSV-2, and has the potential to be applied to other STIs such as HIV.
What Has 30 Years of HIV Vaccine Research Taught Us? Lessons From the Past to Inform the Future

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Soon after HIV was discovered as the cause of AIDS in 1983-4, there was an expectation that a preventive vaccine would be rapidly developed. The molecular structure of the virus was essentially elucidated by 1985, and the first phase I clinical trial of any HIV vaccine was initiated in 1986. The first two generations of candidate vaccines were based on either the neutralizing antibody, or the cytotoxic T-cell paradigms. These paradigms produced important scientific information. Vaccines based on these concepts, however, failed to demonstrate efficacy when tested in large scale human trials. The modest efficacy demonstrated in 2009 by the Thai RV144 trial, finally provided proof-of-concept that an HIV vaccine is an achievable goal. In addition to the exciting science that has been produced over the last 30 years, there are general lessons learned that could be important in accelerating the development of an HIV vaccine: (1) its development is an extremely difficult scientific challenge that needs to be approached in a more systematic and disciplined fashion; (2) the temptation of just following the fashion (or the current paradigm) should be avoided, with more willingness to explore truly innovative concepts; (3) clinical trials, especially efficacy trials, are essential in obtaining critical information to advance the field; (4) HIV vaccine research requires long-term commitment and funding; and (5) any effective vaccine development effort requires sustainable collaborations with multiple partners (for which the Global HIV Vaccine Enterprise was established). The window of opportunity to develop an HIV vaccine may be narrowing. To maintain its relevance within the broader response to the HIV/AIDS pandemic, HIV vaccine research should be conducted with the necessary sense of urgency, and in close coordination with other HIV prevention activities.
OA01.01

Effective CD8+ T Cell Responses Restrict SIV Replication to Follicular Helper T Cells

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Background: HIV and SIV infections are thought to be permanent, even in individuals with elite immunologic control. Although latent infection of long-lived CD4+ T cells contributes to viral persistence, the finding of low-level replication in elite controllers suggests a more active sanctuary where productively infected cells are shielded from immune destruction.

Methods: We used flow cytometric cell sorting to isolate cell populations from lymph nodes (LN) obtained from rhesus macaques (RM) with chronic SIVmac239/251 infection and varying SIV replication set-points, ranging from progressive infection to elite control. In selected RM, LN biopsies were obtained before and after in vivo CD8+ cell depletion with monoclonal antibody M-T807R. Sorted populations were quantified for replication-competent SIV by inductive co-culture and for SIV RNA by qRT-PCR.

Results: In all SIV-infected RM, the frequency of SIV+ cells was highest in CD4+ memory T cells, but the distribution of SIV within CD4+ memory T cell subsets defined by PD1 expression (low, medium and high, with the PD1high subset reflecting follicular helper T cells) was strikingly different depending on the viral replication set-point. In RM with plasma viral load set point >50,000 copies/ml, SIV was similarly distributed among all PD1-defined subsets, but in RM with lower SIV set-points, SIV was increasingly restricted to the PD1high follicular helper T cells such that virus was almost exclusively restricted to this subset in elite controllers (plasma viral load <1,000 copies/ml). CD8+ cell depletion of elite controllers resulted in a transient increase in plasma viremia and an associated transient re-distribution of SIV infection to non-follicular helper cells.

Conclusion: This study shows that productive SIV replication is increasingly restricted to follicular helper T cells as CD8+ T cell immune pressure increases, indicating that the B cell follicle acts as a sanctuary where productively SIV-infected cells are relatively protected from effective CD8+ T cell responses.

OA01.02

The Breadth of Expandable Central Memory CD8 T Cells Inversely Correlates with Residual Viral Loads in HIV Elite Controllers


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Background: Previous studies have shown that elite controllers with minimal T cell responses harbor a highly functional, broadly directed central memory T cell population that is capable of suppressing HIV in vitro. However there is no evidence implicating this cell population in the durable control of HIV replication in vivo.

Methods: We investigated whether there is an association between the breadth of expandable central memory CD8 T cell pool and the level of viremia during chronic HIV infection. Low frequency HIV-specific memory CD8 T cells were expanded using autologous epitopic and variant peptides. Viral load was measured by standard assays and as well as the ultrasensitive single-copy PCR assay.

Results: We show that HIV elite controllers maintain significantly greater breadth of expandable HIV-specific central memory CD8 T cells, which are mainly directed against HIV Gag (Kruskal-Wallis >0.0001). No significant differences were observed in the breadth of this expandable population in treated (Kruskal-Wallis >0.2) and untreated (Kruskal-Wallis >0.5) chronic progressors. Elite controller Gag responses had superior in vitro virus inhibition capacity compared to Nef (p<0.01) and Env (p<0.01) responses, whereas chronic progressors showed diminished inhibitory capacities regardless of HIV protein-specificity (Kruskal-Wallis >0.9). Furthermore, we show that elite controller memory CD8 T cells remain highly functional even after the inducing epitope has escaped. More importantly, we show that the breadth of Gag-specific central memory responses have an inverse correlation with viral load (r=-0.4, p=0.009). This inverse correlation is sustained even when only individuals with suppressed viral loads of between 50 copies to 1 copy are studied (r=-0.5, p=0.02).

Conclusion: Together these data reveal a direct link between the abundance of central memory CD8 T cells and prolonged maintenance of low-level viremia. Our studies highlight a feature of HIV-specific CD8 T cells that would be desirable in vaccine-induced responses.
OA01.03

PD-1 Expression on HIV-1 Specific CD8+ T Cells Is Shaped by Epitope Specificity, TcR Clonotype Usage and Driven by Antigen Load

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Background: Multiple negative and positive regulatory molecules expressed on HIV specific CD8+ T cells define their ability to control natural HIV-1 infection. A second determining factor central for the antiviral activity is the HIV epitope specificity, yet its relationship to antigen load and CD8+ T cell exhaustion during chronic HIV infection remains unclear.

Methods: We used an array of different HLA-B*15:03 and B*42:01 tetramers and studied a total of n=128 HIV CD8+ T cell responses specific for 11 different HIV epitopes to characterize the distribution of multiple surface markers, such as PD-1, CD244, LAG-3, CD57 and CD127 between epitopes and at the TcR clonotype level.

Results: We find that the level of PD-1 expression varies greatly on CD8+ T cells specific for different HIV epitopes (P<0.001), even sampled from the same subject and timepoint. We confirm the previously described correlation between PD-1 expression and viral load setpoint (R=0.21, P=0.02) but find that it is mainly driven by epitopes that do not frequently undergo escape mutation (R=0.37, P=0.03), and not by epitopes prone to escape (R=0.19, P=0.11), suggesting that antigenemia drives PD-1 expression and not vice versa. In addition, we observe an unequal distribution on different TcR Vβ clonotypes recruited within each CD8+ T cell response that is not related to clonotype frequency. Finally we show that the PD-1 high cells are limited to the effector memory population (P<0.001).

Conclusion: Taken together these data suggest that PD-1 expression on HIV specific CD8+ T cells is tracking antigen load at the level of epitope specificity and clonotype usage and may in fact serve as a useful proxy for quantitation of the level of epitopes expressed on the surface of infected cells.

OA01.04

Immunotherapeutic Interventions to Restore HIV-1-Specific CD4 T Cell Help to NK Cells

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Background: PD-1 and IL-10 blockade can restore antigen-specific T cell functions in chronic infections and cancer. In contrast to the significant progress made in understanding mechanisms of CD8 T cell exhaustion, CD4 T cell dysfunction remains much less explored. Here we investigate the impact of combined PD-1 and IL-10 blockade on restoring HIV-specific CD4 T cell help to improve natural killer (NK) cell function.

Methods: We used Luminex arrays to analyze IFN-γ, IL-2, IL-13 and IL-12 secretion in supernatants of CD3- or CD8-depleted PBMC from HIV-infected individuals with progressive infection. Cells were stimulated for 48h with Gag peptide pools in the presence of isotype control antibody, anti-PD-L1 and/or anti-IL-10Rα, anti-IL-12 or anti-IL-2.

We also used intracellular cytokine staining (ICS) to evaluate NK cell function as measured by expression of IFN-γ and CD107.

Results: Contrasting with the moderate effect of single blockade, concurrent PD-L1/IL-10Rα blockade resulted in dramatic increases in IFN-γ production by HIV-specific CD4 T cells (10-fold) and IL-12 secretion by antigen-presenting cells (APCs) (19-fold), which was governed by IFN-γ derived from Thelper cells. ICS assay during the first 12 hours of stimulation showed that CD4 T cells are the only subset producing IFN-γ. ICS assay at 48 hours demonstrated a secondary IFN-γ+ NK cell response that was CD4 T cell dependent. Depletion of CD3 T cell abrogated IFN-γ production from NK cells. Combined PD-L1/IL-10Rα resulted in statistically significant enhancement on the percentage of NK cells producing IFN-γ. Neutralization of IL-12 selectively reduced IFN-γ but not CD107 production from NK cells while neutralization of IL-2 inhibited both functions.

Conclusion: These data provide important evidence on the therapeutic potential of manipulating immunoregulatory pathways to restore HIV-1-specific CD4 T cell help to APCs and NK cell functions, providing evidence for synergistic impact of combined interventions and linking restoration of adaptive and innate immunity.
**Integrative Analysis of Responses to Dendritic-Cell Vaccination Identifies Signatures Correlated with Control of HIV Replication: The DALIA Trial**

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**Background:** We have reported that vaccination with ex vivo generated DC loaded with HIV-lipopeptides in patients (n=19) on antiretroviral therapy (ART) was well tolerated and immunogenic. Vaccine-elicited HIV-specific T cell responses were associated with improved control of viral replication following antiretroviral interruption (ATI from w24 to w48) (CROI 2012 PB440). Here we have performed an integrated analysis of post vaccination immune responses and gene expression measured at w16 with viral parameters during ATI.

**Methods:** Gene expression in whole blood was repeatedly measured by gene expression microarrays (Illumina HumanHT-12 v4) at 14 time points. Post vaccination (w16) immune responses were evaluated using ICS, Multiplex cytokine secretion and Interferon-γ ELISPOT. Peak of viral load was the maximum observed plasma HIV RNA during ATI. Longitudinal analysis of gene expression data was performed using hierarchical models allowing heterogeneity in predefined gene sets (Chaussabel’s functional modules). Data integration of gene expression, immune responses at w16 and peak of viral load during ATI was performed with sparse-Partial Least Square.

**Results:** During vaccination, 69 modules out of 260 varied significantly including T cell (M4.1), protein synthesis (M4.5), apoptosis/survival (M6.6) and inflammation (M4.6) modules. We show an inverse relationship between HIV-specific responses (production of IL-2, IL-13, IL-21, IFN-γ, CD4 polyfunctionality, i.e. production of at least two cytokines) and the peak of viral load during ATI. Those cellular immune responses were positively correlated to genes associated with T cell functional modules (M4.1, M4.15) at w16 and negatively correlated to genes associated with inflammation (e.g. EGLIN1, H2AFY, LTBR). Specifically, IL-21 secretion, that was associated with control of viral replication, was negatively correlated to MFAP3 and TFNR5F1A inflammatory genes.

**Conclusion:** Changes in gene expression profile were associated with vaccine-elicited cellular responses and viral rebound during ATI. Integrated analysis led to identify DC vaccine signatures likely correlated with a better control of HIV replication.

**Effective Antiviral CD8+ T Cells Responses Are Rare in HIV-Positive Step & Phambili Study Participants but Are Targeted to Low Entropy Viral Epitopes**

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**Background:** Reliable correlates of immunological control are critical for HIV vaccine development. We and others have shown the capacity of CD8+ T cells to inhibit HIV-1 replication in vitro is highly predictive of their antiviral efficacy in vivo. The design of immunogens that could elicit effective CD8+ T cell responses is a major challenge.

**Methods:** Ex vivo CD8+ T cell-mediated antiviral inhibitory activity was measured in 36 HIV-1-infected Step and Phambili trial participants (20 vaccinees, 16 placebos, blinded analysis) using PBMC sampled one year post-infection with a panel of 5 viruses representing clades A, B and C. Frequencies of CD8+ T cells to: (i) total proteome using PTE peptides (ii) ‘protective’ peptides (overlapping Gag, Pol, Nef, and Vif peptides (OLPs) preferentially targeted by patients with controlled viraemia) were determined by IFN-γ intracellular staining and Elispot.

**Results:** The majority of study participants lacked potent CD8+ T cell antiviral activity and this did not differ significantly between vaccine and placebo recipients. Despite strong responses to the total proteome (median 1.81% CD8+ T cells), frequencies of IFN-γ-producing T cells targeting the ‘protective’ OLPs were modest overall (median 0.02% CD8+ T cells). CD8+ T cell-mediated virus inhibition was strongly correlated with IFN-γ responses to protective OLP (r = 0.69, p = 0.0001) even after controlling for protective HLA alleles, but not to total proteome responses (r = 0.11, p= 0.64). Viral load setpoint was negatively correlated with CD8+ T cell antiviral activity (r = -0.37, p = 0.05).

**Conclusion:** Sustained potent CD8+ T cell antiviral inhibitory responses were rare in HIV-infected Step and Phambili Study participants but were strongly associated with targeting of ‘protective’, low entropy regions in HIV-1 Gag and Pol. These findings highlight the importance of systematically selecting specific regions within the viral proteome for inclusion in immunogens to avoid targeting of irrelevant epitopes.
**OA02.01**

**Engineering an HIV Envelope Protein to Activate Germline B Cell Receptors of Broadly Neutralizing VRC01-Class Antibodies**

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**Background:** The recent RV144 trial showed ~30% efficacy. Although the protection was modest, the trial indicated for the first time that a vaccine against HIV is possible. Immune correlate analysis suggests that the observed protection was due to non-neutralizing antibody responses. This efficacy might be improved if a vaccine could elicit broadly neutralizing antibodies (bNAbs). Of particular interest for vaccine design are the potent VRC01-class bNAbs targeting CD4 binding site on Env. Unfortunately, a number of studies have demonstrated that recombinant Env proteins do not bind germline-reverted VRC01 class Abs, indicating that current vaccine strategies using recombinant Env are unable to activate progenitor B cells that ultimately give rise to VRC01 class Abs.

**Methods:** We developed reagents and experimental protocols to express functional versions of the mature and germline VRC01 and NIH45-46 BCRs on the surface of B cells, and assays to monitor B cell activation following the antigenic-engagement of these BCRs.

**Results:** Although several recombinant Env proteins were capable of binding to and stimulating B cells expressing the mature BCR forms, no such interactions were recorded with B cells expressing the germline BCR forms. However, we identified key conserved glycosylation sites in the Loop D and V5 regions of a Clade C Env that prevent the binding of the clonally related germline NIH45-46 and germline VRC01 BCRs. Disruption of these glycosylation sites resulted in nM binding affinity to germline BCRs and activation of the corresponding B cells.

**Conclusion:** Our study identifies the earliest roadblock in the elicitation of anti-CD4-BS bNAbs; the lack of engagement of the germline BCR forms of ‘VRC01’ class antibodies by commonly used HIV Env immunogens. Importantly, we have developed a way to overcome this roadblock through the design of an HIV Env that engages and activates B cells expressing the germline BCR forms of ‘VRC01’ class antibodies.

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**OA02.02**

**Focusing Antibody Responses to Specific Protein Surfaces Through Site-Selective Glycan Addition**

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**Background:** Viruses such as human immunodeficiency virus type-1 (HIV-1) and influenza A virus display rapidly mutating or non-neutralizing immunodominant epitopes on their viral glycoproteins to escape antibody-mediated neutralization.

**Methods:** Immunosilent glycoconjugates with terminal sialic acid were added site-selectively to the model protein hen egg lysozyme (HEL), mutant an HIV-1 gp120 or engineered gp120 outer domain. Epitope mapping was subsequently performed on sera from immunized mice.

**Results:** Glycoconjugate addition of HEL led to site-selective loss of antibody binding to epitopes containing modification sites in vitro. Immunization with modified protein led to refocusing of antibody responses from masked to unmasked epitopes in vivo. Application of this sialic acid masking strategy to HIV antigens led to loss of antibody recognition in masked regions whilst maintaining antigenicity of the CD4 binding site which is a target of broadly neutralizing antibodies.

**Conclusion:** Our masking strategy attaches relatively small glycoconjugates to properly folded proteins under mild conditions with near 100% efficiency. This approach circumvents many of the problems, such as improper folding and low occupancy, associated with masking strategies using mutational redistribution of N-linked glycosylation sites. Thus, sialic acid glycoconjugate masking should allow focused targeting of specific antigenic regions for increased B cell recognition, improving vaccine antigen design.
OA02.03

Comparative Antigenicity and Immunogenicity of Indian and South African HIV-1 Subtype C Native and CD4 Liganded Envelope Glycoproteins

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Background: The ability to induce a potent and broadly neutralizing antibody (bNAb) response following vaccination is critical in developing an effective HIV-1 vaccine. This study describes the design and construction of three HIV-1 subtype C South African (derived from founder virus sequences) and three Indian (derived from circulating virus sequences) Env immunogens, and their antigenicity and immunogenicity testing in the presence or absence of liganded CD4, in rabbits.

Methods: Monomeric (gp120) and trimeric (gp140GCN4+) conformations for IN26191, IN25710, IN25925, ZACAP45, ZACAP210 and ZA706010164 were expressed in mammalian cells. Unliganded or 2dCD4 S60C liganded Env glycoproteins were purified by lectin affinity chromatography, followed by conformation and complex purification using size exclusion chromatography. Immunogens/immune complexes were evaluated by ELISA and Surface Plasmon Resonance. Immunogenicity of each conformation alone or complexed to 2dCD4 S60C was evaluated in rabbits. Breadth and potency of each rabbit serum was tested against 12 pseudoviruses (Tiers 1-3) derived from HIV-1 subtype B and C Env using the PhenoSense Neutralizing antibody assay (Monogram Bioscience Inc.).

Results: The antigenicity of Envs against a panel of antibodies showed no significant differences between South African and Indian Envs, as determined by SPR. Minimal neutralizing breadth was obtained from animals immunized with IN25925 and ZACAP210 Env only conformations. However, animals immunized with Env/2dCD4 S60C complexes developed potent bNAbs against all 12 pseudoviruses tested, including tier 2 and 3 pseudoviruses. End-point ELISA titre results revealed that immunizing with Env/2dCD4 S60C produced both Env and 2dCD4 specific titres, but the 2dCD4 S60C titres were on average 10x lower than the 2dCD4 S60C only control group, suggesting an alternative mechanism of neutralization other than a CD4 directed response.

Conclusion: The Env/2dCD4 S60C complex is a super-immunogen and elicits potent and broad NAb responses against Tier 1, 2, and 3 pseudoviruses. Such potent and bNAbs have never been reported previously, and non-human primate studies are underway.

OA02.04

Immunogenicity of a CD207-Targeted Anti-Gag Vaccine and TLR Ligand-Dependent Enhancement

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Background: The targeting of antigens to dendritic cells (DCs) is an attractive concept for the development of new, efficient, safer vaccines. In this aim, better understanding of early events at site of intradermal (ID) vaccine injection is needed for a rational design of future vaccines.

Methods: Non-human primates (NHPs) were immunized with HIV Gag protein associated with an anti-CD207 mAb in fusion protein. Sera were collected from vaccinated animals for the titration of Gag-specific Abs. NHPs underwent ID injection of PBS, R848 or poly(I:C) in presence or not of etanercept (anti-TNFα). Punch biopsies were performed for histology or cell extraction and flow cytometry analysis.

Results: We show that directing HIV-Gag to Langerhans cells (LCs) through langerin (CD207) efficiently primes a specific immune response in NHPs (Ab titer 1.7 log higher than control). This immune response was significantly strengthened (Ab titer 0.53 log higher) by the co-injection of TLR-ligands (TLR-Ls). TLR3-L (poly(I:C)) and TLR7/8-L (R848) induced the local recruitment of polymorphonuclear neutrophils (4 fold increased, p=0.0006) and macrophages (3 fold increased, p=0.0012). This recruitment was directly correlated to LC activation and their migration out of the epidermis. LCs were not directly affected by TLR-L and their activation and migration depended on TNF-α secretion by TLR-L-stimulated inflammatory cells. This was notably demonstrated by inhibiting the TNF-α activity in vivo with etanercept (p=0.0242).

Conclusion: This study highlights new elements of cell activation mechanisms in the skin and shows that targeting a vaccine to specific DC subsets and stimulating specific cells of the innate immune system can increase the efficiency of vaccination.
OA02.05

Impact of TLR9 Stimulation on HIV-1 Env-Specific Immune Responses Elicited in Non-human Primates

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Background: The identification of safe and effective adjuvants is important to promote the development of protective immune responses induced by protein-based vaccines. TLR9 agonists were previously shown to improve responses to existing vaccines, such as the protein-based Hepatitis B virus vaccine formulated in alum analogues. Here, we assessed the capacity of CpG class C, a well-known TLR9 ligand, to quantitatively or qualitatively modulate HIV-1 envelope glycoprotein (Env)-specific B and T cell responses induced by Abisco-100. Abisco-100 is an iscomatrix-based adjuvant frequently used in animal vaccines and currently under evaluation for use in humans.

Methods: Rhesus macaques were inoculated three times with soluble YU2 gp140-F trimers in Abisco-100 with or without the addition of CpG-C. The impact of TLR9 stimulation on the magnitude and durability of antigen-specific antibody titers and memory B cells were determined and antibody isotypes and neutralizing activity against a panel of different HIV-1 strains were assessed. Finally, phenotypic and functional characterization of Env-specific CD4+ and CD8+ T cell responses were performed using multi-color flow cytometry.

Results: Our results show that the specific antibody titers and frequencies of memory B cells displayed similar kinetics and magnitude in the presence and absence of CpG, and there was no apparent difference in HIV-1 neutralizing activity. By using antibodies against specific rhesus macaque antibody isotypes, we found that the Env-specific IgG1 titers were somewhat higher than the IgG2 titers in animals receiving CpG-C, but this difference was not significant.

Conclusion: Therefore, while several other studies have shown that the addition of CpG influences the magnitude or quality of immune responses against other antigen-adjuvant combinations, our results show that in rhesus macaques, the addition of CpG-C to Abisco-100 did not markedly affect the magnitude or quality of HIV-1 Env-specific B cell and T cell responses.

OA02.06 LB

Lipid Components in MPER-Based Immunization Regimens Are Critical for Inducing Broadly Neutralizing Antibody Responses in 2F5 and 4E10 Knockin Mice

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Background: Developing an HIV-1 vaccine is hindered by the inability of immunogens to elicit broadly neutralizing Abs (BnAbs). Using knockin (KI) models expressing the membrane proximal external region (MPER)-specific BnAbs 2F5 or 4E10, we recently identified common hurdles for inducing BnAbs: profound B-cell deletion, and anergy of residual clones. However, residual 2F5/4E10 KI B-cells accumulate at distinct developmental stages, and subsets of each spontaneously escape anergy by selectively losing MPER and lipid specificity, respectively. Since 2F5/4E10’s initial interactions with viral membranes (prior to MPER binding) are required for neutralization, it is key to identify regimens that not only overcome tolerance of 4E10/2F5+B-cells, but also target subsets retaining dual (MPER+lipid) reactivity.

Methods: To dissect components needed to activate anergic, MPER/lipid+B-cells, serum Ab responses were assessed in KI mice immunized with various constituents of a conjugate immunogen comprised of an MPER peptide containing 2F5 and 4E10 epitopes, liposomes, and TLR4/9 agonists oCpG and Monophosphoryl Lipid A (MPLA) respectively, a regimen that elicits high serum BnAb titers in 2F5 KI mice. Additionally, to assess the impact of rescuing 2F5/4E10+B-cells from deletion, Ab responses in WT KI mice were compared to those overexpressing the anti-apoptotic gene bcl2.

Results: In 2F5 and 4E10 KI mice, MPER peptides alone, multimerized on beads, or formulated with oCpG, elicited minimal MPER/lipid+ BnAb titers. In contrast, peptides conjugated to liposomes and formulated with MPLA, elicited robust MPER/lipid+ BnAb titers. However, peripheral B-cell survival signals were required for immunization-induced BnAb responses in 4E10 KI mice, they were dispensable in 2F5 KI mice, consistent with the latter originating from early, T-independent B-cells.

Conclusion: Our results suggest that immunization regimens capable of eliciting BnAbs directed at both 2F5 and 4E10 MPER epitopes require lipid components, and will need to be optimized to target distinct B-cell subsets in these two BnAb lineages.
OA03.01

The HIVconsv Vaccines Induce Polyfunctional and Highly Proliferative T Cells that Control In Vitro HIV Replication: HIV-CORE002 Phase-I Clinical Trial

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Background: A safe and efficacious vaccine is urgently needed to control HIV/AIDS. HIVconsv is a new generation T cell immunogen targeting the most conserved regions of the HIV-1 proteome and thus has great potential as a universal HIV vaccine to protect against the multiple HIV-1 subtypes.

Methods: Immunogenicity of the HIVconsv candidate vaccines pSG2. HIVconsv DNA (D), ChAdV63.HIVconsv (C) and MVA.HIVconsv (M) in various combination regimens (CM, DDDCM or DDDMC) was tested in healthy HIV-1/2-uninfected, low-risk adults in a double-blind, randomized phase I clinical trial. The frequency of vaccine-induced T cells in PBMC was measured by ex-vivo IFN-γ ELISPOT assay. The functional profile of vaccine-induced CD8+ and CD4+ T cells was evaluated in a multi-parameter intracellular cytokine staining assay (ICS) for IFN-γ, TNF-α, IL-2, CD107a and Granzyme-A. Further multi-functional profiling was done by assessing the quantities of secreted cytokines in a Luminex assay. Proliferative capacity of CD4+ and CD8+ T cells was quantified by a flow-based CFSE assay, while the potential of vaccine-induced T cells to control HIV-1 replication was assessed in an in-vitro viral inhibition assay using a panel of eight HIV-1 virus isolates.

Results: The HIVconsv vaccines induced high magnitude IFN-γ ELISPOT responses reaching over 5000 SFU/M cells at peak. ICS and Luminex assays revealed that these vaccine-stimulated T cells comprising both CD8+ and CD4+ populations were highly polyfunctional, secreting several cytokines and signaling molecules such as IFN-γ, TNF-α, IL-2, IL-10, IL-13, MIP-1β and GM-CSF. Additionally, these cells displayed greater proliferation capacity even at 28 weeks post-immunization, and most importantly, they were capable of inhibiting replication of several HIV-1 virus isolates in autologous CD4+ T cells.

Conclusion: HIVconsv vaccines induce polyfunctional and highly proliferative CD8+ and CD4+ T cells with antiviral capacity and could potentially control in vivo HIV-1 infection.

OA03.02

Phase I Clinical Trials of DNA-Protein Vaccine (CombiHIVVac) Containing Artificial Multi-Clade Immunogens

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Background: CombiHIVVac is micelle-like particles, which combine artificial multi-clade polypeptide immunogens - pcDNA-TCI (DNA vaccine) and TCI protein. The TCI contains over 80 optimally selected overlapping epitopes from Env, Gag, Pol, and Nef. The chosen epitopes are highly conservative among HIV-1 subtypes A, B and C. TBI protein contains conserved B- and T-epitopes from Env and Gag.

Methods: The trial was recruiting 30 volunteers who are healthy HIV-negative men and women at low risk for HIV infection. They were randomly divided into two groups. The first group was vaccinated intramuscularly with one dose of CombiHIVVac (75µg of DNApcDNA-TCI and 50µg of TBI protein) at day 0. The second group received one dose of vaccine twice (at days 0 and 28). Combined clinical study and laboratory examination were carried out for each volunteer during one year. Immunogenicity was assessed by Immunoblot, ELISA, IFN-gamma ELISpot, MHC-pentamers and virus neutralization (using HIV-1 Env pseudoviruses). The samples, which were taken before immunization, served as negative controls.

Results: No side effects or negative changes in clinical and biochemical parameters were registered after vaccination. Vaccine was well tolerated, apyrogenic, no inflammatory reactions at the site of administration were found. Neither single nor double intramuscular administration of vaccine had persistent effects on the integral physiological, hematological or biochemical parameters in volunteers. Twofold injection is more efficient and provides developing of more effective immune response. All volunteers from second group had specific humoral immune response (ELISA, HIV-1 New-Lav Blot and preudoviruses) and were IFN-gamma ELISpot reactive.

Conclusion: CombiHIVVac is safe and well tolerated. Vaccination with CombiHIVVac elicited specific as CD8+ T cell and humoral responses. The request for Phase II clinical trials is submitted for approval to the Russian Ministry of Health.
OE03.03

Comparative Analysis of Binding Antibody Responses Elicited by a Cross-Section of Human HIV-1 Vaccine Clinical Trials

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Background: Using a canary pox prime (ALVAC) and protein boost (AIDSVAX B/E) regimen, the RV144 vaccine clinical trial demonstrated 31% efficacy at 42 months. Follow-up studies revealed binding antibodies to the V1/V2 region of HIV-1 as a correlate of infection risk, demonstrating the importance of antibody induction by HIV-1 vaccines. These observations have motivated evaluation of the specificity, breadth, potency, and duration of binding antibodies elicited in previous human vaccine clinical trials in comparison with responses to those elicited by the ALVAC prime-protein boost regimen.

Methods: Nine vaccine trials were analyzed using the Meso Scale Discovery (MSD) system to detect HIV specific binding antibodies to a panel of cross-clade and site-specific antigens that included: trimers from clades A, B, and C, gp120 monomers, V1/V2 (N160-), V3, MPER, and the CD4 binding sites. Comparative analyses were performed on the following trials, which represented a range of immunogens, immunization regimens, and delivery methods: VAX003 and RV114 (protein only), RV132 and RV135 (ALVAC prime, protein boost), RV138 (ALVAC only), RV158 (MVA only), RV262 (DNA prime, MVA boost), RV172A (Ad5 only), and RV172B and VRC008 (DNA prime, Ad5 boost).

Results: Preliminary results demonstrated the inclusion of a protein component within the vaccine regimen increased the potency of binding antibody responses to cognate antigens by five-fold. Ad5, ALVAC, MVA, and DNA alone elicited minimal binding antibody responses, however the combination of prime, boost regimens significantly increased the magnitude of these responses. In addition, increasing the diversity of vaccine inserts increased the breadth of cross-clade binding antibodies.

Conclusion: Using one platform, we compared the capability of multiple vaccine regimens to elicit binding antibodies to a variety of HIV-1 envelope antigens. These results could significantly influence decisions surrounding future HIV vaccine efficacy trials.

OE03.04

Antibody Repertoire Induced by the Multiclade (Env A, B, C) HIV-1 DNA Prime, rAd5 Boost VRC Vaccine

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Background: The phase II DNA prime, rAd5 boost VRC vaccine (HVTN 204) exhibited sufficient safety and immunogenicity to advance into a phase IIb efficacy trial (HVTN 505) in Ad5 seronegative, circumcised, male volunteers in the US. Determining the antibody specificities induced by the VRC vaccine will provide insights in the memory B cell repertoire elicited by this DNA prime rAd5 boost regimen.

Methods: Antibodies were isolated via antigen-specific single memory B cell sorts from 8 subjects who received this VRC vaccine regimen (HVTN 204 and 082 trials), using group M consensus and vaccine-matched Env oligomers, and vaccine-matched MuLV gp70 V1/V2-fusion proteins. Monoclonal antibodies (mAbs) were generated and tested for binding, neutralization, and infectious virus capture.

Results: 235 HIV-1 antibodies were isolated from 8 vaccinees. 92% of the antibodies were gp41-reactive, and only 8% reacted with gp120. Of 217 gp41 antibodies, 64% used VH1-69. Greater than 90% of the VH1-69 gp41 antibodies used the HCDR2-bearing 62L allelic variants. Of 217 gp41 antibodies, 64% used VH1-69. Greater than 90% of the VH1-69 gp41 antibodies used the HCDR2-bearing 62L allelic variants. In contrast, non-HIV-1 antibodies isolated from HIV-1 infected subjects predominantly used the HCDR2 62F allelic variants (P value = 1.8 X10^-25, Fisher Exact Test). Seventeen gp41 mAbs from fifteen different clonal lineages were generated from vaccinees. The gp41 mAbs bound recombinant gp120 protein, but failed to bind gp41 overlapping peptides, suggesting binding to conformational epitopes. Some gp41 mAbs were cross-reactive with commensal bacteria. A subset of gp41 mAbs captured infectious HIV-1 transmitted/founder viruses, but failed to neutralize tier 1A viruses.

Conclusion: This DNA prime, rAd5 boost vaccine regimen induced a dominant gp41-specific antibody response. These data raise the hypothesis that gp41 dominance may be the result of pre-vaccination expansion of B cell precursors that are cross-reactive with a gp41 structure in the Env gp140 vaccine antigen.
OA03.05

A Phase I Clinical Trial of an HIV-1(CN54), Clade C Trimeric Envelope Vaccine Delivered by Parenteral, Nasal and Vaginal Routes of Immunisation

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Background: We conducted a phase 1, randomized, exploratory trial to assess three immunisations of CN54gp140 vaccine formulations, administered in four different regimens at weeks 0, 4 and 8.

Methods: HIV negative female volunteers aged 18-45 years were recruited through two centres and randomised to: (1) Intramuscular (IM) 100µg CN54gp140 adjuvanted in 5µg GLA in an aqueous formulation (GLA-AF) (2) IM 20µg CN54gp140 in 5µg GLA-AF (3) IM priming with 100µg CN54gp140 in 5µg GLA-AF followed by two intravaginal (IVAG) boosts with 500µg CN54gp140 in carbopol gel or (4) Intranasal (IN) 100µg CN54gp140 in chitosan. Vaccine specific humoral responses were assessed in serum and mucosal samples at week 12.

Results: Thirty six women completed all three immunisations and there were no vaccine related serious adverse events. The parenteral groups had significantly more women with serological and mucosal responses at week 12 (p<0.001 and p=0.031 respectively). 9/11 and 9/9 women in the IM-IVAG group had serum IgG. However, responses following additional IM boosting in some nasally primed subjects indicated amnestic responsiveness. Serum IgA was not detected in any volunteers.

Conclusion: The lower dose of CN54gp140 administered IM was as good as the standard dose of CN54gp140, eliciting systemic responses in the majority of subjects, and mucosal responses in just over a third of female volunteers four weeks after the third dose. The mucosal routes failed to induce significant systemic or mucosal responses, in spite of the high IVAG dose of 500µg preceded by IM priming.

OA03.06 LB

HIV-1 Fusion Protein (F4/AS01) and Adenovirus-35 Gag-RT-Int-Nef Induce Potent and Durable T-Cell and Antibody Responses in Healthy African Adults

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Background: Phase I studies show the F4/AS01 (subtype B) HIV vaccine has an acceptable safety profile and induces potent CD4+ T-cell and antibody responses, while the Ad35-GRIN vaccine (subtype A) is safe and induces robust CD8+ T-cell responses. This randomized, double-blind, placebo-controlled study evaluated the safety and immunogenicity of these vaccines in prime boost or co-administration combinations in healthy African adults.

Methods: Healthy adults (n=147) in Uganda, Kenya and Zambia were randomized to receive: F4 fusion protein 10µg formulated in AS01B or AS01E at weeks 0 and 4 followed by Ad35-GRIN 2x1010 vp at week 16, or Ad35-GRIN at week 0 followed by F4/AS01B at weeks 12 and 16, or co-administration of both vaccines (with AS01B) at weeks 0, 4 and 16, or placebo in a 4:1 vaccine:placebo ratio for each group.

Results: The vaccines were well-tolerated in all groups. Systemic reactions were more common with co-administration, but were mostly mild and moderate; all were transient. There were no vaccine-related SAE. IFN-gamma ELISPOT response rates 4 weeks after the last vaccine administration were higher in the co-administration and Ad35-GRIN primed groups (92% and 100%, respectively) compared to the F4/AS01 primed groups (50% and 54%). Potent polyfunctional HIV-specific CD4+ and CD8+ T-cells were observed in all vaccine groups and particularly in co-administration group after only 2 vaccinations. Antibodies against F4-fusion protein were seen in >96% of individuals after complete vaccination. T-cell and antibody responses persisted up to 1 year post last administration. Ad35 neutralization antibodies were detected in 14% and 48% of individuals after 1 versus 3 administrations of Ad35-GRIN and were lower titers.

Conclusion: The F4/AS01 and Ad35-GRIN vaccines show an acceptable safety and reactogenicity profile in sequential prime-boost or coconantam administration. These combinations of vaccines elicit complementary potent, polyfunctional CD4+ and CD8+ T-cell responses and antibodies which persisted up to one year.
OA04.01

PET/CT Imaging of Simian Immunodeficiency Virus Reveals the Dynamics of Viral Replication In Vivo

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Background: The evaluation of HIV replication relies on indirect methods such as plasma viral loads, which does not indicate the anatomic origin of the virus detected. In addition, the suspected residual virus replication seen during HAART or in long-term non-progressors with undetectable plasma viral loads cannot be confirmed or localized. We hypothesized that a method able to capture specific in vivo sites of viral replication would provide a valuable tool to monitor antiviral therapies, determine the site of viral reservoirs and provide clues as to the sites that need to be protected via vaccination to prevent viral progression during the acute infection.

Methods: Using an anti gp120 monoclonal antibody labelled with 64Cu, we have developed a novel strategy to image SIV and SIV infected cells in vivo using positron emission tomography /computer tomography (PET/CT). PET/CT results were corroborated with SIV mRNA levels and in situ detection of SIV by IHC and FISH.

Results: Using SIVgp120 immunoPET/CT, extensive viral replication was seen in the descending colon in most chronically infected, viremic monkeys, with lymph nodes, spleen and nasal lymphoid tissues prominently positive. In contrast, LTNP monkeys showed a more variable distribution of virus signal, with modest signal throughout the intestine including small bowel and various lymphoid organs. Of note, among reproductive organs, a high intensity signal was detected in the epididymis, which was confirmed by in situ detection. Monkeys acutely infected intravenously with SIV showed abundant initial signal in the upper body including lung and NALT, which gradually cleared, while the SIV signal markedly amplified in the lower GI tract during chronic infection.

Conclusion: We submit that we have developed a sensitive real time imaging method to evaluate and localize virus replication in vivo. The method is non-invasive, can be used repeatedly and is readily translatable to the clinic.

OA04.02

Non-human Primate Model to Study the Role of Serum and Vaginal IgA in Acquisition of Infection


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Background: Following the success of the RV144 trial, correlates analysis showed a possible correlation between serum IgA titers and lack of vaccine effectiveness. This data is in contrast to the concept that a protective HIV vaccine will need to induce strong IgA induction to prevent mucosal transmission. To address the role of serum and vaginal IgA in HIV acquisition we utilized an adjuvanted DNA vaccine previously shown to drive IgA induction in a non-human primate vaginal challenge model.

Methods: Groups of 5 Indian rhesus macaques received a pSIVmac239 gag/pol and pSIVe660 gp120 alone, with pCCL27 or pCCL28, mucosal adjuvants, at weeks 0, 6, 12, 18 and 48. Animals were challenged with 500 TCID SIVsmE660 intravaginally twice a week for two weeks.

Results: We observed higher vaginal IgA titers in adjuvanted animals compared with DNA alone. Following challenge, we observed a significant control of set point viremia and chronic viremia in adjuvanted animals (p<0.05). 80% of adjuvanted animals controlled infection compared with only 60% in unadjuvanted animals and 17% in naïve challenge controls. Irrespective of vaccine group, animals that controlled viremia had the highest vaginal IgA and IgG levels post-vaccination. Importantly, there was also no correlation between vaginal and serum IgA

Conclusion: Together these data suggest that vaginal IgA is indeed protective from mucosal HIV transmission. Future clinical studies should incorporate mucosal sampling for correlates analysis because there is likely no correlation between serum and vaginal IgA levels. As is the case for the effective HPV vaccine, the induction of vaginal IgG may also play a role in protection. These data are illuminating in light of the RV144 study, that induction of serum IgA is not predictive of IgA responses at the vaginal site.
Vaccine Induced Epitope Specific Antibodies to the SIV Envelope Are Distinct from Those Induced to the HIV-1 Envelope


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Background: Understanding binding antibody specificities induced in non-human primates (NHPs) following HIV-1 and SIV Env immunization is critical for interpretation of data from preclinical vaccine trials. In this study, we determined whether antibody responses following HIV-1 and SIV immunizations in NHPs can predict antibody responses in human clinical trials.

Methods: We determined binding epitopes specificities in 4 NHP SIV vaccine studies (DNA, MVA, protein, virus particle, and Ad5) and 4 NHP HIV-1 vaccine studies (NYVAC/ALVAC/AIDSVAX and protein). Microarray linear epitope mapping was used to profile Env-specific responses against multiple clades/strains of HIV-1 and SIV, with a peptide library that covers full-length gp160 of multiple HIV-1 and SIV sequences (1942 peptides total). Binding profiles of NHP and human sera against HIV-1 Env, and of NHP against SIV and HIV-1 Envs, were compared.

Results: Antibody specificities developed in NHP following HIV immunizations were similar to that observed in human. However, we observed major differences in epitope specific antibodies from NHP following SIV versus HIV-1 Env immunizations. First, V2-specific antibodies were dominant in SIV-immunized NHPs, whereas V3 specific antibodies were dominant in HIV-1 Env immunized NHPs. In two of the SIV NHP studies, the anti-V2 response accounted for 35-50% of the total binding response, compared to less than 8% for anti-V3 response. Secondly, the anti-V1 antibody response, while not observed in HIV-1 Env vaccinations, commonly developed following SIV Env immunizations, and contributed to 7-15% of total binding response.

Conclusion: We found that although NHPs can develop antibody specificities to the HIV-1 envelope that are similar to those responses induced by human HIV-1 vaccination, epitope specific antibodies to the SIV Env differ from the antibody responses to the HIV-1 Env. Thus, NHP vaccine studies may require inclusion of HIV-1 envelope sequences, instead of SIV envelope, to accurately reflect antibody specificities induced by HIV-1 vaccination in human clinical trials.
OA04.05

Protection of Cynomolgus Macaques from Pathogenic SIV Following Vaccination with Varicella-Zoster Virus Based Vaccines


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Background: Varicella Zoster virus (VZV) is a persistently replicating virus with the potential to deliver life-long immunity in an HIV vaccine setting. As a recombinant vaccine vector, the ability to self-boost may overcome the limited capacity of pox- and adenoviral vectors to generate long-lived immunogenicity.

Methods: We describe a comprehensive preclinical non-human primate challenge trial with recombinant VZV-SIV constructs expressing codon-optimized and safety augmented antigenic targets from SIV as candidate prophylactic HIV vaccines. As a strict evaluation of our approach, a cohort of MHC-typed cynomolgus macaques were administered only a single vaccination with VZV-SIV vaccine or vector alone, and subsequently challenged intra-rectally using a weekly low-dose exposure to highly pathogenic SIV.

Results: Vaccine-induced durable humoral and cellular immune responses were observed with evidence to suggest VZV replication and subsequent reactivation driving progressive increases in anti-VZV IgG titers. There was no significant difference in SIV acquisition rates or challenge doses to infection between vaccine and control groups over the course of multiple low-dose mucosal challenges. Importantly, the vaccine group showed lower peak and set point viral loads (VL) with a subset of vaccinees (~35%) with persistent undetectable VL over 1.5 yrs of follow-up. We saw no evidence of accelerated disease course or increased SIV replication in vaccinees.

Conclusion: A single dose of human VZV-based SIV vaccines was sufficient to provide substantial benefit to vaccinees with apparent early disease modification and preliminary evidence to suggest a similar capacity to induce viral clearance as recently demonstrated by Louis Picker’s related RhCMV-based approach. Future work will be directed to determine the extent of this SIV clearance with subsequent NHP trials designed to augment the immunogenicity and capacity of this approach. The well documented clinical and safety evaluations of VZV vaccines in humans, provides a substantial advantage in developing this platform for human clinical trials.

OA04.06 LB

Therapeutic Efficacy of Potent Neutralizing HIV-1-Specific Monoclonal Antibodies in SHIV-Infected Rhesus Monkeys

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Background: HIV-1-specific monoclonal antibodies (mAbs) with extraordinary potency and breadth have recently been described. In humanized mice, combinations of mAbs have been shown to suppress viremia, but the therapeutic potential of these mAbs has not yet been evaluated in primates with an intact immune system.

Methods: We evaluated the virologic and immunologic impact of infusion of a cocktail of mAbs or individual mAbs in 35 rhesus monkeys chronically infected with the highly pathogenic virus SHIV-SF162P3. 10 mg/kg of the broad and potent HIV-1-specific mAbs PGT121, 3BNC117, and b12, or the isotype control mAb DEN3, were administered.

Results: Administration of the cocktail of HIV-1-specific mAbs, or the single glycan-dependent mAb PGT121, resulted in a rapid and precipitous decline of plasma viremia to undetectable levels in rhesus monkeys chronically infected with SHIV-SF162P3. A single mAb infusion afforded up to a 3.1 log decline of plasma viral RNA in 7 days and also reduced proviral DNA in peripheral blood, gastrointestinal mucosa, and lymph nodes without the development of viral resistance. Moreover, following mAb administration, host Gag-specific T lymphocyte responses exhibited improved functionality. Virus rebounded in the majority of animals after a median of 56 days when serum mAb titers had declined to undetectable levels, although a subset of animals maintained long-term virologic control in the absence of further mAb infusions.

Conclusion: These data demonstrate a profound therapeutic effect of potent neutralizing HIV-1-specific mAbs in SHIV-infected rhesus monkeys as well as an impact on host antiviral immune responses. Our findings strongly encourage the investigation of mAb therapy for HIV-1 in humans and have important implications for our understanding of immune correlates of protection.
**OA05.01**

Structural Basis for HIV-1 gp120 Recognition by a Germ-line Version of a Broadly Neutralizing Antibody


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**Background:** Following infection by HIV-1, the host immune response is unable to clear the virus due to a variety of factors, including rapid viral mutation and the establishment of latent reservoirs. The only target of neutralizing antibodies is the trimeric envelope (Env) spike complex, but HIV-1 can usually evade anti-spike antibodies due to rapid mutation of its two spike glycoproteins, gp120 and gp41, and structural features that allow the spike to hide conserved epitopes. Efforts to design an effective antibody-based vaccine against HIV-1 would benefit from understanding how germ-line B cell receptors recognize the gp120/gp41 envelope spike. Recently isolated from several infected individuals, potent VRC01-like (PVL) HIV-1 antibodies derived from the VH1-2*02 germ-line allele target the CD4 binding site on gp120. These antibodies have unprecedented potency and breadth for HIV-1 neutralization and some protect against HIV-1 infection in animal models. Since the VH1-2*02 germ-line allele is present in up to 95% of the population, it may be possible to elicit by vaccination similar antibodies in other individuals. VH1-2*02 germ-line B cell receptor interactions with gp120 were uncharacterized, which was a bottleneck for designing immunogens to elicit PVL antibodies.

**Methods:** We determined the structures of a VH1-2*02 germ-line antibody alone and a germ-line heavy chain/mature light chain chimeric antibody complexed with gp120 by X-ray crystallography and characterized them through surface plasmon resonance and neutralization experiments.

**Results:** VH1-2*02 residues make extensive contacts with the gp120 outer domain, including all PVL-sigature and CD4-mimicry interactions, but not critical CDRH3 contacts with the gp120 inner domain and bridging sheet that are responsible for the improved potency of NIH45-46 over closely-related clonal variants, such as VRC01.

**Conclusion:** Our results provide insight into initial recognition of HIV-1 by VH1-2*02 germ-line B cell receptors and may facilitate the design of immunogens tailored to engage and stimulate broad and potent CD4-binding site antibodies.

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**OA05.02**

Structural Characterization of Potent, Longitudinally-Defined HIV-1 V1/V2-Directed Antibodies


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**Background:** Approximately 20% of individuals infected with HIV-1 develop neutralizing antibodies that are cross-reactive with a variety of HIV-1 strains. These antibodies generally exhibit extensive affinity maturation and are slow to appear. Antibodies targeted against the V1/V2 region of Env display lower levels of somatic mutation than those targeting other sites of vulnerability and are among the most frequently elicited. Comprehensive analysis of B cell ontogeny of effective V1/V2 antibodies would provide valuable insight into vaccine development aimed at eliciting this class of antibody.

**Methods:** Broadly-neutralizing V1/V2-directed antibodies isolated from donor CAP256 at various time points following infection were isolated, and evolution of antibody development was longitudinally analyzed through deep sequencing and structural characterization.

**Results:** Here we present crystal structures of the antigen-binding fragments from several V1/V2-directed antibodies isolated from donor CAP256. The antibodies contain long heavy chain 3rd complementary determining regions (CDR H3s), a common feature among V1/V2-directed antibodies. The structures reveal a disulfide bond at the base of an extended CDR H3 loop and show that tyrosine sulfation contributes to a negatively charged surface extended away from the framework of the Fab. Deep sequencing data reveal that the length of the CDR H3 is the result of a recombination event and does not extend over time, however specific features of the mature loop such as the disulfide bond are not found early in the lineage.

**Conclusion:** Combinatorial studies with structural characterization and deep sequencing reveal paths to the development of potent and broad V1/V2 directed antibodies. While some structural components appear immediately such as the long CDR H3, affinity maturation plays an important role in stabilizing specific features. The ontogeny of the antibodies presented here reveal that breadth can be achieved rapidly and extensive affinity maturation is not a necessary feature of broadly neutralizing HIV-1 antibodies.
**OA05.03**

**Interplay Between Broadly Cross-Neutralizing V2 Monoclonal Antibodies and Autologous Viral Evolution**

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**Background:** CAP256, an HIV-1 subtype C superinfected individual, developed potent broadly cross-neutralizing (BCN) V2 plasma antibodies that were critically dependent on R166 and K169. Eleven related BCN mAbs targeting V2 have recently been isolated from this individual after 30 months post-infection (p.i.). Here we investigated the relationship between these mAbs, longitudinal CAP256 viruses and previously characterized escape variants.

**Methods:** CAP256 primary transmitted/founder (t/f) (N160 glycan -), superinfecting t/f (N160 glycan +) and 6-, 12-, 21- and 39-month viruses were tested for neutralization by the 11 mAbs. The role of escape mutations at positions 160-171 in V2 and 437 in C4 was investigated using 15 autologous mutants and 3 chimeric viruses.

**Results:** While the primary t/f was only neutralized by 1 mAb, all 11 mAbs potently neutralized the superinfecting t/f. All mAbs were unable to neutralize the 21- and 39-month viruses. mAbs showed differential dependence on individual V2 mutations, however the neutralizing activity of all mAbs was abrogated by R166S or K169E mutations. Deleting the N160 glycan only slightly affected the neutralizing capabilities of each mAb. Finally, a sensitive V1V2 chimeric virus was made resistant to all the mAbs through a P437A mutation in C4.

**Conclusion:** The potent neutralization of the superinfecting t/f but not the primary t/f by all mAbs suggested that the superinfecting t/f was responsible for eliciting these BCN antibodies. The inability of these mAbs (isolated after 30 months p.i.) to neutralize autologous clones after 21 months p.i. suggests they arose before this time point and that this memory B cell family persisted despite viral escape. The presence of circulating viruses with and without the N160 glycan after superinfection explains the independence of the mAbs on this glycan. The drastic effect of the P437A mutation on mAb activity indicates that in the context of the envelope trimer, C4 may modulate V2 conformation.

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**OA05.04**

**A Diverse Antibody Response to the Gp120 N332 Glycan Epitope in an HIV-Infected Donor**

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**Background:** Insight into the development of broadly neutralizing antibodies (bnAbs) in HIV infected individuals is a major goal in HIV vaccine research. bnAbs PGT125-131 were recently isolated from an HIV infected donor and shown to be amongst the most broad and potent anti-HIV antibodies isolated thus far. Biochemical and crystallographic studies have shown that these antibodies are dependent on glycans at positions N301 and N332 as well as residues in the V3 loop.

**Methods:** Here, we use 454 deep sequencing and a novel phylogenetic method to study the diversity of the antibody response to the N332 epitope in this donor and to probe possible pathways of antibody maturation.

**Results:** Sequence analysis and binding characteristics of PGTs125-131 suggest that they originated from a single ancestor and organize into two main branches. These branches differ by the presence of a critical heavy chain insertion and a non-functional light chain deletion. We show that although these branches evolved in the same patient, distinct patterns of viral neutralization, N332 recognition and glycan sensitivity are observed. We show that glycan binding is independent of HIV reactivity and may have evolved as an initial step in the antibody maturation pathway. Further analysis of memory B cells through deep sequencing revealed additional branches that display limited breadth and potency.

**Conclusion:** In summary, in this HIV-infected donor, there appears to be a diverse response to the N332 epitope arising from the same recombination event and against the same envelope sequence. These findings will help immunogen design strategies aimed at re-eliciting this family of potent bnAbs.
OA05.05

MF59 and ALUM, in Combination with an ALVAC-SIV/gp120 Vaccine, Induce Plasmablasts that Differ in the Expression of Homing Markers

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Background: An ALVAC-HIV/gp120 vaccine provided limited but significant protection against HIV-1 acquisition (31.2%) in the RV144 trial. Antibodies against viral envelope were associated with reduced risk of HIV acquisition. The frequency of vaccine-induced antibody secreting cells precursors (plasmablasts) has been recently reported to correlate with protection from SIV acquisition in rhesus macaques. Increasing evidence indicates that chemokines and their receptors have a central role in regional targeting of antibody-secreting cells to hematopoietic, mucosal or inflammatory sites. Thus, the homing of vaccine-induced antibody secreting cells could affect vaccine efficacy.

Methods: Two groups of twenty-seven macaques each were immunized four times with ALVAC-SIV and received two boosts of gp120 formulated either in ALUM or MF59. One month post immunization all animals and forty-seven controls were challenged with repeated low doses of SIVmac251 by intra-rectal route. We studied the vaccine efficacy and the frequency of plasmablasts expressing alpha4beta7, CXCR4 and CXCR3.

Results: Both strategies induced high titer of binding antibodies to SIV-gp120 and a similar frequency of env-specific plasmablasts; nevertheless only ALUM group showed a significantly reduced rate of SIV acquisition when compared to forty-seven unvaccinated controls (Log rank p=0.021). Interestingly, we found that the frequency of plasmablasts expressing alpha4beta7 (a mucosal homing marker) and CXCR4 (a hematopoietic homing marker) was higher in the ALUM group compared to the MF59 group (p=0.049 and p=0.026). In addition, there was a trend (p=0.06) for a higher frequency of plasmablasts expressing CXCR3 (an inflammatory site homing marker) in the MF59 group.

Conclusion: Our data indicate that adjuvants alter the expression of chemokine and integrin receptors on plasmablasts that, in turn, can affect their homing to mucosal, hematopoietic or inflammatory sites. We are investigating whether these phenomena affect vaccine efficacy.

OA05.06

HIV-1 gp120 Vaccination Elicits a Robust and Durable Anti-V1/V2 IgG Response and Yet No HIV-1 Env-Specific IgA Response in HIV-Exposed Infants

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Background: Effective infant HIV-1 vaccination may be impeded by the presence of maternal HIV-1-specific antibodies and the immaturity of the infant immune system. Since the RV144 HIV vaccine trial in adults indicated an association between anti-V1/V2 IgG antibodies and reduced HIV-1 acquisition, it is important to evaluate the elicitation of this potentially protective immune response in vaccinated infants.

Methods: HIV-1-exposed infants in PACTG 230 received four doses of Chiron rgp120 (SF-2 strain) with MF59 (n=47), VaxGen rgp120 (MN strain) absorbed with alum (n=49) or placebo (n=19) between birth and 20 weeks of age; and those in PACTG 326 received ALVAC-HIV + AIDSVAX B/B (n=9) or placebo (n=11) between birth and 12 weeks of age. HIV-1 envelope-binding IgG/IgA responses were measured by BAMA (binding antibody multiplex assay).

Results: Maternally-acquired antibodies measured by anti-gp41 IgG declined to less than 3% of the birth levels at week 52, indicating loss of most transplacental HIV-1 antibodies. Vaccine antigen-specific (MNgp120) IgG responses were higher in all vaccine groups than placebo at week 52 (p<0.001 to 0.008) and were still detected in 56% of Chiron rgp120-vaccinated infants at week 104. The magnitude and responder frequency of anti-gp70 BcaseA2 V1/V2 IgG at week 24 was higher in PACTG 230 vaccinees than placebo (median MFI (frequency), Chiron: 23,924 (98%); Vaxgen: 530 (72%); placebo: <100 (19%), (p<0.001 for all). The frequency of this response also trended higher in ALVAC+AIDSVAX vaccinees than in placebo (86% versus 33%, p=0.06). This response was induced despite detectable maternally-acquired anti-V1/V2 IgG at birth in 72% of infants. Remarkably, at week 52 and 104, respectively, 93% and 63% of infants from the Chiron group had detectable anti-V1/V2 IgG, as compared to 64% in RV144 vaccinees at peak immunogenicity. No vaccine-elicited HIV-1 Env-specific IgA antibodies were detected.

Conclusion: Vaccination of HIV-1-exposed infants can elicit robust anti-V1/V2 IgG responses.
OA06.01

Identifying the Minimal Mutations in VRC01 Required for Neutralization Potency and Breadth to Inform Vaccine Design

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Background: VRC01-class broadly neutralizing antibodies (bNAbs) have been isolated from a number of HIV-infected individuals. Despite substantial differences in primary amino acid sequence, these antibodies all derive from VH1-2 and utilize a nearly identical mode of binding. One of the perceived hurdles to eliciting a VRC01-class response by vaccination is that these bNAbs are highly mutated (~30% from germline). To inform vaccine development, we sought to define the minimal set of somatic mutations needed for broad and potent neutralization of HIV-1 by VRC01-class antibodies.

Methods: Libraries of variants of VRC01 and PGV04 (another VRC01-class bNAb), allowing either germline or mutated residues at each mutated position, were displayed on yeast and sorted for binding to panels of gp120s from different clades. The least mutated library variants that retained high affinity for diverse gp120s were produced as soluble IgG and evaluated for neutralization against a large panel of HIV-1 pseudoviruses.

Results: Using the strategy outlined above, we developed a minimally mutated variant of VRC01 (termed MinVRC01) that maintains the neutralization potency and breadth of VRC01 but is only 9% mutated from germline. MinVRC01 contains several spatial clusters of mutations, and reversion of any single cluster to germline caused significant reduction in potency and breadth. Two of these clusters lie on the surface of MinVRC01 outside the paratope defined by the crystallographic interaction with core gp120, indicating that the VRC01 epitope is more complex than previously recognized. These two clusters suggest specific structural features of HIV Env that may be required in immunogens to induce VRC01-class bNAbs.

Conclusion: Our findings suggest that it may be possible to induce a broad and potent VRC01-class antibody response with substantially fewer mutations than previously thought. Furthermore, we have defined additional, critical components of the VRC01 epitope that likely will be required in vaccines to induce VRC01-class bNAbs.

OA06.02

Structural Organization and CD4-Induced Reorganization in Soluble HIV-1 Trimers

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Background: The Envelope glycoprotein (Env) mediates HIV entry through a series of receptor-triggered conformational changes leading to fusion of viral and host membranes. This trimer of gp120/gp41-subunit heterodimers is the sole target of neutralizing antibodies. The architecture of the pre-fusion trimer as well as its intrinsic dynamics, hypothesized to modulate antigenicity, remain unresolved. Efforts have been made to engineer stabilized, soluble forms of Env (termed “gp140”) as potential next-generation immunogens, including through the introduction of trimerization motifs or the addition of intersubunit disulfide bonds and mutations that favor the pre-fusion state. Here we apply hydrogen/deuterium-exchange mass spectrometry (HDX-MS) to assess the structural organization of these Env constructs.

Methods: HDX-MS provides a detailed map of order and structural dynamics in proteins by measuring the kinetics of local backbone amide solvent exchange. Here, HDX-MS is used to examine monomeric and trimeric SOSIP gp140 (isolate Clade A KNH1144), CD4-bound SOSIP trimer, as well as uncleaved and trimerization motif-stabilized gp140s.

Results: HDX-MS reveals that significant regions of V1/V2, V3 and bridging sheet elements are ordered in SOSIPs, suggesting these sites participate in stable interactions in the pre-fusion trimer. The gp41 subunit exhibits a profile indicative of a trimerization core with additional ordering in the gp41 loop region, which has been proposed to interact with gp120. CD4 binding releases V1/V2 and V3 association reflected by an increase in solvent exchange; changes in gp41 HR1 and fusion peptide proximal regions are also observed. By contrast, uncleaved gp140 constructs exhibit gp41 profiles consistent with a post-fusion, helical bundle organization with loosely tethered gp120 subunits.

Conclusion: Using HDX-MS, we demonstrate that SOSIP trimers represent a reasonably authentic, pre-fusion form of Env. This approach enables detailed analysis of the trimers including characterization of gp41’s organization, identification of regions involved in trimerization, and a detailed mapping of the large-scale reorganization induced by CD4 binding.
**OA06.03**

**Cell-Surface Display and Panning of HIV-1 Derived Envelope Proteins**

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**Background:** Available display systems allow screening of millions of candidate proteins and are the method of choice to identify optimized antigen-antibody binding. To overcome the limitations of prokaryotic expression and presentation in phage display libraries, eukaryotic display platforms were developed. We established a mammalian cell-surface display to present HIV-1 envelope derivatives in a natural, trimeric, membrane bound environment. This allows us to generate affinity enhanced envelope derivatives against broadly neutralizing antibodies (bNAb) in order to select potent Envelope (Env) based vaccine candidates.

**Methods:** An HIV-1 derived lentiviral vector was developed to infect HEK293T cells at a low multiplicity of infection (MOI), in order to correlate phenotype and genotype. The vector was designed and proven to express both GFP and Env in a constant relationship, enabling indirect normalization of produced Env by detecting GFP. After staining with an appropriate bNAb, Env-displaying cells were selected for high affinity binding via FACS sorting.

**Results:** A small model library of Env variants with distinct binding capacities towards the bNAb 447-52D was used for evaluation. In a proof-of-concept experiment, the Env variant with the highest affinity to the applied bNAb could be enriched up to tenfold after two rounds of selection.

**Conclusion:** Hence, the technique provides the possibility to screen for membrane-bound Env variants with high binding capacities towards the bNAb applied. By using bNAb for selection of envelopes out of different libraries new candidates for the potential use in HIV vaccine trials can be identified.

**OA06.04**

**DNA-MVA Prime-Boost Vaccine Eliciting T Cell Specificities Associated with HIV-1 Control Is Highly Immunogenic in Mice and Breaks CTL Immunodominance**

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**Background:** A T cell immunogen guided by human immunogenicity data could help avoid inducing ineffective responses to potential decoy targets. DNA prime followed by recombinant MVA boost regimens have been shown to be an effective schedule for induction of robust T cell activities.

**Methods:** The HIVACAT T cell immunogen (HTI) contains 16 HIV-1 protein segments of 10-70 amino acids in length, which i) are relatively conserved, ii) are targeted by individuals with low viral loads, and iii) elicit responses of higher avidity and broader cross-reactivity. The HTI gene was cloned into a CMV expression vector (pDNA HTI) and then inserted into a modified vaccine virus Ankara (MVA HTI). HTI immunogenicity after 2xpDNA HTI was compared in C57BL/6 mice immunized with a combination of plasmids expressing entire HIV-1 proteins (pDNA GagPolVifNef). Prime-boost regimens combining pDNA HTI and MVA HTI were assessed. Cellular responses were characterized using intracellular cytokine and IFN-γ ELISPOT assays.

**Results:** The HTI covered >50 optimal defined CD4+ and CD8+ T cell epitopes with >40 different HLA restrictions, without overrepresentation of B27/B57/B58 alleles. In C57BL/6 mice, the HTI induced broad CD4+ and CD8+ T cell responses to all segments within Gag, Pol, Vif and Nef compared to a more narrow, Gag-dominated response seen in animals immunized with pDNA GagPolVifNet. These responses were strongly increased both in breadth and magnitude by using heterologous regimens consisting of 3xpDNA HTI and MVA HTI. Booster regimens combining pDNA HTI and MVA HTI were assessed. Cellular responses were characterized using intracellular cytokine and IFN-γ ELISPOT assays.

**Conclusion:** Hence, the technique provides the possibility to screen for membrane-bound Env variants with high binding capacities towards the bNAb applied. By using bNAb for selection of envelopes out of different libraries new candidates for the potential use in HIV vaccine trials can be identified.
**OA06.05**

Detection of Early CTL Escape in Gag and Nef Using Population-Level Approaches

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**Background:** Population-level analyses of chronic infection cohorts have facilitated the identification of HLA-associated polymorphisms in HIV-1, but it is unclear to what extent immune escape can be detected using similar methods during early infection. To address this issue, we compared immune escape signals in early and chronic cohorts of equivalent statistical power.

**Methods:** A cross-sectional early cohort of subtype-B infected individuals sampled a median 88 [IQR 63-120] days following infection (N=221, 203, 233 for Gag, Protease/RT and Nef) was compared against a chronic dataset of equal size and comparable HLA class I distribution for the presence of 400 published HLA-associated polymorphisms derived from an independent cohort of N=1800 chronically-infected individuals. A threshold of p<0.01 (q<0.25) was defined as positive population-level evidence for immune escape.

**Results:** At p<0.01, 148 (37%) of the 400 HLA-associated polymorphisms were detected in early and/or chronic cohorts, yielding an appropriate denominator for this dataset size. Of these, 20 (representing 15%, 3% and 18% of known HLA-associated polymorphisms in Gag, Pol, Nef) were detectable in early infection, albeit at median 35-fold lower odds ratios than chronic infection. Early escape signals mapped to 7 published and 3 predicted CTL epitopes (3 in Gag, 1 in Pol and 6 in Nef). While certain early escape signals were expected (e.g.: B*57:01/T242N exhibited the strongest early escape signal, p=4x10-9), others occurred within understudied or novel epitopes. A minority of early escape signals were not observed in the chronic dataset, suggesting transient CTL escape pathways.

**Conclusion:** Up to 18% of known HLA-associated polymorphisms, notably in Gag and Nef, are detectable at the population level within three months of infection. Results underscore the reproducible nature of HIV-1 immune escape in terms of mutational pathways and timecourse of selection. Population-level approaches can complement longitudinal studies to characterize HIV-host adaptation during this critical infection stage.

**OA06.06 LB**

Neutralizing and Non-neutralizing Antibody Binding to Cleaved, SOSIP-Stabilized and Uncleaved Soluble HIV-1 Env Trimers, Protomers, and gp120

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**Background:** The structure and immunogenicity of soluble HIV-1 trimers (BG505 SOSIP.664 gp140, subtype A), cleaved between gp120 and the gp41 ectodomain, will be described in a Satellite Symposium. In other designs of soluble gp140 trimers, cleavage is usually eliminated. As immunogens, soluble trimers should mimic functional, virion-associated trimers by binding neutralizing but not non-neutralizing antibodies. How do cleaved and uncleaved trimers compare in this regard? And how do these two trimer forms compare antigenically with gp120 monomers and gp140 protomers (gp120 disulfide-linked to the gp41 ectodomain)?

**Methods:** We expressed variants of BG505 Env in 293T cells, and purified them by 2G12-affinity and size-exclusion chromatography. Env was C-terminally D7324-epitope- or His-tagged, which allowed minimally disruptive immobilization to sensor chips for kinetic measurement of MAb binding by surface plasmon resonance (SPR). MAb binding was compared with neutralization of the sequence-matched BG505 virus (Tier 2).

**Results:** MAbs PGV04, VRC01, PG9, PG16, PGT121, PGT123, PGT135, PGT145, and 2G12 neutralized BG505 and bound well to cleaved trimers; b6, b12, 14e, and F240 did not neutralize and bound negligibly. Conversely, b6 and b12 bound well, PG9, PG16, and PGT145 poorly, to uncleaved trimers, gp140 protomers, and gp120. MAbs VRC01 and 2G12 bound similarly to trimers and gp140 protomers. Kinetics-based affinity and stoichiometry measurements for sCD4, PG9, PG16, PGT121, PGT123, PGT128, and 2G12 agreed, where applicable, with calorimetric data and electron micrographs; the latter method also showed cleaved and uncleaved trimers to be morphologically distinct.

**Conclusion:** We found that trimerization has a stronger antigenic influence on gp120 epitopes than hetero-dimerization with the gp41 ectodomain alone. Uncleaved trimers and the monomeric Env forms expose mostly non-neutralization epitopes; the cleaved trimers are better antigenic mimics of functional spikes. The antigenicity and structural integrity of cleaved BG505 SOSIP.664 trimers support their evaluation as immunogens for the induction of bNAbS.
OA07.01

A Role for Glycosylation in HIV Transmission

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Background: Heterosexual transmission of HIV-1 across mucosal tissues involves a complex series of events that are not fully understood. The envelope protein of some transmitted (founder) viruses bear a distinct signature described by a reduced number of potential N-linked glycosylation sites (PNGs). Interestingly, the leader peptides of env from founder viruses have been shown to encode basic residues at specific sites, providing a second “transmission signature”. The specific aim of this study is to determine the manner in which these two transmission signatures influence structural features of HIV-1 envelope (env) and their potential role in transmission fitness.

Methods: Recombinant envelope proteins bearing the two signatures of founder viruses were produced and their reactivity with CD4 and a panel of antigenic and glycan specific probes was evaluated by flow-cytometry and surface-plasmon-resonance.

Results: The removal of PNGs that are frequently missing in founder viruses exerts a pronounced influence on the interaction of the envelope with the CD4 and CCR5 receptors, which functions as the principal docking and fusion receptors utilized by HIV to infect CD4+ T cells. The leader peptide signature was found to influence qualitative aspects of env glycosylation in a manner that also impacts env structure and its interaction with both CD4 and CCR5. In addition leader peptides derived from founder viruses were shown to influence the affinity of the HIV env for DC-SIGN, a receptor expressed on dendritic cells that is believed to facilitate mucosal transmission.

Conclusion: The two distinguishing features of founder envs both impact env glycosylation, which in turn impacts env structure and its interaction with cell-surface receptors critical to transmission. These observations underscore the critical role of glycosylation on HIV env structure. In this way selective alterations in glycosylation may increase the fitness of transmitting viruses.

OA07.02

Role of Integrin α4β7 in HIV Transmission and Pathogenesis

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Background: The integrin α4β7, which mediates the trafficking of T lymphocytes to the gut associated lymphoid tissue (GALT), a site of rapid HIV replication, has been described as an attachment factor for the envelope protein gp120. While differences in binding affinity of early transmitting and chronic gp120s for α4β7 have been noted, this has not translated to any differences in replication. We aimed to investigate what role this binding interaction has in HIV pathogenesis over time.

Methods: All-trans retinoic acid-activated CD4+ T cells were incubated with or without HP2/1 (anti-α4 monoclonal antibody) or Act-1 (anti-α4β7 monoclonal antibody) prior to adding virus. Infectious virus was prepared using matched envelope genes from 8 individuals in the CAPRISA Acute Infection cohort representing the transmitted/founder virus and variants from 1-39 months post infection (p.i.). Replication was monitored by p24 ELISA.

Results: While the transmitted/founder and later viruses from 39 months p.i. were equivalently dependent on α4β7 for viral replication, those from 6 and 12 months p.i. were significantly less dependent on α4β7. The strength of this interaction was negatively correlated with CD4 count at the time that the virus was isolated. Interestingly individuals that developed broadly cross neutralising responses had a lower dependence on α4β7 compared to those that do not.

Conclusion: These data suggests that interactions with α4β7 are preferable for transmission and late chronic infection but are diminished following acute viral expansion. This correlates with HIV pathology; the virus would have a greater dependence on α4β7 important for widespread dissemination during transmission and acute infection, with the need for spread greatly decreased thereafter. The difference between α4β7 dependence in acute and chronic infection in the first year illustrate the selective advantage that α4β7 may offer to transmission. These findings may lead to vaccine and therapeutic opportunities in which dependence on α4β7 for replication may be exploited.
OA07.03

Profound Alterations in Cholesterol Metabolism Restrict HIV-1 Trans Infection of CD4 T Cells in Viremic Controllers

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Background: HIV-1 controllers inhibit infection for years without antiretroviral therapy. We hypothesized that professional antigen-presenting cells (APC), i.e., dendritic cells (DC) and B cells, from HIV-1 infected, viremic controllers (VC), are inefficient in trans infection of T cells due to altered cholesterol metabolism, thus reducing spread of virus and controlling disease progression.

Methods: We assessed HIV-1 trans infection of autologous CD4 T cells by APC from 8 VC, 8 progressors (PR) and 7 seronegatives (SN) in the longitudinal Multicenter AIDS Cohort Study. Total cell cholesterol content and efflux was measured. APC from VC were treated with siRNA to inhibit ABCA1-mediated cholesterol transport, and from SN with nuclear receptor ligands to upregulate cholesterol efflux and Levastatin to inhibit cholesterol synthesis.

Results: Strikingly, APC from none of the VC (0/8) trans infected CD4 T cells with HIV-1, whereas APC from 8/8 PR and 7/7 SN demonstrated efficient trans infection. There was no difference in direct (cis) HIV-1 infection of T cells from the 3 groups. Importantly, APC from VC had impaired trans infection both prior to and after primary HIV-1 infection (seroconversion), whereas APC from PR had this capacity both before and after seroconversion. APC but not T cells from VC showed significantly lower cholesterol content and higher efflux compared to SN and PR. Alteration of cholesterol metabolism reduced trans infection by APC from SN, while interference with the ABCA1 pathways in VC restored their ability to transmit virus.

Conclusion: We show that APC from VC completely lack the ability to trans infect T cells. This was associated with profoundly enhanced cholesterol metabolism that appears to be an inherited trait. Our results provide important new clues to control of HIV-1 infection.

OA07.04

Highly Potent Broadly Neutralizing Antibodies Lack Potential to Inhibit HIV-1 Cell-to-Cell Transmission

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Background: HIV-1 utilizes two different ways to transmit between cells: free-virus and cell-to-cell transmission. While the contribution of the two transmission modes in natural infection has not yet been defined, there is compelling evidence that cell-to-cell transmission allows the virus to evade antiretrovirals and broadly neutralizing antibodies (bnAbs). However, only few reagents have been assessed for their capacity to inhibit cell-to-cell transmission and we urgently need information how bnAbs, promising candidates for vaccine design, function in that process.

Methods: Our group has recently developed assay systems that allow for unambiguous discrimination between free-virus and cell-to-cell transmission using CD4- and CCR5-expressing cell lines or PBMC, either infected with replication competent virus or transfected with pseudotyped virus. Target cells overexpress rhesusTRIM5α which efficiently restricts free-virus but not cell-to-cell transmission. (Abela et al., 2012)

Results: Using these assays, we probed the efficacy of the highly potent PG and PGT bnAbs in comparison to gp120-directed (b12, VRC01) and gp41-directed (T20, 10E8) inhibitors to block cell-to-cell transmission of a range of Tier-1 and Tier-2 strains. Strikingly, we found for all strains tested that PG9, PG16, PGT121, PGT128, PGT130, PGT135 and PGT145 showed a dramatically decreased inhibition capacity during cell-to-cell transmission in contrast to MPER-specific bnAbs maintaining their activity during cell-to-cell transmission. Time-of-addition studies showed that unlike MPER bnAbs, the PGT bnAbs are capable to block HIV only before CD4-engagement but not afterwards. This highlights that CD4-engagement is the rate limiting step during cell-to-cell transmission and difficult for gp120-specific bnAbs to interfere with.

Conclusion: Our studies confirm that free-virus and cell-to-cell transmission of HIV are not equally susceptible to different entry inhibitors. In particular gp120-specific antibodies lack activity during cell-to-cell transmission. As HIV initially spreads locally in the newly infected host, presumably involving cell-to-cell transmission, vaccines and inhibitors targeting both free-virus and cell-to-cell transmission should be actively sought for.
Highly Pathogenic Adapted HIV-1 Strains Limit Immune Responses and Dictates Rapid Disease Progression in Early Infection


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Background: The study of HIV-1 Rapid Progressors (RP) has been limited so far to particular case reports. Nevertheless, a broad functional analysis of viral and host factors leading to RP will provide crucial information for an accurate description of the correlates of disease outcome.

Methods: With this aim, we study a previously unreported large group of HIV-1 RP (n=46) and compared to infected individuals with average disease progression, or Standard Progressors (SP, n=46) early after HIV-1 seroconversion (≤ 1 year). Viral factors were tested in terms of virus coreceptor usage, replicative capacity and sequence variation. Additionally, HLA immunogenetics markers, host CD8+ T cell responses and plasma neutralizing activity were determined.

Results: Our data demonstrate the existence of particularly pathogenic HIV-1 strains in RP during recent infection. Viral strains were characterized by preferential X4/Dual-Mixed coreceptor usage (26.3% RP vs. 2.8% SP, p=0.006), and high in vitro replicative capacity (median 81.5% vs. 67.9%; p=0.025). Host immunogenetic markers revealed an increased frequency of common Caucasian haplotypes (RP vs. 2.8% SP; p=0.006), and high in vitro replicative capacity (p<5e-7), or were predicted to be de novo HLA escape mutations in the TSP (p<5e-7). As predicted by the model, three well-established risk factors—TSP viral load, seroconverter gender (female) and genitourinary infections—independently reduced the transmission selection bias, polymorphisms were less likely to be transmitted if they had a large impact on in silico protein stability (p<1e-3), had putative compensatory mutations (p<5e-7), were in viruses with low in vitro replicative capacity (p<5e-7), or were predicted to be de novo HLA escape mutations in the TSP (p<5e-4). As predicted by the model, three well-established risk factors—TSP viral load, seroconverter gender (female) and genitourinary infections—independently reduced the transmission selection bias (p<1e-4). A sequence-derived transmissibility index significantly segregated the TSPs in the cohort from risk-matched chronically infected individuals (173) who had not transmitted to their partners (p=0.01).

Conclusion: These data indicate an uncommon convergence of viral factors in rapid progression in recent infections. Consequently, widespread of particularly aggressive HIV-1 strains leading to rapid disease progression should be taken into account to implement future immunotherapeutic and vaccine strategies.
OA08.01

Gut-Homing of Plasmacytoid Dendritic Cells Persists in the Absence of HIV/SIV Replication and Contributes to Residual Chronic Immune Activation

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**Background:** Lentivirus infections are characterized by a dramatic loss of mucosal CD4+ T cells, breakdown of the gut mucosa, and subsequent chronic immune activation. Residual immune activation persists even during ART therapy and is the single greatest cause of ongoing disease morbidities.

**Methods:** Plasmacytoid dendritic cells, primary producers of IFN-α, from naive patients and rhesus macaques, chronically HIV-infected persons and SIV-infected macaques, ART-treated humans and macaques, and elite controllers of HIV were analyzed in blood and tissues using polychromatic flow cytometry. Cells were evaluated functionally by intracellular cytokine staining and spatially by immunohistochemistry.

**Results:** During both HIV and SIV infections blood pDCs were depleted compared to naive subjects. However, correlating with plasma viremia, the remaining pDCs upregulated the gut-homing marker, α4β7. Even during ART and Elite Controllers' pDCs remained reduced in blood and α4β7 expression was still significantly upregulated compared to naive animals. Absolute numbers and frequencies of pDCs in jejunum and colon were 3-fold greater in infected compared to naive macaques. pDCs accumulating in the mucosae during infection secreted high levels of IFN-α, MIP1-β, and TNF-α, resulting in net increases in cytokines in mucosal tissues. In both humans and macaques, pDC trafficking to the gut mucosa was associated with an increase in PAMPs — microbial translocated LPS and CpG-DNA — in plasma, regardless of ART treatment or elite control status. Furthermore, pDC trafficking to the gut was associated with increased markers of activation on circulating T cells.

**Conclusion:** Here we show a novel mechanism by which pDCs are not depleted during lentivirus infections, but rather traffic to and accumulate in the gastrointestinal mucosa. This accumulation occurred even in the absence of HIV and SIV replication and was associated with chronic immune activation. These data suggest that alternative stimuli contribute to pDC activation and gut-trafficking as a prime source of residual chronic immune activation.

OA08.02

HIV-1 Infection Induces Potent Type I IFN Signatures in Conventional Dendritic Cells from HIV-1 Elite Controllers

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**Background:** Recent data suggest that in most HIV-1-infected individuals, cell-intrinsic immune responses to HIV-1 are blocked by host proteins such as Samhd1 and trex1 in conventional dendritic cells (cDC). Elite controllers (EC) control HIV-1 replication in the absence of treatment, but immune defense mechanisms in these patients are not well understood. Here, we investigated cell-intrinsic innate immune responses to HIV-1 in cDCs from these specific patients.

**Methods:** PBMC from EC, untreated chronic progressors (CP), HAART-treated and HIV negative subjects were ex vivo infected with HIV-1. cDCs were isolated and expression of viral replication products, Samhd1, Trex1, type I IFNs and 30 IFN stimulated genes (ISGs) were subsequently analyzed by qPCR. Type I IFN transcriptional signatures were obtained by unsupervised hierarchical clustering and co-expression analyses.

**Results:** cDC from HIV-1 negative persons were moderately susceptible to HIV-1, while cDC from CP only weakly supported HIV-1 replication, likely due to high-level Samdh1 expression and impaired reverse transcription. cDC from EC also showed low susceptibilities to productive HIV-1 infection, but reverse transcription in these cells was largely unaltered, while restriction of viral replication seemed to preferentially occur at the level of viral integration. Importantly, in contrast to CP, HIV-1 infection of cDCs from EC induced a unique transcriptional signature of type I IFN-stimulated genes, and a selective upregulation of four putative cell-intrinsic sensors of microbial DNA. Functionally, these altered patterns of viral restriction and potent IFN signatures in cDC from EC were associated with increased cellular immune activation, and improved abilities to prime T cell responses in vitro.

**Conclusion:** cDC from EC can mount cell-intrinsic immune responses against HIV-1 thought effective sensing of HIV-1 DNA, which may support the generation of highly effective HIV-1-specific T cell responses in these patients.
OA08.03

Acute-Phase Response Proteins Are Overexpressed in Vaginal Mucosa and Plasma of HIV-Resistant Women upon Viral Challenge

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Background: The primary receptive portals of entry for HIV are through mucosal surfaces. Our previous studies show that HIV-exposed uninfected individuals (HESN) have unique innate responses associated with resistance to HIV infection, including increased anti-viral antiprotease expression coupled with a reduced immune activation phenotype. However, we do not know whether this immune state is static or induced upon viral exposure. To answer this question we challenged HESN women with a live attenuated Flumist vaccine and analyzed their mucosal and systemic immune responses using a systems biology approach.

Methods: HESN women (n=10) and HIV-susceptible controls (n=10) were challenged with an intranasal Flumist vaccine, and clinical samples (cervicovaginal lavage (CVL), plasma) were collected at Day 0, 1, 7 post-challenge. Mucosal/plasma were analyzed by a combination of label-free tandem mass spectrometry, hierarchical clustering, and pathway analysis.

Results: HESN women exhibited significant changes both mucosally and systemically upon vaccine challenge 1 and 7 days post-exposure. Of the >450 proteins identified in CVL, 62 were overexpressed (p<0.05), and 48 overexpressed in plasma (of 220 proteins) (p<0.05), over that of controls. Expression profiles showed significant correlations between compartments. Hierarchical clustering identified two major functional pathways distinguishing HESN individuals, including the acute phase and LXR-RXR response pathways (p<1x10^-17). Many of these factors include antiproteases (serpins), apolipoproteins, complement components, and SAA proteins which have known inhibitory properties against HIV.

Conclusion: As the acute phase response pathway has been implicated as important for controlling inflammation and early stage viremia in HIV-infected individuals, overexpression of this pathway supports the hypothesis that these factors are contributing to reduced susceptibility to infection. Understanding the role of these pathways in mucosal susceptibility to HIV may help guide existing microbicide/vaccine strategies against HIV.

OA08.04

Immune Complex – Fc Receptor Interactions Reduce Inflammatory Responses at the Genital Mucosa as a Correlate of SIV Vaccine-Induced Protection

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Background: Previous studies of mucosal transmission have implicated that an efficacious prophylactic HIV/SIV vaccine should be able to prevent viral replication/expansion at the genital mucosa. So far, this scenario has only been achieved experimentally using live-attenuated SIV vaccines. Thus, great interest lies in identifying correlates and mechanisms of such a protection.

Methods: A global genomics approach, tissues analysis, and explant cultures were used to reveal important components of protective responses in the female genital mucosa of SIVmac239Δnef-vaccinated animals.

Results: Genome-wide transcriptional analysis revealed a downregulation of inflammation-related genes, but a selective enrichment of an anti-inflammatory program in the genital mucosa upon virus exposure in Δnef-vaccinated animals. The inhibitors of NF-kB and MAPK pathways, COMMD1 and SPRED1, were upregulated in the mucosal epithelium. Additionally, the Δnef vaccine induced a progressive accumulation of SIV-specific non-neutralizing antibodies (mostly IgGs) at the female genital mucosa. Surprisingly, these antibodies were concentrated at the mucosal epithelium, colocalized with FcRn. Moreover, the inhibitory receptor FcγR2b is the only FcγR expressed on rhesus genital epithelium. Therefore, we hypothesized that SIV-specific immune complex-FcγR2b interaction induced inhibitory signaling in mucosal epithelium, and examined its role in suppressing proinflammatory responses to virus exposure. The exposure of cervical epithelium to SIV-specific immune complexes, formed by pre-incubating SIV with either vaccinated sera or rhesus anti-SIVgp41 mAb 4.9C, increased the expression of COMMD1 and SPRED1 in genital epithelial cells, while did not alter proinflammatory cytokine expression. This effect was abrogated by an FcγR2b-blocking Ab, antibody deglycosylation, and conditions disrupting immune complexes (a mild acid or base treatment).

Conclusion: The SIV-specific immune complexes-FcγR2b interaction suppressed proinflammatory responses in the genital epithelium; therefore, contributed to the vaccine-induced protection. These highlighted an unappreaciated, non-neutralizing role of SIV-specific Abs at the mucosal surface and implicated mucosal epithelium as a critical, initial immunological sensor that determines the outcome of viral transmission.
OA08.05

Optimization of Systemic and Mucosal Immune Responses to SIV and HIV-1 Antigens Using Different Protocols of DNA and Protein Immunization in Macaques

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Background: Understanding the quality of immune responses developing after different vaccine regimens is essential for improving the prospects of AIDS vaccines. We analyzed different regimens of DNA/protein immunization with SIV or HIV-1 antigens to identify magnitude, breadth, mucosal dissemination as well as their protective ability. We compared DNA vaccination by electroporation in the presence of IL-12 DNA, a protocol that showed strong responses in DNA vaccinated humans, to DNA and protein vaccine regimens.

Methods: Rhesus macaques were vaccinated using electroporation with either DNA, DNA/protein co-immunization, or DNA prime followed by protein boost. Humoral (including neutralizing, linear and conformational antibodies) and cellular responses were followed over time. Vaccinated macaques were challenged to determine immune correlates of protection.

Results: Although DNA vaccination achieved strong humoral responses, protein boost after DNA vaccination greatly increased Ab levels. Importantly, a co-immunization strategy of DNA/protein injected in the same muscle the same time induced highest and broad humoral responses. Similar data were obtained using either purified Env proteins or inactivated viral particles. Inclusion of DNA in the vaccine promoted persistence of plasma antibody levels for greater than 2 years. SIV DNA/protein vaccination induced: higher SIV Env-specific IgG in saliva; more responders with higher SIV Env-specific IgG in rectal fluids; higher and longer-lasting plasma bAb and Nab to homologous and heterologous Env; and Ab to V1/V2. Systemic and mucosal vaccine-induced Ab responses against SIVsmE660 correlated with slower virus acquisition upon challenge. In addition, vaccinated macaques showed strong protection against chronic viremia compared to controls. Similar to SIV, HIV DNA/protein (purified Env) vaccination also induced higher Ab levels compared to DNA only, including significant higher levels of V1/V2 Abs.

Conclusion: DNA/protein co-delivery increases the magnitude and longevity of systemic and mucosal humoral immune responses in immunized rhesus macaques.

OA08.06 LB

Immune Correlates of a Functional Cure Following Therapeutic Vaccination of SIV-Infected Rhesus Macaques

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Background: Therapeutic vaccines that increase T cell responses may improve treatment of HIV. We previously showed that therapeutic immunization of SIV-infected macaques with a DNA vaccine during antiretroviral drug therapy prevented viral rebound in 9/14 animals for at least 6 months after withdrawing drugs.

Methods: To determine if the protection from viral rebound was durable or required re-vaccination, 5 of the 9 protected animals were re-vaccinated and four were left untreated. To define what immune responses contributed to protection from viral rebound, we compared mucosal and systemic CD4+ and CD8+ T cell responses in animals that maintained viral control for > 18 months after stopping ART to responses in a set of animals that exhibited viral rebound within 6 months post-ART.

Results: Re-vaccination resulted in distinct but transient increases in the magnitude, function and breadth in the specificity of the T cell response. However, most of the animals, regardless of re-vaccination, continued to exhibit durable protection from viral rebound (<100 viral RNA copies/ml) and no evidence of disease for the duration of the study (2 years after stopping ART). A higher frequency of multifunctional CD8+ T cell responses, higher CD4+ T cell proliferation, and broader specificity in the mucosal SIV-specific T cell response in the gut correlated with long term protection from viral rebound and significant reduction of residual virus in the gut.

Conclusion: These results indicate that a single series of DNA vaccinations was sufficient to provide long term viral control and furthermore, suggest that vaccines that induce both systemic and mucosal T cell responses during HAART may be an effective approach to achieve a functional cure.
**Poster Sessions**

**Poster Session 01**
Tuesday, 8 October  | 17:30 – 19:00 | Odd-numbered posters

**Poster Session 02**
Wednesday, 9 October  | 17:30 – 19:00 | Even-numbered posters

All posters are available for viewing throughout the conference and will be displayed in two locations at the CCIB.

Poster Presentation schedule and floorplan map is available on the inside back cover.

**L1, 111 – 112**

P01: Adjuvants, Immunogens and Inserts .......................... 81–90
P02: Animal Models and Preclinical Trials ................................ 91–109
P03: B Cell Immunology and Antibody Functions .................. 110–148
P04: Clinical Vaccine Trials and Trial Site Challenges .............. 149–171
P05: HIV Transmission and Viral Diversity ............................ 172–190

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P06: Immunogenetic Factors ........................................... 191–201
P07: Innate Immunity .................................................. 202–212
P08: Mucosal Immunity .................................................. 213–226
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P13: Vaccine Concepts and Design .................................. 271–311
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L1, Room 111 – 112. Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L1, Room 111 – 112.

**P01.01**

A Novel PD1 Isoform Exhibits Potent Intramolecular Adjuvant Activity in Potentiating HIV-1 Specific Protective Immunity

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**Background:** Inducing effective and specific immunity is important in the protection and elimination of HIV-1 infection. Programmed death-1 (PD1) up-regulation during HIV-1 chronic infections results in “exhausted” function of CD8+ T cells, but is restored by blockade of the PD1/PD-L pathway with antibodies or a soluble form of (s)PD1. Apart from sPD1, the other three spliced variants previously identified currently have no known function.

**Methods:** In this study, we identified a new isoform of human PD1, named Δ42PD1, that contains a 42-nucleotide in-frame deletion located at exon 2 near its IgV domain found expressed in PBMCs by RT-PCR. We then characterized the biological function of Δ42PD1 both in vitro and in vivo.

**Results:** We found that Δ42PD1 is distinct from PD1 because it does not engage PD-L1 or PD-L2 expressed on dendritic cells (DCs). It, however, is capable of inducing the production of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) especially in DCs. We, then, used Δ42PD1 as an intramolecular adjuvant to construct a DNA fusion vaccine with HIV-1 Gag p24 antigen. Following immunization in Balb/c mice, a significantly enhanced level of anti-p24 antibody titer and p24-specific CD8+ T cell responses were elicited that persisted for at least 7.5 months. Importantly, vaccinated mice were protected against pathogenic vaccinia-Gag virus and lethal tumor challenges, likely due to the improved proliferative and cytotoxic functions of the elicited CD8+ T cells.

**Conclusion:** Our findings not only identified a novel human PD1 isoform that has immunoregulatory function but also demonstrated a novel vaccine strategy in enhancing host protective immunity against HIV-1 infection.

**P01.02**

Design and Evaluation of Chimeric HIV-1 Envelope Glycoproteins with Embedded Cytokine Domains to Target B Cells

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**Background:** One reason that we do not have an HIV-1 vaccine is because several factors limit the quantity and quality of the antibodies raised against the viral envelope glycoprotein complex (Env). The immunogenicity of Env can be increased by fusion to co-stimulatory molecules and we have shown that embedding a cytokine protein domain such as GM-CSF within the Env sequence enhanced Env-specific antibody and T cell responses in mice.

**Methods:** A stabilized Env trimer was engineered harboring the interleukin-21 (IL-21) within the V1/V2 domain of gp120. The expression of Env-IL21 was analyzed by SDS-PAGE. We performed probing studies with a panel of neutralizing antibodies and receptor mimics in a trimer ELISA to investigate the conformation of Env. The functionality of the IL-21 was tested on human B cells by immunoglobulin ELISA and flow cytometry.

**Results:** We hypothesized that targeting Env vaccines directly to B cells, by fusing them to molecules that bind and activate these B cells, would improve Env-specific antibody responses. We describe soluble Env trimers with embedded IL-21 domains, designed to activate B cells that recognize Env. Env-IL21 is expressed efficiently and antibody probing with neutralizing antibodies revealed that the Env components are folded correctly. Furthermore, IL21-conjugated Env trimers potently activated human B cells in vitro and induced the secretion of IgA, IgG and IgM and enhanced plasmablast formation. In addition to IL21, we have been exploring the use of APRoproliferation-Inducing Ligand (APRIL). Env-APRIL induced higher anti-Env antibody responses in rabbits, and neutralizing antibodies against Tier 1 viruses.

**Conclusion:** We have shown that chimeric HIV-1 Env trimers with costimulatory domains show improved immunogenicity compared to Env alone and we work on further improving the design. These studies should guide the further design of chimeric proteins and assist the development of an HIV-1 vaccine inducing protective humoral immunity.
P01.03

Cyclophilin A as Genetic Adjuvant, Induced Broader and Long-Lasting Gag-Specific Cellular Immune Response Based on CyPA-Gag Specific Interaction

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Background: It is reported that, when dendritic cells succumb to HIV-1 infection co-transfected with SIV Vpx protein, HIV-1 induces DC maturation, stimulate an antiviral type I interferon response and activation of T cells. These processes are dependent on the interaction of newly synthesized HIV-1 capsid with cellular cyclophilin A (CyPA). In this study, we investigated the suitability of CyPA as a genetic adjuvant for an HIV-1 Gag DNA vaccine.

Methods: We designed single expression cassette that including Gag and CyPA gene respectively; and also constructed dual expression cassette that expressed both Gag and CyPA gene in one plasmid. 6-week-old female BALB/C mice were administrated thrice intramuscularly with variance DNA vaccine at two weeks interval. Gag-specific IFN-γ ELISPOT and ELISA detected cellular and humoral response after two weeks following the last immunization.

Results: In CyPA specific adjuvanticity analysis, we found CyPA could not enhance Env or irrelevant antigen immunity but augment HIV Gag-specific cellular immune responses. In the dual expression cassette regimen, the cellular immune responses showed that, Gag/CyPA could stimulate high level Gag-specific cellular immune responses, compared with Gag alone. Moreover, based on CyPA mutations Co-IP analysis, we assume adjuvant effect of CyPA is based on Gag-CyPA specific interaction. In assessing the potential effect of CyPA on the breadth of T cell responses in mice, CyPA broadened the T cells’ response spectrum of Gag peptides, with 8 pools showing significant enhancement through CyPA use. In the longitudinal analysis of immunogenicity, Gag-specific cellular immune responses induced by CyPA were maintained at high level from 210 days after the final inoculation, compared with Gag alone.

Conclusion: There is firstly report that Cyclophilin A could augment HIV-1 Gag specific cellular immune response as genetic adjuvant in multiplex DNA immunization strategies. And this adjuvanticity is specific, broad, long-lasting and based on Gag-CyPA interaction.

P01.04

Engineered Immunogens that Raise Antibodies to a Single Epitope

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Background: A broadly effective HIV vaccine will have to elicit broadly neutralizing antibodies to conserved epitopes from which the virus cannot easily escape. The CD4 binding site (CD4bs) is one such epitope against which several antibodies (e.g. b12, VRC01) have been isolated. Passive immunization experiments in macaques demonstrate that prophylactic administration of CD4bs-directed antibodies is protective, whereas therapeutic administration is not.

Methods: Yeast surface display is a powerful method for rapidly engineering complex glycoproteins. We begin with a stripped core gp120 scaffold and use directed evolution by yeast surface display to generate a panel of immunogens that present a functional CD4 binding site but are otherwise highly diverse. We also develop an accompanying suite of tools with which to map conformational epitopes of neutralizing antibodies and monitor the specificity of serum following immunization.

Results: Mice immunized with these diversified immunogens in series elicit CD4bs-directed antibodies to the exclusion of all others. The elicited antibodies were isolated from hybridomas, sequenced, and tested for neutralization. The antibodies did not neutralize HIV in a TZM-bl assay, and they did not bind to intact trimeric envelope.

Conclusion: Though the elicited antibodies were non-neutralizing, we have demonstrated a generalized method of generating diversified immunogens that share a common, complex epitope. When administered in series, these immunogens elicit antibodies to the shared epitope. This method should be adaptable to scaffolds based on trimeric gp160 that more accurately mimic the native viral envelope.
**P01.05**

**Increase in HIV-1 Envelope Incorporation into Virions Mediated by Genetic Modification of the Cytoplasmic Tail of Envelope**

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**Background:** Vaccine-elicited antibody responses against HIV Env are characteristically weak and short-lived. One method to induce potent antibody responses uses proteins presented as an array on viruses or virus-like particles (VLPs). However, this approach has proven challenging for HIV, which expresses Env at low levels on virions. Although Env incorporation into virions can be increased by removal of the cytoplasmic tail, this effect is modest, typically with only a 2-3-fold increase in Env incorporation relative to wild-type HIV. Based on rational manipulation of trafficking signals in the Env cytoplasmic tails of HIV and SIV, which can have positive or negative effects on Env incorporation, we sought to derive virion-based immunogens with greatly enhanced levels of Env trimers.

**Methods:** The cytoplasmic tail of HIV-1 was modified 1) to remove all known endocytosis signals and 2) to incorporate a segment from the SIV Env cytoplasmic tail that can positively affect Env incorporation. We generated virions from HEK 293T cells and quantified Env incorporation via the Env-Gag ratio, as measured by antigen-capture ELISA for p24 and gp120.

**Results:** Envelopes that lacked a cytoplasmic tail and/or contained mutations that ablated known endocytosis signals resulted in a ≤2-fold increase in Env content on virions. However, when HIV Env also contained a 13 amino acid segment from the proximal region of the SIV Env cytoplasmic tail, HIV Env incorporation could be increased 3- to 8-fold. In contrast to other reported approaches to increase HIV Env incorporation, viruses containing these changes could establish a spreading infection in CD4+ T cell lines.

**Conclusion:** The novel Env modifications we describe here are effective across different HIV isolates and clades and can be applied in combination with other approaches to increase the surface density of Env on inactivated virus or VLP vaccines. The effect of these modifications on immunogenicity is currently under investigation.

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**P01.06**

**In Vivo Tracking of an Anti-HIV Vaccine by Near-Infrared Fluorescence Imaging in Non-human Primates**


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**Background:** Non invasive and longitudinal imaging approaches are required to study the dynamics of skin antigen presenting cells (APCs) migration to the draining lymph nodes (LNs) following immunization in order to better understand the mechanisms leading to the induction of cellular and humoral immune responses. Here we evaluated the dynamic of migration of a fluorescent anti-langerin antibody fused to HIV antigen using in vivo near infrared fluorescence (FNIR) imaging after intradermal injection with or without resiquimod (R-848), an agonist of Toll-like receptor 7 and 8, in non-human primate (NHP).

**Methods:** Anti-langerin-HIVgag-FNIR (exc. 682nm; em. 710nm) was injected intradermally, with or without R-848 in NHP (n=3). In vivo NIR fluorescent imaging was performed prior to and at different times post-injection (FluobeamTM, Fluoptics). The fluorescent intensity was measured using ImageJ software. Confocal time-lapse microscopy was then performed on whole skin biopsies to track fluorescent APCs in both dermis and epidermis. Cell tracking and quantification were performed by using Volocity software (Perkin elmer).

**Results:** The NIR fluorescent-labelled anti-langerin-HIVgag vaccine was visualized by in vivo imaging for up to 48 hours post-injection in NHP. At 2h post-injection, the signal intensity at the injection site of anti-langerin-HIVgag-FNIR was 46.8 ± 2.3% of the injected fluorescence whereas the one measured at the injection site of α-langerin-gag-FNIR and R-848 was lower (28.89 ± 11.9%). Confocal videomicroscopy confirmed that the anti-langerin-HIVgag-FNIR specifically targeted Langerhans cells, which were also stained by anti-HLADR and anti-CD1a monoclonal antibodies.

**Conclusion:** In vivo NIR fluorescence imaging can be used to monitor the effect of a vaccine and/or an adjuvant on APC from the skin to the LNs in NHP. This approach will allow better characterization of the early cellular events in different experimental settings such as vaccination and immune therapeutic interventions.
P01.07

Local Immunomodulation of TLR3-Triggered Immune Activation by Single-Stranded DNA Oligonucleotides in Non-human Primates

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Background: Among other pathways, HIV initiates immune responses through TLR3 engagement. It was recently shown that single-stranded DNA oligonucleotides (ssDNA-ODN) can modulate the immune response triggered by Poly(I:C), a TLR3 ligand (TLR3-L). Therefore, its potency as a novel skin adjuvant to prevent TLR3-exacerbated inflammation was tested in vivo in non-human primates.

Methods: Cynomolgus macaques (n=12) were injected intradermally on the back with PBS on one side and with Poly(I:C) alone or with ssDNA-ODN on the other side. After 24h, skin biopsies were performed to study local cellular events (flow cytometry), cytokine secretion (luminex) and perifollicular ODN on the other side. After 24h, skin biopsies were performed to study local cellular events (flow cytometry), cytokine secretion (luminex) and expression arrays (Agilent system). Statistical significance was tested by Kruskal-Wallis followed by Dunn’s multiple comparison tests and Fisher’s exact test.

Results: As expected, i.d. injection of TLR3-L induced skin inflammation with infiltration of polymorphonuclear cells (PMN, p<0.05) and maturation of skin DC subsets (CD80/86 expression, p<0.01). HLA-DR - CD14 + macrophages were also recruited in dermis (fold increase (FI)=9) and a group of HLA-DR + CD1a - cells accumulated (FI=31 and p<0.01) in epidermis. Addition of ssDNA-ODN appeared to dampen maturation of Langerhans cells (p<0.05) and reduced infiltration of dermal macrophages and epidermal HLA-DR + CD1a - cells (FI=2 and 9, respectively). Microarray data revealed that Poly(I:C) injection induced innate immunity with significant induction of many interferon regulated genes (p<0.05) (fold change (FC) range 35-2640 at 10% FDR). ssDNA-ODN presence reduced pro-inflammatory cytokines transcription as compared to Poly(I:C) injection alone. In parallel, mRNA expression of immune-regulatory cytokines, such as IL-10 family members and IL-17A, was increased. Luminex analyses performed on supernatants from skin biopsies explants confirmed IL-10 induction (FI=15) and reduced IL-6 production after addition of ssDNA-ODN. However, several interferon regulated innate HIV-1 restriction factors (p<0.01, FC>4) were induced even in the presence of ssDNA-ODN.

Conclusion: These results demonstrate local ssDNA-ODN effective modulation of TLR3-induced immune response and support future clinical investigation in non-human primates.

P01.08

Improving Cell Surface Expression and Immunogenicity of HIV-1 Envelope by Modification of Residues Within the Cytoplasmic Tail

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Background: HIV-1 Envelope (Env) protein is an ideal target for an HIV-1 vaccine, but has been poorly immunogenic in clinical trials to date. Env contains endocytosis motifs within its cytoplasmic tail (CT) that may decrease Env expression on the surface of infected cells and reduce immunogenicity. HIV-1 and Simian Immunodeficiency Virus (SIV) Env proteins contain a conserved endocytosis signal represented by the sequence GYxxΦ (where G=Gly, Y=Tyr, x=any amino acid, and Φ=an amino acid with a bulky hydrophobic side chain, such as Leu). Previous studies using SIVmac Env showed that expression was increased on the surface of transfected cells when this endocytosis motif was disrupted. This increase was augmented when the CT was truncated to remove distal endocytosis signals. However, in the context of HIV-1 Env, these mutations do not significantly increase surface expression. Therefore, we hypothesized that the CT of SIVmac Env contains positive signals for surface expression. HIV-1 Env constructs containing SIVmac sequences may be ideal candidates for optimizing cell surface expression of Env, with the end goal of improving immunogenicity of Env through increased dendritic cell-mediated antigen presentation to B cells.

Methods: In this series of experiments, we used HIV-1 Env derived from multiple virus isolates and clades (89.6, JRFI, R3A, and 1086C), and generated a series of constructs containing the SIVmac segment with various CT mutations. 293T cells or primary human dendritic cells were transfected or infected with vaccinia virus encoding Env, and surface expression was measured by immunostaining and flow cytometry.

Results: Incorporation of SIVmac CT sequences into HIV-1 Env resulted in up to a 30-fold increase in Env expression on the surface of 293T and dendritic cells.

Conclusion: CT-modified HIV-1 Env constructs are promising candidates for improving immunogenicity in the context of an HIV vaccine; these constructs are currently being tested in small animal models.
Background: A major obstacle to obtaining protective immunity against HIV is defining adjuvants that induce high affinity and broadly neutralizing antibody responses against envelope (Env) proteins. Our data demonstrated that a combination of TLR4 ligand and TLR7 or 7/8 ligands delivered using synthetic nanoparticles strikingly enhanced the magnitude, quality and durability of humoral and cellular immune responses against protein immunogens in mice and rhesus macaques (Nature, 2011 Feb 24;470(7335):543-7). We hypothesized that such a combination of ligands could significantly enhance humoral and cellular immunity against SIV immunogens in rhesus macaques with implications for HIV-1 vaccine development in humans.

Methods: Nanoparticles containing a combination of TLR4 and TLR7/8 ligands (NP adjuvant) were used to adjuvant virus-like particles (VLPs) and recombinant antigens (gp140), both derived from the SIVmac239 virus. Immune responses were compared with those elicited by antigen delivered in alum, an adjuvant currently used in humans. Rhesus macaques were immunized four times at eight-week intervals. Innate and adaptive immune responses were monitored at early and late time points post vaccinations.

Results: NP adjuvant significantly expanded subsets of monocytes and dendritic cells in peripheral blood at early time points post vaccination. NP adjuvant induced sustained polyfunctional Env specific CD4+ T cell responses in the presence of gp140 but not in the presence of VLPs. NP adjuvant in the presence of gp140 induced robust Env specific plasmablast responses in peripheral blood after each booster immunization and induced long-lived plasma cells in bone marrow and draining lymph nodes. NP adjuvant in comparison with alum, induced significantly higher Env specific binding antibody responses (IgG and IgA) in serum, vaginal secretions and rectal secretions.

Conclusion: Our results indicate that synthetic nanoparticles carrying a combination of TLR4 ligands offer a novel approach to engineer robust and durable Env specific humoral affinity in rhesus macaques with implications for HIV-1 vaccine development in humans.

Background: The glycan-V3 loop on gp120 is one of the major sites of vulnerability on the HIV-1 spike. Several broadly neutralizing human antibodies that target this site have been isolated. One of them, PGT128, achieves broad and potent neutralization of over 70% of HIV-1 strains. Structural studies revealed that PGT128 interacts with the backbone of V3 in addition to glycans on the V3 loop. The elicitation of PGT128-like antibodies with structurally designed V3-peptides and the resolution of immunogenic determinants of such designs, as potential immunogens in animal models are fascinating questions, which have yet to be explored.

Methods: To create PGT128-specific immunogens, we performed structure-based design to transplant the epitope of interest containing the three N-linked glycans and a segment of V3, onto various scaffold proteins. In addition, various combinations of glycan-knockout mutants were created to explore the importance of each glycan. To further explore the relationship between antibody recognition and conformational flexibility, a non-scaffolded V3 peptide was also created and tested as a control.

Results: We characterized these designed immunogens with biochemical, biophysical and structural approaches. Our results indicate that these small scaffolded proteins are readily secreted by mammalian cells in multiple glycoforms. Surface plasmon resonance binding data revealed that these 11 designed immunogens were able to bind PGT128 with high affinities, ranging from 2 nM to 35 nM. Binding assays performed with glycan mutants and non-scaffolded V3 peptide confirmed the importance of glycan binding and conformation. We also included various combinations of glycan-knockout mutants to explore the importance of each glycan.

Conclusion: Structure-based design of proteins was able to produce immunogens, with high affinity and specificity to PGT128. The ability to tailor immunogens with specific antigenicity and conformation using structural biology, represents a first and important step in the activation, expansion and maturation of PGT128-like B cell lineages.
**P01.11**

**Solution Structural Analysis of the Secondary Structure for HIV-1 gp120 Outer Domain by NMR Spectroscopy**

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**Background:** The heavily glycosylated outer domain (OD) of HIV-1 gp120 has been proposed as a minimal immunogen to elicit broadly neutralizing antibodies. Immunization with a number of different OD variants has thus far failed to elicit neutralizing antibodies. Solution structure of the unliganded OD and an understanding of its dynamic properties may assist in the use of OD as an immunogen.

**Methods:** NMR spectroscopic characterization of proteins requires the production of protein samples enriched in 15 N/13 C and/or 2H stable isotopes. The heavily glycosylated OD is especially problematic to express using conventional prokaryotic expression systems. We developed a method to enrich glycoproteins with 15 N/13 C isotopes using a mammalian expression system that exploits the high level of protein expression obtained from an adenoviral vector. We employed heteronuclear NMR spectroscopy to obtain structural and dynamic information of unliganded OD. Three dimensional NMR experiments for sequential and side-chain assignments along with 15 N and 13 C edited NOESY experiments were recorded on uniformly labeled OD as well as on an OD sample selectively enriched in 15 N/13 C for Ile, Leu and Val.

**Results:** We successfully produced isotopically enriched OD samples, suitable for NMR analysis. Standard triple resonance NMR experiments on the uniformly labeled OD were combined with backbone and isotope edited experiments recorded on an OD sample that was selectively enriched in 15 N/13 C for Ile, Leu and Val to assign HN, C', C alpha, and N backbone resonances in 128 of the 220 residues of OD. These preliminary backbone assignments were further validated using HC(C)H and (H)CCH TOCSY side-chain experiments.

**Conclusion:** We succeeded in assigning ~1/2 of the backbone for unliganded OD with triple resonance and side-chain NMR experiments. Extension of these assignments with NOESY experiments is now proceeding. Our results indicate that solving a solution structure of the HIV-1 gp120 OD by NMR spectroscopy is feasible.

**P01.12**

**Enhancement of SIV-Specific Cell Mediated Immunity by Co-administration of Soluble PD-1 and Tim-3 as Molecular Adjuvants in Mice**

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**Background:** An HIV/SIV vaccine that contains multiple antigens may confer better effective protection. The magnitude and breadth of immune responses may be limited, especially for the subdominant antigens.

**Methods:** In this study, we evaluated the immunogenicity of a multiple antigenic SIV vaccine (gag, pol, env, nef, vpx, vpr, vif, raf, tat) in combination with two molecular adjuvants, soluble PD-1 (sPD-1) and soluble Tim-3 (sTim-3), harbored in recombinant adenoviral vectors. The use of sPD-1 and sTim-3 is to block the inhibitory receptors PD-1 and Tim-3, and thus to maximize the activation of CD4 and CD8+ T cells by MHC-epitope complex.

**Results:** Although the mice immunized with SIV vaccine produced antigen specific cell mediated immunity (CMI), mice immunized with SIV vaccine co-inoculated with sPD-1 and/or sTim-3 showed significantly stronger CMI, reflected by the generation of more IFN-γ+CD4+T and CD8+T cells and the enhanced proliferation of antigen specific T lymphocytes. Interestingly, the CMI elicited by non-structural proteins vif and tat were significantly enhanced in both magnitude and breadth.

**Conclusion:** Our study provided a strategy to enhance vaccine induced cellular responses, which may be adopted to improve the vaccination effects against SIV/HIV or other chronic infections. Future study will be carried out using a SIVmac239 model in rhesus macaques.
**P01.13**

Mimetic Peptide Selection from Phage-Display Libraries Using Patient Sera Evidence Epitope Structural Features for the Apex of V3 Loop in gp120

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**Background:** The crown region of the V3 loop in HIV-1 that contains the conserved amino acid sequence GPG/R/G is known as the principal neutralizing determinant due to the extraordinary ability of antibodies to this region to neutralize the virus. Apparently it is one of the most vulnerable viral target for which synthetic peptide models are explored for vaccine candidates. Peptide mimics of the epitope recognized in random phage-display libraries by patient anti-V3 antibodies could be of high utility but were not so far obtained due to the heterogeneity of the antibodies that V3 produces.

**Methods:** The screening of random phage-display libraries of high complexity including linear 12mer and constrained 7mer peptides was developed using polyclonal antibodies from HIV-1 subtype B-infected Mexican patients.

**Results:** The short constrained peptides selected presented two conserved amino acid sequences: PR-L in N-terminus and GPG in the C-terminus representing the two known epitope binding sites. In an interesting way the GPG triad has the same function as the V3 crown GPGR sequence but without the involvement of the R despite its being considered as the signature of the epitope in B-subtype viruses. In the case of the PR-L motif, it contains a proline not existing in the epitope, this amino acid is postulated to induce a beta-turn in the backbones of all peptides and create a spatial element mimicking the N-terminal conformational variable binding site. As immunogens, polyclonal antibodies to these mimotopes induced in rabbit recognized a panel of V3 peptides and moderately decreased the fusion between HIV-1 Env- and CD4-expressing Jurkat cells.

**Conclusion:** The identified structural features of V3 mimotopes are sources of information on the fine structure-function properties of HIV-1 principal neutralizing domain and contributing to rational design of anti-HIV-1 immunogens.

**P01.14**

Epitope Competition Could Influence the Kinetics of Specific CD8+ T Cell Responses During Repeated DNA Vaccination

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**Background:** Previous study suggested that specific T cell responses against HIV-1 Gag were suppressed when being co-expressed with HIV-1 Env. Epitope competition was postulated to be one of the underlying mechanisms. The objective of this study is to elucidate the influences of epitope competition on specific T cell responses during repeated DNA vaccination.

**Methods:** DNA vaccines expressing Gag92, Env203 or Gag92/Env203 fusion epitope (Gag92 and Env203 were mouse T cell epitopes identified in our previous work) were constructed. C57BL/6 mice were immunized with single epitope DNA vaccines (pSV-gag92 or pSV-env203), fusion gene DNA vaccine (pSV-gag92/env203) and the mixed single epitope DNA vaccines (pSV-gag92+pSV-env203). Epitope specific CD8 T cell responses were analyzed by the method of intracellular cytokines staining.

**Results:** We assayed the epitope specific T cell response after 3-time intramuscular immunization, and found that the frequency of Env203-specific IFN-γ+CD8+ T cell [(1.001±0.2676)\%] was significantly higher than Gag92-specific IFN-γ+CD8+ T cell [(0.1563±0.09369)\%] in pSV-gag92/env203 immunizing group. While, no significant difference was observed in the group immunized with mixed DNA vaccines (pSV-gag92+pSV-env203). Moreover, the mixed single epitope DNA vaccines could induce higher rate of Gag92-specific IFN-γ+CD8+ T cell response [(0.4220±0.4497)\%] than pSV-gag92/env203.

Furthermore, dynamic observation showed the specific IFN-γ+CD8+ T cell responses against non-dominant epitope(Gag92) could not be efficiently boosted during repeated vaccination of pSV-gag92/env203 fusion gene vaccine and only dominant epitope(Env203) specific IFN-γ+CD8+ T cell responses could be improved. While, the mixed single epitope DNA vaccines could induce much more balanced CD8+ T cell responses against both epitopes.

**Conclusion:** Epitope competition could significantly decrease the frequency of specific T cell response against non-dominant epitope. This is true not only at the end but also during the process of repeated DNA vaccination. And separate expression of different epitopes may help to balance the magnitudes of T cell responses among different epitopes.
P01.15

Multi-envelope Transmitted Founder DNA Vaccine Induces Potent Cross Clade Humoral Responses in Guinea Pig and Rabbits

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Background: It has been reported that guinea pigs vaccinated with transmitted founder gp140 envelope proteins are able to induce low but broad neutralizing antibodies to both tier 1 and tier 2 viruses. This general induction of coverage may be ideal for a priming immunization, establishing a response which is able to be boosted with the addition of either chronic or consensus envelopes. Since the DNA vaccine platform is seen as a priming regimen, we aimed to investigate if similar broad response could be induced using primary transmitted founder (TF) gp160 immunogens from clades A, B, and C.

Methods: Initial immunogenicity studies of individual constructs were performed in guinea pigs. Additional combination studies were performed in rabbits with between 4 to 6 immunizations followed by in vivo electroporation.

Results: All guinea pigs seroconverted with two immunization of individual plasmid expressing clade A TF envelope. Rabbits sequentially immunized with different clade A TF envs developed limited binding titers to primary gp120s. However, if these same plasmids were grouped together and immunized, all rabbits showed robust binding titers to cross-clade primary gp120s after two immunizations and limited tier 1 neutralization. Including TF envelopes from different clades increased binding titers and neutralization breadth and potency. Antibody epitope mapping revealed vaccination was inducing primarily V3 binding.

Conclusion: We have shown that DNA plasmids expressing TF gp160 immunogens are both expressed and induce a potent immune response. We have observed for the first time that exposure of the immune system to multiple envelopes at one time can dramatically change the immune phenotype induced. This finding has profound implications for development of an HIV vaccine. Further studies into how this priming response can be broadened are currently under investigation. We are also determining the role of T follicular helper cells driving humoral responses to DNA vaccines.

P01.16

Stability of an Acute HIV-1 Tanzanian Subtype C gp145 Envelope Protein for Clinical Development

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Background: There has been renewed interest in a vector prime and protein boost strategy since the RV144 clinical trials. One of the lead candidates to be used as a protein boost is a CHO expressed gp145 trimer envelope protein derived from an acute Tanzanian HIV-1 isolate (CO6980v0c22), which is currently being manufactured for clinical trials. A number of questions have arisen as to the best formulation buffer and the stability to lyophilization and multiple freeze-thaws.

Methods: The gp145 envelope protein was expressed in a stable CHO cell line, purified and placed in PBS, pH 7.4. The stability of gp145 was tested in PBS or after dialysis with Tris-saline buffer, pH 7.4. Samples frozen once and stored at 4°C were compared with samples that were subjected to three -80°C freeze-thaw cycles. Samples were lyophilized from PBS or Tris-saline and reconstituted. The gp145 subjected to the various treatments was analyzed by ELISA for binding to various monoclonal antibodies, VRC01, PG9, PG16 and pooled plasma from patients chronically infected with HIV clade C. The gp145 was also analyzed on blue native gels followed by Western blotting with these antibodies.

Results: No significant difference was observed in binding with each of the antibodies to the gp145 formulated in PBS when stored at 4°C, lyophilized or repeatedly frozen and thawed. However, changing the buffer from PBS to Tris-saline caused 30-50% reduction in the binding of VRC01, PG9 and PG16. No changes in binding were observed with the clade C plasma. However, lyophilization of the gp145 from Tris-saline further reduced the binding of each of the antibodies an additional 10-20%.

Conclusion: These data demonstrate that use of PBS as a buffer for gp145 is preferable to Tris-saline. The gp145 protein in PBS can be lyophilized and reconstituted and can undergo multiple freeze-thaws with no loss of antibody binding ability.
P01.17

Comparable Antigenicity and Immunogenicity of Multimeric Forms of a Novel, Acute HIV-1 Subtype C Gp145 Envelope for Clinical Development


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Background: Broadly reactive neutralizing antibodies (NAbs) that target the quaternary structure of the V1/V2, V3 and CD4 binding domains of the HIV-1 Env trimer highlight the importance of representing the functional Env spike in immunogen design. Considering the novel properties and global representation of subtype C, a trimeric C Env (CO6980v0c22) derived from an acute Tanzanian HIV-1 isolate was developed as a candidate vaccine.

Methods: The CO6980v0c22 gp145 Env was expressed in a stable CHO cell line. Purified oligomers were fractionated into dimers, trimers and higher order multimers using size exclusion chromatography. The trimeric structure of gp145 was evaluated by cryo-electron microscopy. Antigenicity was determined by BiaCore and Octet Biosensor analysis. Rabbits were vaccinated with individual protein fractions; the rabbit sera were assessed for binding antibodies by ELISA, and for NAbs using PBMC-based and TZM-bl pseudovirus assays.

Results: Expressed gp145 was found to contain 60% physical trimers. Larger multimeric, trimeric and dimeric fractions were purified to 95%, 92% and 94% homogeneity, respectively. The gp145 trimer bound NAbs that target the MPER (4E10), CD4bs (VRoC1, b6, b12), V2/V3 epitopes (PG9, PG16) and the V3 crown (447-52D), as well as the cell-expressed alpha4beta7 receptor. After three immunizations, rabbits developed strong binding antibody titers (up to 10^6) to several Env antigens, including the V1V2gp70 MuLV scaffold protein used in RV144 correlates discovery. Neutralization was observed against Tier 1 subtype B and C pseudoviruses, and against Tier 1 and Tier 2 IMC from three different clades. No differences in antigenicity or immunogenicity were observed between the various oligomeric protein fractions.

Conclusion: These data demonstrate the antigenic and immunogenic features of a novel acute subtype C HIV-1 Env that warrants further testing for clinical development. GMP production of the gp145 trimer is ongoing.

P01.18 LB

Increased HIV-1 Immunogenicity of Replication Competent NYVAC

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Background: The human RV144 efficacy trial revealed a 31% efficacy rate against acquisition of HIV infection using the ALVAC/vcp1521/AIDSVAX gp120 B/E prime boost regimen. In addition to a modest efficacy rate, the duration of protection was relatively short-lived, suggesting that both vaccine components must be improved. The Copenhagen vaccinia derived replication deficient NYVAC has been shown to be highly immunogenic in combination with DNA priming in both non-human primates (NHPs) and human clinical trials.

Methods: Host range genes K1L and C7L were inserted in NYVAC vectors expressing HIV-1 clade C Env, and Gag-Pol-Nef to restore replication in human cells. This replication competent NYVAC-KC was compared head to head with the parental replication deficient NYVAC-C vectors for immunogenicity in rhesus macaques using two different prime-boost vaccine schedules; a) 2x NYVAC at week 0 and 4 followed by 3x NYVAC+gp120/120/MFS9 at week 12 and 24 and 48; b) 3x DNA at week 0, 4 and 8 followed by 1x NYVAC at week 20 and 3x gp120/MFS9 at week 28, 32 and 48. T-cell responses were assessed by ICS and IFNγ ELISPot and humoral responses by anti-Env binding IgG antibodies, neutralizing antibodies, Env V1V2 IgG and ADCC.

Results: The DNA prime/NYVAC boost regimen induced vigorous polyfunctional CD4+ and CD8+ T-cell responses (IFN-g+IL-2+TNF+ by ICS and ±5000 SFUs/10^6 cells at peak response). In the absence of DNA priming T-cell responses were significantly higher within the NYVAC-C KC (>1 log) as compared to NYVAC-C. T-cell responses were significantly lower compared to NYVAC-C. T-cell responses were significantly lower compared to DNA primed groups. Marked IgG Env antibody responses including IgG V1V2 responses were detected in all groups although there was a trend to higher responses in the absence of DNA priming.

Conclusion: Replication competent NYVAC-KC significantly increased immunogenicity as compared to NYVAC thus providing the rationale for moving NYVAC-KC to clinical development.
A Trimeric HIV-1 gp140-BAFF Fusion Construct Enhances Mucosal Anti-trimeric HIV-1 gp140 IgA in Mice


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Background: Although trimeric HIV-1 gp140 can mimic native HIV-1 envelope (Env) spikes on viral surface, derived vaccines still can not induce potent broadly neutralizing antibodies (bNabs). HIV-1-Env specific IgA at mucosa may block HIV-1 transmission through mucosal surface. The tumor necrosis factor superfamily members, APRIL, BAFF, and CD40L, were previously shown to promote antibody responses, including mucosal IgA. We tested whether APRIL, BAFF or CD40L conjugated HIV-1 gp140 constructs could enhance anti-HIV-1-Env mucosal IgA responses.

Methods: FLAG-tagged codon-optimized HIV-1 YU2 gp140 sequence was linked to an exogenous trimerization domain, foldon, which was then fused with mouse APRIL, BAFF, and CD40L sequence. FLAG-tagged codon-optimized HIV-1 YU2 gp140 with or without foldon were constructed as controls. Following expression in HEK-293T cells, the constructs were assessed for proper expression, gp140 folding, and trimerization, via protein gel electrophoresis/western blotting, immunoprecipitation, and Native-PAGE, respectively. Protein production was scaled up in bioreactors and proteins were isolated using FLAG-tag affinity purification. Balb/c mice were randomly divided into 6 groups (4 mice per group): 1. Gp140; 2. Gp140-foldon; 3. GP140-APRIL; 4. Gp140-BAFF; 5. Gp140-CD40L; 6. Naïve. Mice were primed intramuscularly 3 times with DNA plasmids and boosted intraperitoneally 2 times with the proteins (Phosphate-buffered saline instead of DNA/protein was injected into naïve group mice). Vaginal lavage and fecal pellet were collected before and then every 2 weeks after vaccinations. Samples from the same group were pooled together and anti-HIV-1 trimeric gp140 IgA in the pooled samples were detected by kinetic ELISA, using trimeric HIV-1 YU2 gp140 as coating antigen.

Results: The fusion constructs formed stable trimers consistent with proper folding of HIV-1 Env. Presently, we tested samples from 4 weeks after the last boost. Gp140-BAFF enhanced anti-HIV-1 trimeric gp140 IgA in fecal pellet and vaginal lavage, which was 4.8 and 10 fold, respectively, of that induced by gp140-foldon control. Gp140-APRIL and gp140-CD40L did not show enhancement. Further work is in progress to analyze the induced antibodies.

Conclusion: The trimeric HIV-1 gp140-BAFF fusion construct enhanced mucosal anti-HIV-1 trimeric gp140 IgA and deserves to be studied further as a possible HIV-1 vaccine candidate.
Isolation of mAbs from Sorted Single B Cells by RT/PCR for Analysis of B Cell Responses to HIV-1 MPER Epitope in Vaccinated Rhesus Macaques


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Background: Rhesus macaques are important models for evaluation of HIV-1 vaccines. Analysis of immunoglobulin (Ig) VH and VL genes derived from single B cells is a powerful technology for definition of Ig repertoires to viral infections and vaccination. We have produced a large panel of recombinant mAbs from immunized rhesus macaques to ask if a HIV-1 gp41 immunogen could induce an antibody lineage with binding characteristics of a broadly neutralizing antibody (BnAb).

Methods: Primers were designed for amplification of rhesus Ig VH and VL gene segments from sorted single memory B cells by RT/PCR. The isolated VH and VL gene segments were analyzed using a newly assembled and annotated database of rhesus germline gene segments, and used to produce mAbs using linear antibody gene expression constructs generated by PCR without cloning. PBMC from rhesus macaques that were immunized with HIV-1 JRFL Env and MPER peptide-liposomes in a 2F5-like 2-step binding mechanism.

Results: A total of 195 unique VH and VL gene pairs were isolated from sorted single memory B cells and expressed as IgG1, of which, 106 mAbs reacted with HIV-1 Env antigens. We found 34.4% of these antibodies belonged to 23 clonal lineages. Antibodies in the CH167 and 2F5-negative antibody lineage (5 members) have VH mutation frequencies ranging from 1.4%–4.2% and bound to HIV-1 gp41, MPER epitope with a footprint involving the 2F5-nominal epitope, DKW. MAbs bound to MPER peptide-liposomes in a 2F5-like 2-step binding mechanism.

Conclusion: A streamlined strategy for amplification and expression of rhesus Ig VH and VL genes has been developed for study of the rhesus macaque antibody repertoire in response to immunization with HIV-1 vaccines. Our study showed that immunization with HIV-1 JRFL Env and MPER peptide-liposomes induced an antibody clonal lineage with characteristics of precursors of an MPER BnAb.

Replicating VSV-SIV Chimeric Viral Vector Immunogenicity in Indian Rhesus Macaques


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Background: Live attenuated vaccines have been developed to prevent viral diseases including smallpox and measles; however, for HIV this is not practical. Therefore, we developed replication-competent vesicular stomatitis virus (VSV) vectors in which VSV glycoprotein (G) was replaced with SIVenv and SIVgag producing chimeric viruses (VSV-SIV).

Methods: Chimeric vaccines were tested in two NHP studies to compare systemic and mucosal immunization routes. NHPs were primed with VSV-SIV and boosted with adenovirus-5 containing SIVgag and SIVenv (Ad5-SIV). In the first study, VSV-SIV was administered intramuscularly (IM) and in the second, VSV-SIV was delivered by combined intranasal (IN) and oral (OR) inoculation. Additionally, to improve mucosal uptake, some animals in the second study were vaccinated with VSV G-pseudotyped chimeric virus.

Results: In the 1st study, NHPs were primed 3 times, IM 8 weeks apart with 10 4, 10 6 or 10 8 pfu of VSV-SIV followed by an Ad5-SIV boost. Humoral immune responses were dose dependent with the highest dose inducing SIVenv antibodies after each prime. Following the Ad5 boost, antibody titers increased 3-5 logs and pseudovirus-neutralizing activity was detected in the 10 6 and 10 8 groups. In the 2nd study, 10 8 pfu VSV-SIV was administered mucosally (IN/OR) and in one group, VSV-SIV was pseudotyped with VSV-G. Mucosal immunization induced humoral responses of similar magnitude to IM while G-pseudotyped virus enhanced Env-specific antibody by 4-logs and pseudovirus neutralization 1-2 fold. Gag antibody titers were low after VSV-SIV but priming was evident with a 5-log increase in titers following boost. T cell responses were low but detectable and increased significantly following Ad5-SIV boost indicative of priming by the VSV. NHPs in the 2nd study are being

Conclusion: VSV-SIV chimeras are safe and immunogenic at 10 8 pfu when administered to macaques systemically and mucosally. VSV-SIV immunization induced pseudovirus neutralizing antibodies and immunogenicity is improved by G-pseudotyping broadening the VSV cell tropism.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L1, Room 111 – 112. Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L1, Room 111 – 112.

**P02.03**

**Immunogenicity of HIV Virus-Like Particles in Rhesus Macaques by Intra-Nasal Administration**


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**Background:** The development of vaccination strategies able to elicit protective systemic and mucosal immune response represent a major goal in the HIV vaccine field. To this aim we have evaluated the immunogenicity of HIV-VLPs in Rhesus Macaques by a sequential combination of mucosal (intra-nasal, i.n.) and systemic (intra-muscular, i.m.) routes, according to homologous (VLP + VLP) or heterologous (DNA + VLP) prime-boost schedules.

**Methods:** 24 female Rhesus Macaques were equally divided in four experimental arms. Groups 1 and 2 were immunized by the i.n. route, using the homologous prime-boost protocol in the absence (Group 1) or in the presence (Group 2) of the Eurocine L3 nasal lipid adjuvant. Group 3 was immunized using the heterologous prime-boost protocol in the presence of Eurocine L3 and N3 adjuvants. Additionally, group 2 received two further boosting doses of VLPs by the i.m. route, 22 weeks after the last i.n. administration. Group 4 was the control group.

**Results:** The data show that i.n. administration of HIV-VLPs, in either homologous or heterologous prime-boost protocol, does not elicit measurable serum Ab titers. Moreover, it does not efficiently prime the systemic immune system, since two subsequent i.m. injections were needed to elicit serum Ab titers. Similarly, i.n. administration of HIV-VLPs, in both prime-boost protocols, does not elicit measurable mucosal Ab titers. However, it seems to prime the mucosal immune system which, six months after the last i.n. boost, is able to respond after the two i.m. immunizations.

**Conclusion:** HIV-VLPs are immunogenic in NHPs by homologous and heterologous prime-boost schedules. The i.n. administration was not fully effective but primed the mucosal immune response for subsequent boosting dose by i.m. route. Results are encouraging for elicitation of immune response at mucosal port of entry but further investigation is required.

**P02.04**

**CDV Vectors as Vaccine Candidates**

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**Background:** The development of a live attenuated HIV vaccine is not considered to be an option at this time. Our approach is to use unrelated live viruses as vectors to deliver an AIDS vaccine by developing a replication-competent, recombinant canine distemper virus vectors expressing HIV genes. As a proof of concept we developed two rCDV vectors containing simian immunodeficiency virus (SIV) genes encoding Gag and Env proteins (CDV-SIV).

**Methods:** Non-human primates were immunized intranasally (IN) at weeks 0 and 6. Two animals received CDV-SIV vectors at 10⁷ pfu and a control animal received empty CDV vector. All animals received an Ad5-SIVGag and Ad5-SIVEnv boost and were subsequently challenged with SIVmac239 by intrarectal route.

**Results:** CDV/SIV genome copies could be detected in intestinal tissue, cells from bronchalveolar lavage and in the oral cavity (4 weeks post 2nd immunization). Low level Env specific T cell responses were detected after each CDV immunization. Env antibodies were detected following the 1st CDV immunization and were further boosted following 2nd CDV immunization. After Ad5-SIV boost, a robust increase in Env and Gag specific T cell responses was seen in both BAL and PBMCs. Env antibody titers increased and Gag antibodies were detected following Ad5-SIV. All animals were intrarectally challenged with increasing doses of pathogenic SIVmac239. One immunized monkey became infected after one challenge. The remaining animals, resistant to two subsequent IR challenges, were ultimately infected intravenously. One CDV-SIV immunized NHP showed a peak viral load 3 logs lower than the control and although being infected both the immunized NHPs have maintained low viral loads for several months compared to the control animal.

**Conclusion:** rCDV vectors were shown to be safe and induce immune responses in a prime/boost regimen resulting in sustained control of SIV replication to low levels. A repeat study is currently underway to investigate these results further.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L1, Room 111 – 112.

Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L1, Room 111 – 112.

P02.05

Optimization of HIV-1 Envelope DNA Vaccine Candidates Within Three Different Animal Models, Guinea Pigs, Rabbits and Cynomolgus Macaques

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Background: HIV-1 DNA vaccines have many desired features. Evaluation of HIV-1 vaccine candidates often starts in small animal models before macaques and human trials. Here we selected and optimized DNA vaccine candidates by systematic testing in rabbits for induction of broadly neutralizing antibodies (bNAb) and compared different animal models guinea pigs, rabbits and cynomolgus macaques.

Methods: Env from the prototype Bx08 and two elite neutralizers were selected. Codon-optimized genes encoded secreted gp140 or membrane bound gp150, or were modified for stabilized soluble trimer products (SOSIP-R6-I2 mutations), and delivered individually or mixed. Specific IgG after repeated i.d. inoculations with electroporation confirmed in vivo expression and immunogenicity.

Results: Evaluation in rabbits and guinea pigs displayed similar results. The optimal DNA construct in rabbits was a trivalent mix of non-modified codon-optimized gp140 envs (SSImix). Despite potent and broad NAb responses in guinea pigs and rabbits, the DNA vaccinated macaques displayed less bNAb activity.

Conclusion: It is concluded that a trivalent mix of non-modified gp140 genes from rationally selected clinical isolates is optimal to induce high and broad NAb in the rabbit model, but this optimization does not directly translate into cynomolgus macaques. This suggests species-specific differences in the quality of immune response to HIV-1 env DNA.

P02.06

Testing Efficacy of DNA/IL-12 Delivered by Electroporation with Vector Boost in the SIVmac239 System

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Background: We have previously demonstrated that vaccination with SIV ORFs encoding the full SIV genome (9 gene) with plasmid IL-12 (pIL-12) delivered via electroporation (EP) followed by an Adenovirus-5 SIV ORFs (Ad5-SIV) boost, can protect non-human primates (NHPs) from infection or reduce viral load of NHPs that do become infected. We undertook the study below to further investigate the utility of EP DNA/IL-12 and Adenovirus prime/boost regimen.

Methods: Five groups of NHPs: 1) SIV-DNA plus Ad5-SIV 9 gene, 2) SIV-DNA plus Ad5-SIV 4 gene (Gag, Pol, Env, Nef) 3) SIV-DNA plus Ad35-SIV 4 gene, 4) SIV-DNA alone 9 gene and 5) Controls were immunized 3 times by EP 8 weeks apart with pIL-12 followed by Ad5-SIV or Ad35-SIV boost.

Results: SIV-DNA prime induced low level T cell responses in all SIV-DNA groups predominantly against Gag, Env and Nef proteins. Gag, Env, Pol and Nef antibody titers and SIV pseudovirus neutralizing antibodies were of similar magnitude for 9 and 4 gene groups. Following Adenovirus-SIV boost there was significant increase in T cell responses. While all groups demonstrated similar quality of response for CD4 and CD8’s, producing IFNγ, TNFα and IL-2, the frequency of these responses varied, with both 4 gene groups demonstrating higher frequencies of multifunctional CD4 T cells compared to other groups. Mucosal surfaces and serum demonstrated increased levels of SIV specific mucosal IgG and IgA and serum antibodies in both Ad5-SIV and the Ad35-SIV group compared to DNA alone and control groups. We also observed significant increases in neutralizing antibody titers in both 4 and 9 gene groups to Tier 1A and 2 pseudoviruses.

Conclusion: Results demonstrate induction of effective cellular and humoral immune responses can be maintained when the number of SIV vaccine immunogens delivered is reduced and alternative adenoviruses are capable of inducing immune responses of the same or greater magnitude as Ad5.
P02.07

Adaptation of HIV-1 Envelope to Macaque CD4 Affects Antibody Recognition of the V1V2 Region

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Background: Species-specific differences between the human and macaque CD4 receptor restrict the ability of HIV-1 envelope variants to infect macaque cells. Two independent mutations, in the C2 region (A204E) and V3 loop (G312V) of gp120, confer efficient use of the macaque CD4 receptor to diverse HIV-1 envelope variants. Changes in the HIV envelope required for infection of macaque cells are particularly relevant to understanding the biological and antigenic properties of pathogenic SHIVs, which have been selected to replicate and persist in macaques.

Methods: To assess the effect of these changes on the antigenic properties, neutralization sensitivity to broadly neutralizing monoclonal antibodies (Mabs) was determined for 5 HIV-1 envelopes incorporating the A204E and G312V mutations and for a HIV-1 envelope from the pathogenic SHIV AD8. The Mabs tested recognize the CD4 binding site (VRC01), the V1V2 region (PG9/16) and the V3 loop (PGT 121/128/145). IC50 values were determined for each envelope variant/Mab combination using the TZM-bl assay.

Results: Introduction of A204E and G312V had only a modest (<10-fold) effect on the neutralization sensitivity to the CD4 binding site antibody VRC01 and V3-specific Mabs (PGT 121/128/145), except for one wild type and mutant pair where there was an increase in IC50 for all three V3-specific Mabs. In contrast, for approximately half of the envelope pairs, particularly those encoding A204E, there was a dramatic effect (>100-fold increase) in the IC50 value for antibodies recognizing the V1V2 region (PG9/16). Interestingly, HIV-1 envelope from SHIV AD8 exhibited a similar pattern of resistance to V1V2 antibodies without change in sensitivity to VRC01 and PGT Mabs when compared to the parental envelope.

Conclusion: These data suggest that adaptation of HIV-1 envelopes to macaque CD4 results in structural changes that affect recognition at epitopes distal to the CD4 binding site.

P02.08

Development of an Early Stage Investigator Scholar Program for Preclinical Researchers

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Background: Key themes from the March 2008 NIAID Vaccine Summit were 1) to strengthen linkages between preclinical and clinical researchers to collaboratively tackle key areas of scientific inquiry, and 2) to attract and retain new, talented investigators to the HIV vaccine field.

Methods: The HIV Vaccine Trials Network (HVTN) and the Center for HIV/AIDS Vaccine Immunology (CHAVI), with funding from NIAID, created a novel mentored research program for early stage investigators (ESIs) doing HIV vaccine research in non-human primate (NHP) models. From 2008–2012, HVTN and CHAVI recruited, reviewed, and selected 14 scholars during 3 application cycles; scholars proposed 1-2 year projects under guidance from senior preclinical and clinician scientists. HVTN provided training workshops on host genetics, challenge models, starting a lab, seeking funding, and other professional development topics. Post-program surveys were collected from 11 of 14 scholars and 15 of 24 mentors; key milestones (e.g., publications, positions, grants) were tracked for all scholars.

Results: Scholars presented their final results at 10 HVTN and CHAVI conferences. Training programs were well received (mean score 4.6 on scale 1-5 with 5 as high score). Combined, the 14 HVTN/CHAVI NHP ESI Scholars published 69 peer-reviewed manuscripts and presented 55 abstracts based on their awarded project results. Together they have received 11 promotions or appointments and 29 grants summing more than $28.4 million for future HIV research. Nine of 11 surveyed scholars intend to stay in the field of HIV vaccine research. All mentors reported a plan to continue mentoring their scholar beyond the program period.

Conclusion: A novel mentored research program that aims to strengthen the linkage between preclinical and clinical HIV vaccine researchers produced important scientific advances and bolstered the careers of young investigators. Future investment in targeted mentored research programs to advance HIV vaccine science is warranted.
**P02.09**

**Altered Immunodominance Hierarchy and Increased T Cell Breadth upon HIV-1 Conserved Element DNA Vaccination in Macaques**

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**Background:** We tested the hypothesis that a vaccine candidate composed of highly Conserved Elements (CE) of the HIV proteome excluding the variable regions would help overcome problems of viral sequence diversity and potential negative effects of immunodominance, two hurdles in the development of an effective AIDS vaccine. CE were selected based on both stringent conservation and association of specific elements with immune control. A prototype HIV DNA vaccine expresses 7 CE identified in p24 gag as a single protein and was able to induce robust cross-clade specific immune responses in mice.

**Methods:** Macaques were immunized by IM injection followed by electroporation with two DNA plasmids providing potential epitopes found in >99% of all HIV-1 M group sequences. Cellular immune responses were compared to those obtained upon vaccination with gag DNA only.

**Results:** CE DNA vaccination induced robust immunity in all 10 vaccinated macaques, whereas full-length gag DNA vaccination elicited responses in only 5 of 11 animals targeting fewer CE per animal. CE DNA vaccination elicited highly cytotoxic T cells against CE, capable of Granzyme B production and degranulation, desired features for an effective vaccine. Importantly, boosting CE-primed macaques with DNA expressing full-length p55 gag increased both magnitude of CE responses and breadth of Gag immunity, demonstrating altered immunodominance hierarchy in the presence of pre-existing CE-specific responses.

**Conclusion:** Combination of conserved elements and full-length immunogen provides a novel strategy to increase the magnitude and breadth of immune responses to Gag, and allows for the development and expansion of subdominant responses. This vaccine allows the immune system to target the Achilles heel of the virus, for which few escape pathways exist. Inclusion of a conserved element immunogen provides a novel and effective strategy to broaden responses against any highly diverse pathogen by avoiding decoy epitopes, while focusing responses to critical and invariable viral elements.

**P02.10**

**Ancestral vs. Consensus vs. Polyvalent: Envelope Effects on Vaccine Efficacy in an Equine Lentiviral Attenuated Vaccine Model**


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**Background:** Env is a determinant of lentiviral vaccine efficacy. We previously demonstrated a significant, inverse, linear relationship between EIAV Env divergence and protection from disease, utilizing our EIAV<sup>an</sup> attenuated vaccine and variant challenge strains, EV0, EV6, and EV13 (homologous, 6%, and 13% Env divergence, respectively). We further explore the relationship between Env evolution, diversity, and vaccine efficacy by directly comparing three approaches toEnv immunogen development in our attenuated vaccine model.

**Methods:** Ancestral, consensus, and polyvalent vaccines were evaluated in the EIAV<sup>an</sup> backbone substituted with defined, controlled Envs based on natural isolates from which EV0, EV6, and EV13 are derived. A consensus Env was engineered utilizing approximately 90 isolates detected throughout all stages of disease. To create the consensus Env, amino acid and nucleotide sequences were aligned, hand-edited, synthesized, and cloned into the EIAV<sup>an</sup> backbone (ConD9). To create a polyvalent EIAV<sup>an</sup> backbone substituted with defined, controlled Envs based on natural isolates from which EV0, EV6, and EV13 are derived, an ancestral Env was engineered utilizing approximately 90 isolates detected throughout all stages of disease. To create the ancestral Env, amino acid and nucleotide sequences were aligned, hand-edited, synthesized, and cloned into the EIAV<sup>an</sup> backbone (ConD9). To create a polyvalent EIAV<sup>an</sup> backbone substituted with defined, controlled Envs based on natural isolates from which EV0, EV6, and EV13 are derived, an ancestral Env was engineered utilizing approximately 90 isolates detected throughout all stages of disease. To create the ancestral Env, amino acid and nucleotide sequences were aligned, hand-edited, synthesized, and cloned into the EIAV<sup>an</sup> backbone (ConD9). To create a polyvalent EIAV<sup>an</sup> backbone substituted with defined, controlled Envs based on natural isolates from which EV0, EV6, and EV13 are derived, an ancestral Env was engineered utilizing approximately 90 isolates detected throughout all stages of disease. To create the ancestral Env, amino acid and nucleotide sequences were aligned, hand-edited, synthesized, and cloned into the EIAV<sup>an</sup> backbone (ConD9). To create a polyvalent EIAV<sup>an</sup> backbone substituted with defined, controlled Envs based on natural isolates from which EV0, EV6, and EV13 are derived, an ancestral Env was engineered utilizing approximately 90 isolates detected throughout all stages of disease. To create the ancestral Env, amino acid and nucleotide sequences were aligned, hand-edited, synthesized, and cloned into the EIAV<sup>an</sup> backbone (ConD9). To create a polyvalent EIAV<sup>an</sup> backbone substituted with defined, controlled Envs based on natural isolates from which EV0, EV6, and EV13 are derived, an ancestral Env was engineered utilizing approximately 90 isolates detected throughout all stages of disease. To create the ancestral Env, amino acid and nucleotide sequences were aligned, hand-edited, synthesized, and cloned into the EIAV<sup>an</sup> backbone (ConD9).

**Results:** Attenuated strains replicated at normal levels without EIAV-associated illness. Each group experienced different levels of disease upon challenge. TriD9 vaccines demonstrated the highest levels of protection (P<0.0001) while the ConD9 the lowest level of protection (P=0.0002). Further analysis demonstrated a significant trend for the complexity of immunogen and protective efficacy (Logrank test for trend, P=0.02).

**Conclusion:** Results directly compare for the first time an ancestral, consensus, and polyvalent Env, utilizing an attenuated vaccine (which to-date has demonstrated the highest levels of vaccine immunogenicity) and validate an Env protection scheme: polyvalent>ancestral>consensus.
P02.11

Analysis of Early Antibody Response in Macaques Infected with SHIV-1157 Virus, with Introduced V1/V2-Dependent Quaternary Neutralizing Epitopes

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Background: A number of highly potent neutralizing antibodies directed against quaternary epitopes (QNEs) in HIV envelope proteins have been recently described. To analyze the development of antibodies and antibody-dependent neutralization responses after infection in macaques we generated a mutant clade C-derived virus, SHIV1157ipd3N4 (Q170K/I192R) that expressed a high affinity QNE. Here we describe the Env-specific binding and neutralizing antibody activities induced in the early stages of viral infection.

Methods: Sera samples from macaques during the first 24 weeks after infection were screened by ELISA for antibody binding to various rgp120, rgp140, V1/V2, V3 and CD4bs protein constructs. Neutralization activity was determined in pseudovirus luciferase assay. Anti-gp120 and V1/V2-specific antibodies were isolated from infected sera by immunofinity chromatography on gp120 and gp70-V1/V2 columns and further characterized for binding and neutralizing activity against SHIV and HIV-1 pseudoviruses.

Results: High-titered, anti-gp120/gp140-binding antibody response developed shortly (~2-4 weeks) post-infection. Most animals developed anti-V1/V2 antibody responses by week 8 and many possessed specific anti-V3 antibodies. A robust autologous neutralizing activity appeared in several macaques during weeks 12-16, which plateaued over the following few months. High-titered cross-reactive neutralizing antibodies were also present against several Tier 1 viruses. Immunoaffinity depletion of infected sera indicated that much of the neutralizing activity was directed against targets in the V1/V2 region.

Conclusion: The immune response in early stages of HIV infection is of particular interest for vaccine development. The generation of a pathogenic SHIV expressing high affinity forms of QNEs provides a model system for examining the development of antibody response against these epitopes. This virus retains the Tier 2 phenotype, so the induction of autologous neutralizing activity is of particular interest. These animals are being followed to see whether the specificity of this response broadens with time, and whether this system will provide insight into the development of broadly neutralizing antibody specificities.

P02.12

Protection from SIVmac251 Acquisition by an ALVAC/SIV/gp120 Regimen Is Not Improved by the Increase of Anti-Envelope Antibodies and T Cell Responses

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Background: A prime-boost regimen of recombinant canarypox priming (ALVAC-HIV) with HIV (Clade B and E)-gp120 proteins boosting formulated in Alum has resulted in significant but limited protection from HIV acquisition in the Thai Trial (RV144). We hypothesized that the use of the Th1/Th2- adjuvant MF59 as an alternative to the Th2-Alum, could improve the protection elicited by the ALVAC-SIV/gp120 vaccine platform, by increasing antibody response to the envelope as well as T cell responses.

Methods: We vaccinated 54 macaques four times (0, 4, 12 and 24 weeks) with the identical ALVAC backbone used in RV144, expressing SIV mac251 Gag-pro and gp120TM (instead of the HIV-1 Envs used in RV144), and then we boosted 27 macaques twice (12 and 24 weeks) with both SIVmac251- and SIVsmE660- gp120 proteins formulated either with Alum or with MF59. A total of 24 adjuvanted or naive animals were included as concurrent controls and additional 23 as historical controls. All the animals were exposed to 120 TCID50 of SIVmac251, by the intra-rectal route, once a week, at 4 weeks after the last immunization.

Results: ALVAC-SIV gp120/Alum (RV144-like) resulted in significant protection from SIVmac251 acquisition (log rank p=0.021). MF59 significantly increased binding antibody titer, ADCC, neutralizing antibodies, and CD4+ T cell producing cytokines to the SIV-envelope. Surprisingly, however, MF59 did not improve the efficacy of the ALVAC-gp120 regimen (log rank p=0.56). In addition, ALVAC-SIV gp120/MF59 had significantly higher number of transmitted founder variants compared to ALVAC-SIV gp120/Alum regimen.

Conclusion: In conclusion, the combination of ALVAC-SIV vaccine and gp120/MF59 was more immunogenic than that of gp120/Alum; however it did not increase protection against SIVmac251 acquisition.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L1, Room 111 – 112.
Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L1, Room 111 – 112.

**P02.13**

**Latent SIV Turns Over Surprisingly Quickly Off Therapy: Implications for HIV Cure Studies**

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**Background:** Understanding latent HIV is essential to cure the infection. However, the dynamics of the turnover of latent HIV in subjects not on combination anti-retroviral therapy (cART) is poorly understood, as latent HIV is often defined in the presence of cART.

**Methods:** We further advanced a pigtail macaque model of SIV infection to quantify immune escape and compensatory mutations in serial samples of plasma SIV RNA and FACS-sorted resting CD4<sup>+</sup> T cell SIV DNA. We studied 3 CTL epitopes (KP9, KVA10, KSA10) and a mutation that compensates for KP9 escape (V145A). All epitopes examined escape with unique patterns and time-frames in 20 macaques, using real-time PCR (for KP9) and Roche 454 sequencing.

**Results:** In the setting of high viral loads (>10<sup>e5</sup> copies/ml), there was rapid penetration of KP9 (Gag) CTL escape mutation from plasma RNA into resting CD4<sup>+</sup> T cell SIV DNA, suggesting a rapid turnover of latent SIV DNA (~1 day). In the setting of low viral loads, similar to cART treatment, turnover of latent SIV DNA was much longer (up to 10<sup>e5</sup> days). There was a significant inverse correlation between viral load and turnover of latent SIV DNA, by both real time PCR and deep sequencing. Escape at the 2 Tat CTL epitopes and the compensatory mutation occurred in a less predictable pattern, often evolving over time to settle on a fixed mutation, often at time points beyond acute infection. This results in a complex and delayed pattern of penetration of escape mutations from plasma RNA into resting CD4<sup>+</sup> T cells.

**Conclusion:** The rapid turnover of resting CD4<sup>+</sup> T cell SIV DNA with high viral loads in this model suggests the latent reservoir of SIV may be more vulnerable to “purging” at the initiation of cART rather than after long-term cART. This has implications for clinical trials to cure HIV.

**P02.14**

**Delayed Onset of Graft Versus Host Disease in Immunodeficient HLA-DQ8 Transgenic Immune Deficient Mice Repopulated by Human PBMCs**

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**Background:** Hematopoietic humanization of mice is frequently used to study the human immune system and its reaction upon experimental intervention. Immune compromised NOD-Rag1<sup>-/-</sup> mice, additionally deficient for the common gamma chain of cytokine receptors (yc) (NOD-Rag1<sup>-/-</sup> yc<sup>-/-</sup> mice), lack B, T and NK cells and allow for efficient human peripheral mononuclear cell (PBMC) engraftment. Yet, a major experimental drawback for studies using these mice is the rapid onset of graft versus host disease (GvHD).

**Methods:** In order to elucidate the contribution of the xenogenic murine MHC class II in this context, we generated immune deficient mice expressing human MHC class II (HLA-DQ8) on a mouse class II deficient background (A<sup>B</sup>-/-). We studied repopulation and onset of GvHD in this mouse strain following transplantation of DQ8 haplotype matched human PBMCs.

**Results:** Surprisingly, the presence of HLA class II significantly promotes the repopulation rates in these mice and virtually all of the engrafted cells were CD3<sup>+</sup> T cells. Also, the presence of HLA class II did not advance B cell engraftment, such that humoral immune responses were undetectable. However, the overall survival of DQ8-expressing mice was significantly prolonged, compared to mice expressing mouse MHC class II molecules, and correlated with an increased time span until onset of GvHD.

**Conclusion:** Our data thus demonstrate that this new mouse strain is useful to study GvHD and the prolonged animal survival makes it superior for experimental intervention following PBMC engraftment.
P02.15

Persistently SIV-Seronegative Macaques Elicit Mucosal Immune Responses Following Serial Low-Dose SIV Mucosal Challenge

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Background: Multi-low dose mucosal SIV challenge of NHPs has become widely adopted to improve the physiological relevance of the model. This approach also improves our ability to observe efficacious SIV vaccines that may otherwise have been deemed non-protective following a single high dose parenteral challenge.

Methods: We immunized a cohort of cynomolgus macaques intratracheally with recombinant vectors expressing SIV Gag/Pol/Env and Nef/Tat/Rev immunogens or empty vector. One year post-vaccination, animals were challenged weekly with SIVmac239 intra-rectally following a dose escalation regimen. Peripheral blood was collected to determine systemic vaccine- or challenge-specific cellular and humoral responses. Rectal biopsies were collected mid-way through the challenge phase to evaluate mucosal vaccine- and challenge-specific T lymphocyte as measured by expression of IFN-γ, IL-2, TNFα and CD107a.

Results: Multiple sub-infectious challenges elicited detectable SIV specific cellular immune responses in T lymphocytes in rectal mucosa. Moreover, modest cellular responses were also detectable in blood. Cumulative CD8+ cytokine responses in rectal tissue correlated with apparent resistance to infection as measured by # of challenges, total dose and infecting dose. Individually, CD8+ TNFα responses showed the strongest correlation with this protective effect. Mucosal polyfunctional responses were detected in vaccinees and controls against SIV determinants not present in the vaccine construct. An increase in polyfunctional CD4+ responses correlated with higher doses and number of SIV challenges to infection, and a lack of these responses negatively influenced peak and set-point VLs. SIV-specific antibodies were detectable in serum of seronegative animals after 16 weeks of challenge.

Conclusion: We show that multiple low dose challenges with pathogenic SIV can induce cellular and humoral responses in the absence of detectable infection by real time PCR and SIV co-culture. Further studies may reveal how immune responses generated as a consequence of low dose challenges may affect disease course/ pathogenesis following SIV infection.

P02.16

HIV and SIV Vaccine Antigens Expressed by Live Attenuated Rubella Vectors Elicit Durable Immunity in Rhesus Macaques

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Background: Live attenuated viral strains are among the most successful vaccines. Unfortunately, for HIV, an attenuated strain could present safety concerns due to insertional mutagenesis and reversion to wild type.

Methods: We have used live attenuated rubella vaccine strain RA27/3 as a vector to express HIV MPER and SIV Gag antigens. The vaccine strain has demonstrated safety and immunogenicity in millions of children, and one dose protects for life against rubella. Rubella readily infects rhesus macaques. This was the first successful trial of rubella vectors in macaques, in combination with DNA vaccines in a prime and boost strategy.

Results: Rubella vectors have replicated to high titers in cell culture, however, replication in vivo, immunogenicity, and antibody durability were unknown. The rubella vectors replicated robustly in rhesus macaques, while expressing SIV and HIV antigens, and they were highly immunogenic. The anti-Gag antibody titers elicited by immunization were greater than or equal to those induced by natural SIV infection. The antibodies have persisted for over 9 months. Repeat immunization with rubella vectors boosted the titer of anti-rubella and anti-Gag antibodies, indicating the induction of memory B cells. In addition, rubella vectors elicited a strong antibody response to the HIV MPER insert. Priming with DNA vaccine and boosting with the vector resulted in increased levels of Gag specific CD8+ T cells.

Conclusion: Rubella vectors combine the growth, immunogenicity, and safety of rubella vaccine with the antigenicity of the inserts. By presenting these antigens in the context of an acute infection, at a high level and for a prolonged duration, these vectors may stimulate a stronger and more persistent immune response, including production of high affinity antibodies and maturation of memory B cells. Rhesus macaques will provide an ideal animal model for demonstrating immunogenicity of novel vectors and protection against SIV or SHIV challenge.
Creating a Novel Non-human Primate Model for Preclinical Vaccine Efficacy Studies Using a Synthetic Swarm of SIVmac239

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Background: Mucosal HIV-1 transmission typically results in establishment of systemic infection with one or a few variants, an important feature to recapitulate in developing authentic models of mucosal transmission of SIV in NHPs. However, infection with a single variant from a diverse viral isolate may increase variability between animals since distinct variants within the stock may differ functionally. To decrease variability between animals while retaining the ability to conveniently enumerate the number of transmitted/founder variants, we modified SIVmac239 to generate 10 unique clones that differ by 2-3 synonymous mutations (molecular-tags), thereby providing a biologically homogeneous virus population distinguishable by sequence analysis.

Methods: A molecularly-tagged virus stock was produced by pooled transfection of all 10 clones with or without subsequent short-term expansion in RhPBMC. Indian-origin rhesus macaques were challenged intrarectally, intravaginally, or intravenously. The proportion of each variant in each inoculum and in plasma from infected animals was determined by single genome amplification (n=5,346).

Results: All 10 variants were detected in nearly equal proportions for transfection and RhPBMC-expanded stocks. Intrarectal challenge with transfection-derived virus led to productive infection in 6/6 animals with a median of 2 variants. Following intravaginal challenge, this same stock infected 5/9 animals with a median of 4 variants (range 2-8). For the PBMC-expanded stock, 12/12 animals became infected within 11 repeated low-dose intrarectal challenges with a median of 1 variant per animal. Using this same stock intravenously, 5IU and 1IU each infected 3/3 animals following a single challenge with 2-5 and 1-2 variants, respectively, and 0.2IU infected 3/3 (1-2 variants) within three challenges.

Conclusion: Both transfection and PBMC-expanded stocks of molecularly-tagged SIVmac239 were transmissible and the number of variants conveniently discernible by sequence analysis. This model establishes methods to enumerate transmitted/founder variants while maintaining the consistency of using an infectious molecular clone, which may benefit pre-clinical vaccine development in NHP.

Combination of Therapeutic Vaccination and Chemotherapy Conferred Partial Control of SIV Infection in SIVmac239-Infected Rhesus Macaques

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Background: Antiretroviral chemotherapy could substantially reduce viremia in HIV-infected people or SIV-infected macaques. However, life-long usage of chemotherapeutic drugs possesses problems of economic burden, drug resistance, and other side effects. Therapeutic vaccination may provide an alternative approach for a "functional cure", defined as control of viral load without or with less drug administration.

Methods: In this study, we treated six SIVmac239-infected Chinese rhesus monkeys with reverse transcriptase inhibitor tenofovir/PMPA and emtricitabine/FTC followed by immunization with a previously reported AVIP (adenoviral vector infected PBMCs) vaccination (JV86:11031, 2012).

Results: The result showed that this strategy could delay viral rebound, partially suppress viral load by ~1 log in set-point and significantly improve the survival rate and quality of life. All macaques appears to be healthy and without symptoms of AIDS, while most untreated animals were dead due to AIDS by 3 years after infection. These macaques have the induction of SIV-specific immune responses with more IFN-γ+CD4+T cells, polyfunctional CD4+ and CD8+ T cells, and higher proliferation capacity of antigen specific CD4+ and CD8+ T cells.

Conclusion: Our results indicated that combination of antiretroviral chemotherapy with AVIP vaccination warrants further study to explore its potential for effective control against HIV/SIV infection.
**Amino Acid Mutations Around Protease Cleavage Sites of SIVmac239 Correlate with Lower Viral Load and Higher CD4+ Counts in Cynomolgus Macaques**

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**Background:** The protease of HIV-1 is a small 99-amino acid aspartic enzyme that mediates the cleavage of Gag, Gag-Pol and Nef precursor polyproteins. This process is highly specific, temporally regulated and essential for the production of infectious viral particles. A total of twelve proteolytic reactions are required to generate a viable virion, thus, a vaccine targeting the sequences around the 12 protease cleavage sites (PCS) of HIV-1 can be used to prevent infection and disease progression.

**Methods:** We have conducted a proof of concept study using Cynomolgus macaques and pathogenic SIVmac239 as a model. The macaques were immunized with recombinant VSVs (rVSV) expressing 12 20-amino acid peptides overlapping 12 PCS of SIVmac239 and boosted with Nanopackaged peptides. The macaques were intrarectally challenged with an increased dose of SIVmac239 (1000, 2000, 4000, 4000, 4000TCID50). Viral infection, viral load, CD4+ counts were conducted to evaluate the effect of the vaccine in protection from infection and disease progression.

**Results:** Antibody and T cell response to the PCS peptides correlated with protection against higher dose of SIVmac239 challenge (p=0.005,R2=0.42). The vaccinated macaques maintained higher CD4+ counts than the controls (p=0.0002). Viral sequence analysis detected extensive sequence variations around the PCS and correlation analysis showed amino acid mutations around the 12 PCS (-5/+5, -10/+10, and regions just outside -10/+10) correlated with lower viral load (p<0.0001).

**Conclusion:** Targeting sequences around the 12 PCS is an effective vaccine approach against HIV-1, a virus attacking a key component of immune system, CD4+ T cells. Because a single failed cleavage reaction is sufficient to generate noninfectious virions, targeting 12 PCS attacks HIV protease function from 12 different directions. Furthermore, the sequences around the PCS are relatively conserved; targeting 12 PCS can deal with viral diversity and take the advantage of viral mutation.
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**P02.21**

Characterization of Antibodies Targeting the Receptor and Co-receptor Binding Region of SIV Envelope

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**Background:** Non-human primate models of SIV infection are useful for studying vaccine mediated and immune correlates of protection. Some studies suggest antibody mediated correlates of protection against SIV but little is known about binding or neutralizing epitopes on SIV Env. Thus, we sought to study SIV infection of rhesus macaques to identify phenotypic and functional characteristics of protective SIV-specific antibody responses.

**Methods:** Trimeric SIVgp140 probes and J08-scaffolded V1V2 probes were used to isolate putative CD4-binding site (CD4bs)- and V1V2-specific B cells, respectively. Individual CD4bs- and V1V2-specific B cells were isolated by indexed cell sorting and matched immunoglobulin heavy and light chain genes from individual B cells were amplified by RT-PCR, cloned and expressed in vitro and purified monoclonal antibodies (mAbs) tested for SIV-specific binding and neutralization.

**Results:** We sorted 160 CD4bs- and 152 V1V2-specific B cells (0.02% and 0.09% of total CD20+ B cells) from SIV vaccinated and challenged rhesus macaques. Four of six mAbs cloned from putative CD4bs-specific B cells bound to monomeric and trimeric SIV gp140, were confirmed to be CD4bs-specific by competition ELISA and had potent but varying neutralizing activity against SIVmac and SIVsm Tier 1 and 2 isolates. We also screened 15 mAbs cloned from V1V2 probe-sorted B cells. One mAb did not bind monomeric or trimeric SIVgp140 while 10 mAbs bound only monomeric SIVgp140, 2 of which had detectable neutralizing activity against SIVsm Tier 1. The remaining 4 mAbs bound both monomeric and trimeric SIVgp140, had potent neutralizing activity against Tier 1 SIVmac251 and detectable neutralizing activity against Tier 1 SIVsm.

**Conclusion:** We have isolated and characterized SIV CD4bs-specific and V1V2-specific mAbs. Use of additional probes will facilitate identification and characterization of the range of protective SIV Env-specific epitopes which can be used to optimize HIV vaccine immunogens, adjuvants and/or vaccine regimens.

**P02.22**

Multiclade E-DNA Prime Enhances the Functional Antibody Response Induced by a Recombinant gp120 Boost in Non-human Primates

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**Background:** The partial success of the RV144 trial provided the critical benchmark for HIV vaccine design and testing showing for the first time a vaccine was capable of inducing antibody responses that correlated with risk of infection in humans. The study also suggests that a prime-boost strategy may be required for a successful vaccine.

We have focused on improving the antibody induction with our E-DNA vaccine approach and tested potency of a multi-clade prime-boost strategy in non-human primates.

**Methods:** Initial studies were performed in rabbits receiving multi-envelope DNA prime at weeks 0, 3, 6, and 9 followed by a boost with multi-valent gp120 at week 45. Groups of 5 Indian Rhesus macaques were vaccinated at weeks 0, 6, 12 and 18 with pVax gag/pol/env A, B, C, D, A/E. Monkeys were boosted at week 32 with SF162 gp120. An additional 4 monkeys received only the protein boost.

**Results:** In rabbits, the DNA prime induced potent cross clade binding titers after two immunizations. After protein boosting these levels increased over 100 fold. In monkeys, the multi-envelope (ME) DNA prime was capable of inducing potent cross clade cellular and humoral response. The ME DNA prime also generated tier 1 neutralizing titers great than 1:100. The recombinant protein boost enhanced these titers 50-100 fold while single dose protein alone induced lower binding titers and no neutralization activity. Importantly, antibody dependent cellular cytotoxicity (ADCC) was also only detected following the DNA prime/ protein boost.

**Conclusion:** Improvements in immune potency have been achieved using these E-DNA vaccines. These vaccine regimens have binding and neutralizing titers in non-human primates and serves as an effective prime platform for a recombinant protein boost, which further enhances the magnitude and functional profile of responses.
**P02.23**

**Profiling SIV Envelope Variants from Breakthrough Infections in a DNA/MVA Vaccination Trial**


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**Background:** A SIVmac239 based recombinant DNA prime/MVA boost vaccine with and without GM-CSF adjuvant was previously tested in rhesus macaques along with an MVA only regimen. After heterologous SIVsmE660 challenge, 71% protection was observed in the GM-CSF group, which was positively associated with antibody avidity. We hypothesized that comparing envelope (Env) glycoproteins from SIV variants that established infections in vaccinated macaques to those from unvaccinated macaques, and between immunization groups, could reveal signatures of vaccine induced immune pressure that favoured transmission of select SIV variants.

**Methods:** Post-challenge plasma samples were collected from infected macaques in each vaccine group at weeks 1 or 2 post infection. Viral RNA was reverse transcribed and single genome PCR was used to amplify the entire Env coding region. Multiple SGA Env sequences from 14 macaques were analysed by aligning amino acids, generating phylogenetic trees and using the Los Alamos HIV Database Highlighter program.

**Results:** Highlighter analysis of SIV Env sequences revealed that a single founder variant established infection in each macaque, regardless of the vaccination group. Phylogenetic analysis demonstrated that the Env sequences from the SIVsmE660 challenge stock and founder viruses from the macaques in the vaccine and control groups intermingled and exhibited less than 3% diversity overall. Amino acid alignments showed that the changes were focused in the gp120 V1V2 domain and gp41 cytoplasmic tail. However, no distinct genotypic signatures indicating vaccine-induced selective pressure emerged from these analyses.

**Conclusion:** The results indicate that a single variant established infection in each macaque; however, evidence for selective transmission of a particular SIVsmE660 Env variant based on vaccination induced immune pressure was not apparent from the genotypic analyses. Phenotypic characterization of founder EnvS could elucidate whether the DNA/MVA with GM-CSF regimen resulted in selective transmission of neutralization resistant viruses from the SIVsmE660 challenge quasispecies.

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**P02.24**

**Use of NOD/SCID/β2 Humanized Mice to Test a Novel Lentiviral-Based HIV-1 Vaccine**

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**Background:** Humanized immunodeficient mice have been used as small animal model to recapitulate HIV-1 infection and pathogenesis and to evaluate vaccine prototypes. We used hu-NOD/SCID/β2 mice to evaluate the immunogenicity of a novel CAL-SHIV-IN- lentivector HIV vaccine. This vector was derived from the genome of the highly pathogenic SHIV-KU2, lacking the integrase gene and driven by the constitutive LTRs of the naturally attenuated caprin arthritis encephalitis lentivirus (CAEV).

**Methods:** Plasmid DNA was transfected into HEK-293-T cells by lipofection and viral protein assembly was examined by electron microscopy. Virus in culture medium was assayed for infectivity in the human CD4+ T cell line M8166. BALB/c and hu-NOD/SCID/β2 mice were IM-injected by a single dose CAL-SHIV-IN- DNA. Mice were euthanized at week 2 and 4 post-immunization and plasma and Splenocytes were isolated. INF-γ ELISPOT and multi-parametric FACS assays were used to examine Ag-specific T cells producing cytokines and proliferating. ELISA was used to detect the presence of vaccine-induced antibodies.

**Results:** CAL-SHIV-IN- induced specific immune responses in both BALB/c and hu-NOD/SCID/β2 mice, with increased responses at week 2 post-immunization. Interestingly Ag-specific T cells and antibodies induced by the vector were significantly higher in humanized hu-NOD/SCID/β2 compared to BALB/c mice. These results provided the demonstration that the additional cycle of replication in human target cells has significantly improved the antigenicity of the novel vector.

**Conclusion:** These prove of concept in the hu-SCID mouse model provided the basis to instigate the immunogenicity of this lentivector in NHP model (see Arrode et al.). The new NSG and NOG SCID models that have been demonstrated to be more efficient should provide enhanced data.
A Combined Saavi MVA-C and Novartis Gp140 Env Protein Vaccine Boost Elicits Robust HIV Cellular and Humoral Responses in a Non-human Primate Model

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Background: We previously reported induction of cellular responses by SAAVI DNA-C/SAAVI MVA-C (DNA/MVA) prime/boost immunisation. These vaccines express matched gag, rt, nef, tat and env genes based on HIV-1 clade C. In view of the moderate protection reported in RV144 efficacy trial in which a combined poxvirus/Env protein regimen was used, we included a protein vaccine in the present study.

Methods: Seven rhesus macaques which had received a DNA/MVA prime/boost vaccine regimen >3 years previously were re-vaccinated with MVA followed by a boost with Novartis TV1 gp140ΔV2 protein in MF59 adjuvant (Env protein) and finally with a combined MVA/Env protein vaccination. A control group (n=3) was vaccinated with Env protein only. HIV responses were quantified in the blood at various time points and lymphoid tissues at experimental endpoint using an IFN-γ ELISPOT assay. Binding (bAb) and neutralising (nAb) antibodies were measured and Env-specific IgG-secreting cells were quantified in a B cell ELISPOT assay.

Results: Peak cumulative IFN-γ ELISPOT responses were achieved in the blood 1 week after re-vaccination with MVA. Robust IFN-γ ELISPOT responses were detected in inguinal, mesenteric, iliac and bronchial lymph nodes and spleen following combined MVA/Env protein vaccination. A single boost with protein in primed animals induced strong nAb responses against multiple tier 1 viruses and sporadic weak responses against multiple tier 2 viruses, whereas no nAbs were detected post prime. Moderate improvement in the magnitude of responses to tier 1 viruses and breadth against tier 2 viruses was seen after the combined MVA/Env protein boost in these animals. Similar responses were seen after 2 inoculations with protein only. Env-specific bAb and IgG-secreting cells were detected in the blood after MVA, Env protein and combined MVA/Env protein vaccinations.

Conclusion: These data demonstrate the potential of poxvirus-vector and protein candidate HIV vaccines in generating cellular and humoral immune responses when used in combination.

Priming with Adenovirus26 Vaccines Affects ALVAC-SIV/gp120 Vaccine Efficacy Tested in a SIVmac251 Challenge Model Performed One-Month Post-Vaccination

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Background: The 31.2% efficacy afforded by ALVAC-HIV/gp120 vaccines (RV144 Thai trial) needs to be improved. ALVAC-SIV/gp120 vaccines tested in a SIVmac251 macaque model, demonstrated a similar immunogenicity and 40% vaccine efficacy at each challenge. We used this model to test whether priming with DNA or adenovirus 26 could improve the immunogenicity and efficacy of ALVAC/gp120 vaccines.

Methods: Twenty-seven macaques were given four vaccinations with ALVAC expressing SIV genes. A second cohort of twenty-four macaques had the two initial ALVAC vaccinations replaced with either DNA or adenovirus 26 protein in alum. Four weeks post immunization, vaccinated macaques and 41 controls were given repeated low-dose intra rectal challenges with SIVmac251. The immunogenicity and efficacy of each regimen was evaluated.

Results: DNA-ALVAC/gp120, Ad26-ALVAC/gp120 and ALVAC/gp120 vaccine regimens induced equivalent gp120 binding antibodies that recognized the V1/V2 region. The Ad26 primed group demonstrated significantly greater neutralization of SIV tier 1 viruses and increased SIV specific IFNγ responses. When the rate of SIV acquisition of each vaccination regimen was compared to controls, priming with DNA or ALVAC resulted in an equivalent significant protection from SIV acquisition (51.2 and 40% efficacy respectively). Intriguingly, despite increased immunogenicity, the rate of SIV acquisition was not significantly different between the Ad26-ALVAC/SIV+gp120 regimen and controls.

Conclusion: Priming with DNA or ALVAC resulted in a significant reduction in the rate of SIV acquisition. Surprisingly, we observed a lack of protective efficacy when Ad26 was combined with ALVAC-SIV/gp120. This finding differs from other studies that demonstrated protective efficacy using Ad26-MVA regimens with challenges 3-9 months post vaccination. The time between vaccination and challenge may affect efficacy. Understanding the evolution of protective responses and the resulting virologic consequences in macaque models, could inform the design of human trials that use these vaccine modalities.
P02.27

**Newly Developed gp140 Trimeric Formulations Induce Inter-subtype HIV-1 Neutralization When Administered After DNA Prime Regimen to Cynomolgus Macaques**


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**Background:** New immunogens capable of inducing broadly neutralizing antibodies (NAbs) are a priority in HIV-1 vaccine research. The NGIN consortium developed a set of new gp140 immunogens of HIV-1 subtype A and B derived from the virus of infected individuals, who had developed broadly neutralizing antibodies (NAbs) during follow-up. The gp140 trimeric proteins inducing the best NAbs in immunized rabbits were forwarded for immunization of cynomolgus macaques (NHP).

**Methods:** Immunization was performed with a prime-boost regimen including a trivalent gp140 DNA delivered by electroporation followed by the gp140 trimeric protein boost given by intramuscular injection with the adjuvant CAF01. NGIN pursued three immunization strategies with the prime-boost being: 1) intra-subtype (B DNA – gp140), 2) inter-subtype (B DNA – A gp140), and 3) inter-virus group (HIV B DNA – SIV gp140). The third approach was introduced to test whether boosting with a heterologous virus would favour antibody responses to highly conserved regions.

**Results:** In all immunization strategies HIV-1 gp120 specific IgG but not IgA were detected by ELISA in the serum of NHP after the DNA immunizations and further enhanced after each protein boost. Low titer subtype specific NAbs were detected after DNA immunization. Protein boost enhanced intra-subtype NAbs to higher titers, and broadened the NAB reactivity to inter-subtype Tier 1 viruses. No vaginal mucosal NAbs were detected. Peptide microarray analysis showed inter-subtype antibodies to the gp41 immunodominant regions and env V3 loop in all vaccination strategies used, whereas antibodies directed to C2 and C5 were detected only in the HIV-1 immunization strategies (1 and 2).

**Conclusion:** In conclusion, the NGIN consortium has developed a new set of unique gp140 trimers. Although only neutralizing activity against Tier 1 viruses was induced, the elicited humoral immunity showed great breadth in reactivity across viruses from different subtypes. Work supported by EC-FP7-grant NGIN_201433.

P02.28

**Rhesus Full Length Single Chain (rhFLSC) Protects Rhesus Macaques from Heterologous Intrarectal Challenge with Multiple Low Doses of SHIV162P3**

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**Background:** We evaluated whether a rhesus variant of Full Length Single Chain (rhFLSC) could protect against a multiple low dose, heterologous, mucosal, SHIV challenge model and identify potential correlates to any protection observed.

**Methods:** Groups of 6 rhesus macaques were inoculated with either gp120(Bal) or rhFLSC formulated in either Iscom matrix, a saponin containing iscom, or RC529-SE, a stable emulsion of squalene, glycerol, phosphatidyl choline and a synthetic monophosphoryl lipid A, a TLK-4 agonist. Untreated naïve animals were included as controls. All animals were challenged rectally at weekly intervals with 50 TCID50 SHIV162P3 and monitored for infection. Cellular and humoral immunity was assessed throughout the experiment.

**Results:** Analysis of the immune responses vs the rate that the immunized animals became infected during the challenge phase generated several key observations. (1) Slower acquisition rates were observed in animals that presented titers to rhFLSC that were 2x higher than gp120 (rhFLSC/gp120 titer >2); competitive epitope titers to a CD4i MAb, N12-i2 >1:100; higher titers to transmitted founder clade C envelope derived from HIV-11086.C (AUC titers > 10,000). (2) Similar to the pattern observed in RV144 (4), protection in Rh048 was highest through the early challenge phase (first 4 challenges) after which all of the naïve controls became infected, and waned thereafter. (3) Increased acquisition rates and risk were associated with higher T cell responses as assessed by IL-2 and IFN-g ELISPOT. Polyfunctional FACS analysis of the envelope specific CD4+ and CD8+ responses revealed that the T cells responses were primarily single function but little to no evidence of T cells with 2 or more functions.

**Conclusion:** Based on these results, we conclude that immunization with rhFLSC can provide sterilizing protection but that an overabundance of single function T cells may provide targets for HIV infection that negate the efficacy provided by rhFLSC.
**P02.29**

**DNA Prime/Subunit Boost Using SIV (E660) Based rhFLSC Yields 75% Efficacy Against Cross Clade SIVmac251 Intrarectal Challenge**

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**Background:** The rhesus version of the FLSC protected macaques against repeat rectal challenges with SHIV162P3 but the level of efficacy was low. To improve efficacy, we evaluated a DNA prime with different genetic adjuvants including IL-12 followed by a subunit boost in the SIVmac251 challenge model.

**Methods:** Groups of 8 rhesus macaques were immunized with multiantigen pDNA expressing gag/pol(SIVsmE543) and rhFLSC(SIVsmE660) coadministered with plasmids expressing either LTA1, IL-12 or LTA1+IL-12. Control groups received no adjuvant plasmid or were left untreated. At week 42, vaccinated animals received rhFLSC (SIVsmCG7V) formulated in Al(OH)3. All animals were then rectally challenged weekly for 10 weeks starting at week 44 with a cross-clade SIVmac251 stock.

**Results:** DNA prime + IL-12 followed by a subunit boost generated 75% efficacy as compared to the same protocol without IL-12. Although antibody binding titers and neutralizing titers to Teir 1 SIVmac251 were >10(4) in all vaccinated groups, efficacy did not correlate with either. The regimen using IL-12+LTA1 generated superior CMI responses throughout the study; however, this group did not exhibit superior protection. While correlates of protection remain to be defined in this study, this level of efficacy is we observed with a DNA prime + IL-12 followed by a rhFLSC subunit boost is consistent with the best that was observed by others that used prime/boost regimens.

**Conclusion:** Using a protocol of a SIVsmE543 multiantigen DNA prime that included rhFLSC(SIVsmE660) adjuvanted with plL-12, followed by a boost with rhFLSC(SIVsmCG7V) subunit formulated in Al(OH)3, we were able to generated 75% efficacy as compared to the same protocol without IL-12 against a stringent cross-clade multiple low dose intrarectal challenge with SIVmac251.

**P02.30 LB**

**Vectored ImmunoProphylaxis with VRC07 Protects BLT Humanized Mice from Vaginal Transmission of Founder HIV**

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**Background:** Recently, a number of antibodies capable of broadly neutralizing HIV have been isolated from HIV infected patients, stimulating efforts to elicit their production in naive individuals. As an alternative to vaccination, we recently described vectored immunoprophylaxis (VIP) as an approach capable of generating high serum concentrations of a desired monoclonal antibody in mice following a single intramuscular injection of a specialized adeno associated viral vector (AAV). Mice that received VIP encoding b12, VRC01 or VRC07 antibodies demonstrated long-term circulating antibody expression in serum, and VIP-treated humanized mice exhibited remarkable protection against high dose, intravenous challenge with CXCR4-tropic HIV. However, most human infections are initiated by transmission of CCR5-tropic strains through mucosal tissues.

**Methods:** To measure the efficacy of VIP against clinically relevant strains, we humanized VIP-treated mice by adoptive transfer of peripheral blood mononuclear cells (PBMC) and challenged these animals with CCR5-tropic HIV strains including JR-CSF, as well as REJO.c, a transmitted molecular founder. To determine the ability of VIP to prevent mucosal transmission of clinically relevant HIV, we developed a repetitive intravaginal challenge model in VIP-treated BLT humanized mice that were challenged weekly with JR-CSF or REJO.c.

**Results:** PBMC humanized mice expressing b12 or VRC01 were protected from intravenous challenge with JR-CSF. In contrast, the b12-resistant REJO.c strain readily infected PBMC humanized mice expressing b12 antibody, while mice expressing VRC01 demonstrated nearly complete protection following challenge. Intravaginally challenged BLT animals expressing luciferase as a control all became infected over the study period while a majority of animals expressing VRC01 or VRC07 had no detectable HIV infection despite repeated intravaginal challenges with JR-CSF or REJO.c.

**Conclusion:** VIP is capable of protecting humanized mice from challenge by diverse HIV strains and can prevent vaginal transmission. These findings warrant continued development of VIP as a novel approach for HIV prevention.
**P02.31 LB**

**A Single Immunization with Integrase Defective Lentiviral Vector Expressing gp140 Induces Persistent and Functional Immune Response in Rhesus Monkeys**

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**Background:** Integrase defective lentiviral vector (IDLV) expressing antigens induces long-lasting and protective immune responses in mice after a single immunization. IDLV persists at the site of inoculum in the absence of integration, resulting in a prolonged antigen expression. The aim of this study was to demonstrate in monkeys that immunization with IDLV delivering the prototype clade C transmitted founder HIV-1 Env 1086.Cgp140 (IDLV-Env) induces sustained and functional anti-Env antibodies (Abs) and T cell responses.

**Methods:** Six rhesus monkeys were primed with IDLV-Env and boosted at 1 year after priming. An analysis of Ab response was assessed over time. Binding, epitope mapping, neutralizing activity and ADCC of anti-Env Abs were evaluated at sequential time points. IFNg ELISPOT was performed to evaluate persistence and presence of functional Env-specific T cells.

**Results:** A single immunization with IDLV-Env induced strong and prolonged immune responses. Anti-Env Abs peaked between 2 and 6 weeks and were still present at 1 year after the vaccination. All monkeys showed neutralizing Abs in serum samples starting from 6 weeks and peaking at 14 weeks after immunization. Three out of 6 monkeys showed ADCC in serum samples and 4 out of 6 had detectable anti-V1V2 Abs. High levels of IFNg producing T cells were elicited in all monkeys, decreasing over time, but still detectable up to 1 year after immunization. Abs and T cell responses showed a significant increase after the boost.

**Conclusion:** This is the first demonstration that an IDLV-based vaccine expressing HIV Envelope is able to induce functional, comprehensive and persistent immune responses in non-human primates. These results support the further evaluation of IDLV as a delivery system in the context of a HIV-1 vaccine.

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**P02.32 LB**

**Viral Evolution Following Infection with a Derivative of SHIV1157ipd3N4 Sensitive to V2-Dependent, QNE-Specific, Broadly Neutralizing Antibodies**

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**Background:** PG9 and PG16 are founding members of a class of broadly neutralizing antibodies (BNAbs) that target V2-dependent quaternary neutralization epitopes (QNEs) that includes the conserved N-linked glycan at position N160. CAP256 sera contain related highly potent BNAbs that target an overlapping epitope not dependent on N160. Commonly used SHIVs (SHIV-SF162P3, SHIV-1157ipd3N4, SHIV-AD8, and SHIV-BaL) poorly express these epitopes. Two changes in SHIV-1157ipd3N4 Env, Q170K and I192R, conferred sensitivity to QNE-specific NAbs. This mutant is infectious and pathogenic in pigtail macaques.

**Methods:** Tracking the course of infection with SHIV1157+QNE should allow the recapitulation of steps leading to the production of QNE-specific BNAbs. We monitored the development of antibody responses and evolution of Env sequences in infected macaques. SGA was used to amplify sequences; representative sequences were cloned for analysis of neutralization sensitivity.

**Results:** Autologous neutralizing antibodies were detected at week 16 post-infection (PI). At week 16 PI, 12/13 sequences contained mutations at position K143 that conferred resistance to sera from weeks 16 - 32 PI. These viruses were sensitive to week 35 sera, indicating the development of a new antibody response. At week 24, only ~half of the sequences had K143 mutations, and the remaining had V1 deletions and mutations elsewhere in Env, including the K169E mutation that in other Env contexts conferred sensitivity to QNE-specific NAbs. This mutant is infectious and pathogenic in pigtail macaques.

**Conclusion:** In this macaque, the initial antibody response targeted V1 and/or V2, and escape mutants were readily selected. Neutralization of the escape mutants by week 35 sera indicates evolution of a new antibody response that broadens neutralizing activity. This suggests that this SHIV should be useful for studies of the evolution of V2-dependent, QNE-specific BNAbs in macaques. This SHIV should also prove useful as a challenge stock for vaccine trials attempting to induce these antibodies.
Acute SIV Infection Induces Hypercytotoxicity and Massive Numerical Loss of Innate Lymphoid Cells in the Gastrointestinal Tract

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Background: Studies from our lab and others have identified a novel NK cell-like population restricted to mucosal tissues and distinguishable from classical NK cells by high expression of NKP44 and RORγt. So-called innate lymphoid cells (ILCs) play a key role in mucosal defense and homeostasis, but are depleted from colorectum during chronic SIV infection. However, nothing is known about the kinetics of ILC loss, or if it occurs systemically.

Methods: This planned sacrifice study included a total of twenty-one rhesus macaques — six naïve; six infected with SIVmac239 sacrificed at 14 days post-infection; and 9 infected with SIVmac239 sacrificed in chronic stage disease. Tissue from colon, jejunum and mucosae-draining lymph nodes were collected from all animals. ILCs and classical NK cells were enumerated and analyzed phenotypically by surface and intracellular flow cytometry, as well as immunohistochemistry, and mononuclear functions were quantified by intracellular cytokine staining.

Results: During acute SIV infection a massive up to 8-fold loss of Nkp44+ ILCs was observed in all mucosal tissues, but classical NK cells were maintained in number. Both ILCs and NK cells exhibited significantly increased levels of apoptosis, but while classical NK cells also showed increased proliferation, ILCs did not. Interestingly, ILCs, which are normally noncytolytic, dramatically upregulated cytotoxic functions in acute and chronic infection and acquired a polynuclear phenotype secreting IFN-γ, MIP1-β, and TNF-α, but decreased production of the prototypical cytokine, IL-17. Classical NK cells had a less dramatic change in function, but exhibited upregulated perforin expression and increased cytotoxicity.

Conclusion: Herein we demonstrate the first evidence for acute, systemic, and permanent loss of mucosal ILCs during SIV infection. The massive reduction appears to involve apoptosis without compensatory de novo development/proliferation, but the full mechanism of depletion and the impact of functional change so early in infection are unclear.

Optimized Multidrug-Resistant HIV-1 Reverse Transcriptase Induces Strong Immune Responses in DNA-Immunized Mice

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Background: Development of multigene DNA-vaccines against HIV faces a problem of low immunogenic performance of enzymes genes, in particularly reverse transcriptase (RT). Under the pressure of antiretroviral drugs, RT accumulates mutations. Mutated protein escapes the effect of drugs, but at the same time becomes more immunogenic. Basing on this, we designed a new DNA-immunogen encoding RT with multiple drug-resistance mutations, and tested its performance in preclinical trials.

Methods: RT gene cloned earlier from HIV-patient with multiple drug resistance mutations (RT1.14) was expression-optimized and mutated further to deprive RT of the enzymatic activity generating RT1.14opt-in. Genes were expressed in HeLa cells, expression level and proteolytic stability was assessed by immunoblotting. BALB/C mice (n=4 per group, in two repeats) were injected intradermally or intramuscularly with plasmids. Injections were followed by electroporation (DermaVax). Immune responses were assessed by IFN-g/IL-2 Fluorospot (Mabtech) and indirect ELISA. Gene delivery and dynamics of expression was followed by in vivo imaging using a co-delivered gene of the reporter protein luciferase.

Results: Gene optimization led to a 2-fold increase in protein expression. Half-life of active RT1.14 in HeLa cells was 2-3h, of RT1.14opt, 6-8h and of RT1.14opt-in, 2-3h. RT1.14opt-in gene induced significant IFN-g, IL-2 and dual IFN-g/IL-2 responses; RT1.14opt gene, a significant IL-2 response; and viral RT1.14, a weak IFN-g response. Only optimized genes induced anti-RT IgG of G1, G2b subclasses in titter 80000, and RTopt-in gene, also IgG2a in titter 30000 indicating a mixed Th1/Th2 type of immune response. In vivo imaging demonstrated that the immune response induced by RT1.14opt and RT1.14opt-in genes cleared the expressing cells from the injection sites.

Conclusion: Thus, the immune responses induced by novel genes significantly exceeded cellular responses to any previously tested RT gene variant. In conclusion, codon-optimized gene of multidrug-resistant RT represents a good candidate for inclusion into a therapeutic multi-gene HIV vaccine.
Rapid Appearance of Vaccine-Induced Potentially Protective Anti-Env Antibody Responses with Early Co-administration of gp120 with NYVAC or DNA

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Background: The RV144 study demonstrated that anti-Env antibody responses (including V1V2 IgG) were scarcely detected before gp120/AIDSVAX administration, e.g. during the first 3 months and waned rapidly by month 12, e.g. 6 months after the last ALVAC/AIDSVAX boost. These results suggest that early appearance and longer persistence of V1V2 IgG antibodies may be associated with a better vaccine protection.

Methods: The newly generated DNA-C and NYVAC-C were used in different prime/boost combinations with bivalent clade C gp120 (TV1 and 1086) proteins formulated in MF59 at week 0, 4, 12, 24 and 48. DNA and NYVAC were used either as a prime alone at week 0 and 4 or co-administered with the bivalent gp120 at all time points. One arm of the study evaluated DNA+bivalent gp120 at all time points. Each study group consisted of 8 mature rhesus macaques. Vaccine-induced T-cell responses were assessed by binding antibody multiplex assays (including V1/ V2 IgG, peptide microarray, neutralisation assays, and ADCC assays. Humoral responses were assessed by binding antibody multiplex assays (including V1/ V2 IgG, peptide microarray, neutralisation assays, and ADCC assays. Antigen-specific cellular immune responses were assessed by ICS and IFNg ELISpot. Humoral responses were assessed by binding antibody multiplex assays (including V1/ V2 IgG, peptide microarray, neutralisation assays, and ADCC assays.

Results: Administration of the bivalent gp120 protein in combination with DNA or NYVAC at week 0 and 4 was consistently associated with high levels of binding IgG anti-Env including V1V2 antibodies by week 6 (2 weeks after the 2nd protein administration) as compared to the study Groups receiving NYVAC or DNA alone as a prime. In these latter Groups, antibody responses were only detected at week 14 (2 weeks after the 1st protein administration in combination with NYVAC). The influence of early bivalent gp120 administration on the long-term persistence of binding IgG anti-Env and neutralizing antibodies is currently being assessed and will be presented at the conference.

Conclusion: Early bivalent gp120 protein co-administration with NYVAC or DNA was associated with rapid appearance (week 6) of vaccine-induced potentially protective antibody responses.

Intradermal DNA Immunization with HIV-1 Protease Followed by Electroporation Improves Immunogen Delivery and Potency in Mice

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Background: DNA-vaccines can be effective against many infectious diseases due to their safety, stability, and ease of manufacture. However, they still lack in immunogenic performance, especially compared to live attenuated viruses, recombinant proteins, or viral vectors. A considerable improvement in DNA-vaccine immunogenicity was achieved with the development of immunization technique, specifically application of in vivo electroporation. In this study we evaluated the capacity of a plasmid encoding inactivated HIV-1 protease (PRin) (Hallengärd,2011) to direct antigen expression and induce immune responses in BALB/c mice.

Methods: PR DNA and a reporter luciferase gene were delivered by intradermal (ID) or intramuscular (IM) injections followed by electroporation (DermaVax). Expression kinetics was monitored by in vivo quantification of reporter luminescence (Caliper). Antigen-specific cellular immune responses were assessed as in vitro secretion of IFNg- and IL-2 by murine splenocytes stimulated with PR, peptides representing Immunodominant Immunodominant CD4+ and CTL epitopes of PR and control antigens (Fluorospot, Mabtech). Antibody response to PR (NIBSC) was assessed by ELISA.

Results: Compared to IM, ID delivery resulted in a 2-fold higher initial reporter expression which peaked earlier (day 1 compared to 4 for IM) and demonstrated significantly lower endpoint levels. PRin gene injected ID induced 3 times greater number of IFNg- and IL-2-secreting splenocytes recognizing CD4+ and CTL epitopes of PR than PRin gene injected IM. Immunization route had no effect on the epitope hierarchy: in both groups IFNg response to CTL was stronger than to CD4, while magnitude of IL-2 and IFNg/IL-2 responses to CD4+ and CTL epitopes were the same (Wilcoxon test). Anti-PR antibody responses triggered via both routes were low.

Conclusion: These data show that the correct immunogen delivery can strongly improve the magnitude of immune response induced by DNA vaccines. With further improvements, the ability of DNA-vaccines to induce an immune response would reach the level required for human applications.
Immunization of Humanized BLT Mice Against Gag Alters Early HIV-Specific CD8+ T Cell Responses and Transiently Suppresses Viremia

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Background: Humanized BLT mice appear to accurately reflect HIV-specific CD8+ T cell responses observed during natural infection (Dudek et al, Science Translational Medicine 2012). However, little is known regarding the potential of this model to elicit vaccine-induced immunity.

Methods: Four sets of BLT mice reconstituted from distinct human donors were immunized with a PLGA microparticle prime and a replication-defective HSV vector boost. Mice were then challenged intravaginally with a moderate dose of HIV (Groups 1 and 2) or intraperitoneally with a high dose (Groups 3 and 4) and bled every two weeks to measure cellular immune responses, viral loads and sequence evolution.

Results: Following intravaginal challenge, 3/6 mice from Group 1 successfully suppressed viremia for up to 6 weeks with a mean difference in viral loads of 1.7 log₁₀ (p ≤ 0.03) during this period. Post-challenge Gag-vaccinated mice exhibited a more rapid and stronger Gag-specific T cell response compared to mock-immunized mice. Alternatively, in Group 2, expressing a distinct HLA haplotype, only 1/9 mice delayed viremia for up to 4 weeks and had an overall 0.4 log₁₀ mean difference at week 6 post-challenge (p = 0.03). With the more demanding high-dose intraperitoneal challenge, a transient 0.5 log₁₀ difference in the mean viral loads was observed at week 6 post-challenge (p < 0.04; Groups 3 and 4).

Exploring a mechanism for the immune-mediated control of HIV in Group 1, deep sequencing revealed increased rates of immune selection pressure in p17 by 6 weeks post-infection in vaccinated mice (3/6 Gag-vaccinated vs 1/4 mock-immunized), supportive of enhanced Gag-specific immunity. Importantly, Gag-vaccinated mice that exhibited control showed delayed viral escape in this epitope, suggestive of preservation of this response.

Conclusion: These results suggest that humanized BLT mice support vaccination studies aimed at elucidating the importance of altering CD8+ T cell immunodominance hierarchies against various HIV targets.
**P03.01 D**

**Heterogeneity of Anti-V2 ADCC Ab Responses and Implications for Vaccine Development**


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**Background:** Anti-V1/V2 HIV-1 envelope (Env) binding antibodies (Abs) correlated with decreased risk of HIV-1 infection in the RV144 clinical trial. We isolated four anti-V2 monoclonal Abs (mAbs) from vaccine recipients and characterized their ability to recognize HIV-1 Env on the surface of infected cells and to mediate ADCC. We also determined whether anti-V2 Abs synergize with vaccine-induced anti-C1 conformational Abs to bind HIV-1 infected cells and mediate ADCC.

**Methods:** HIV-1 Env-specific mAbs were isolated from memory B cells of vaccine recipients enrolled in the RV135 and RV144 trials. CD4+ target cells infected with clade AE (CM235), clade B (Bal), and clade C (1086.C) infectious molecular clones were used to evaluate the breadth of mAb binding. The ability of the anti-V2 mAbs to synergize with vaccine-induced anti-C1 antibodies for the recognition of infected cells and ADCC was evaluated on clade AE CM235-infected target cells.

**Results:** All four RV144 vaccine-induced V2 IgG mAbs displayed unique profiles for binding HIV-1 infected cells and mediating ADCC. Env binding characteristics of CH58 mAb most closely recapitulate those of Ab responses correlated with lower risk of infection. Interestingly, some combinations of anti-V2 mAbs (i.e. CH58, HG107) and anti-C1 mAbs displayed functional synergy. Functional synergy is defined as enhanced binding of anti-V2 mAbs to infected cells, higher peak ADCC activity, and improved ADCC endpoint titers to levels similar to those detectable in the plasma of RV144 vaccine recipients.

**Conclusion:** RV144 vaccine-induced anti-V2 mAbs are of diverse fine specificities and recognize different conformational structures of Env present on the surface of infected cells. This specificity impacts the ability of these mAbs to mediate ADCC, and to act in synergy with vaccine-induced anti-C1 mAbs. Understanding how to induce mAbs with fine specificities capable of functional synergy will inform future vaccine design. (NIH-U19 AI067854-07, NIH AI073922).

**P03.02 D**

**Modalities of Broad and Potent Antibody Neutralization at the CD4-Binding Site on HIV-1 gp120**

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**Background:** The functionally conserved site of CD4-attachment on HIV-1 gp120 is one of the major sites of vulnerability to neutralizing antibodies. Broad and potent human antibodies that target this site, represented by antibody VRC01, have been found in a half a dozen donors. VRC01-class antibodies utilize a common V-gene origin VH1-2’02 and achieve broad neutralization by partially mimicking CD4. Recently, antibodies with non-IGVH1-2’02 origin and relatively long CDRH3, some capable of neutralizing as effectively as VRC01, were isolated from 4 donors. Elucidation of the mechanisms used by different antibodies to achieve effective neutralization is of great importance to vaccine design.

**Methods:** To define the modalities of antibody recognition of the CD4-binding site (CD4BS), we crystallized VRC01-class antibodies from 6 donors and non-VRC01 class antibodies from 4 donors in complex with HIV-1 gp120, and analyzed the structures for common and special features in HIV-1 recognition.

**Results:** Structures of VRC01-class antibodies revealed a convergence in viral recognition with Vgene-encoded CDRH2 and framework H3 mimicking the action of CD4 CC’ ridge, despite variations in antibody sequences and donor origins. In contrast, structures of non-IGVH1-2’02 antibodies showed canonical V(D)J-encoded CDRH3 recognition at the CD4BS without CD4 mimicry. Comparison of the Vgene-encoded and V(D)J-encoded modalities of gp120 recognition, however, indicated that these antibodies recognize a common set of residues that forms a ‘super site’ of recognition at the CD4BS. Despite differences in the Vgene-encoded and V(D)J-encoded modalities, the orientations of CD4BS antibodies relative to the viral spike appear to be confined within 45 degree of freedom indicating restricted antibody accessibility to the CD4BS on viral spike.

**Conclusion:** Broad and potent neutralization of HIV-1 can be achieved through multiple modalities of recognition at the CD4-binding site. However, the accessibility to this site on viral spike may be restricted to certain range of approach for antibodies to achieve effective neutralization.
**P03.03 D**

**Vaccine-Elicited CD4 Binding Site-Directed Antibodies Utilizing the Macaque Equivalent of the Human VH1-2 Heavy Chain Variable Gene Segment**

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**Background:** Potent broadly neutralizing antibodies (bNAb)s isolated from HIV-1 infected individuals have yielded information about their interaction with conserved determinants, such as the CD4 binding site (CD4bs), on the envelope glycoproteins. One common feature of many CD4bs-directed bNAbS is their use of the same heavy chain variable gene segment, VH1-2, suggesting that activation of B cells expressing this V-segment is important for the elicitation of CD4bs-directed bNAbS. Therefore, we asked if the macaque homologue of VH1-2 is used by CD4bs-directed Abs elicited by subunit Env vaccination.

**Methods:** Using a well-defined Env immunogen, we previously reported detailed analyses of HIV-1 Env vaccine-elicited antibody responses in rhesus macaques. Here, we identified the macaque equivalent to human VH1-2. We then isolated CD4bs-specific antibodies utilizing this V-segment by single-cell sorting followed by PCR of matched heavy and light chain transcripts. Isolated monoclonal antibodies (MAbs) were evaluated for Env binding and neutralization activity. Epitope mapping was performed for the most potent MAb, GE356, and the Ab paratope was characterized by alanine scanning of the CDRs of the heavy and light chains and directly compared to VRC01.

**Results:** Of 154 heavy chain sequences initially screened we produced 4 functional clonally unrelated CD4bs-directed MAbs utilizing the macaque equivalent of human VH1-2. All MAbs were capable of neutralizing Tier 1 viruses and one MAb, GE356, displayed improved neutralization compared to previously isolated vaccine-elicited CD4bs-directed Abs of diverse VH usage. Alanine scanning of GE356 showed that amino acid residues in the CDR3 and to a lesser extent CDR1 were critical for neutralization, demonstrating its differential mode of interaction with HIV-1 Env compared to VRC01.

**Conclusion:** Using single-cell sorting of macaque memory B cells, we isolated CD4bs-directed MAbs utilizing the macaque equivalent of the human VH1-2 representing the first demonstration that B cells expressing this V-segment are activated by Env immunization in primates.

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**P03.04**

**Enhanced DNA Vaccine with Protein Boost Delivered by EP Expands B- and T cell Responses and Neutralizing Phenotype In Vivo**

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**Background:** An effective HIV vaccine will most likely require the induction of strong T cell responses, broadly neutralizing antibodies (NAbs), and the elicitation of antibody-dependent cellular cytotoxicity (ADCC). Previously, we demonstrated the induction of strong HIV/SIV cellular immune responses in macaques using consensus DNA immunogens delivered via electroporation (EP). While these results demonstrated the increased magnitude and breadth of cellular responses using DNA immunogens, DNA’s ability to induce high antibody titers and neutralization has remained limited.

**Methods:** Here, we investigate the immunogenicity of consensus Envelope DNA constructs encoding gp140 sequences from HIV-1 subtypes A, B, and C in a DNA prime-protein boost vaccine regimen. Guinea pigs were primed with single- and multi-clade DNA via EP and boosted with recombinant gp120 protein. Sera were then analyzed for gp120 binding and neutralizing antibody activity.

**Results:** Immunization with recombinant Env protein alone induced low-titer antibodies while a prime-protein boost protocol was able to induce significantly higher antibody titers. Sera from prime-protein boost groups were also able to neutralize a high percentage of viruses against a panel of tier 1 clade B viruses as well as multiple tier 1 clade A and clade C viruses.

**Conclusion:** This study suggests that optimized DNA constructs and improved delivery modalities may be able to induce the B cell activation and antibody production that has eluded DNA vaccines in the past. Further investigation of the impact of this approach on immune breadth is underway.
**P03.05**

**Systematic Profiling of Polyclonal HIV Antibodies and Prediction of Effector Functions**

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**Background:** Antibody-dependent effector functions (ADEF) with the ability to recruit the innate immune response may play an important role in combating the spread of HIV infections. ADEF are mediated by the antibody Fc and depend on antibody class and glycosylation status. We describe the systematic profiling of the Fc-regions of HIV-specific antibodies isolated from different patient sera.

**Methods:** Using a LuminexTM-based suspension array, HIV antibody fractions binding to various HIV antigens are captured on beads and probed for binding to (1) antibody class-specific binding reagents, (2) Fc receptors, (3) complement proteins and (4) different lectins. The obtained binding profiles are correlated with other measures of effector function and may help to understand which ADEF are crucial to provide protection.

**Results:** We demonstrate comparable results to many current gold standard measurements including ELISA and Biacore using monoclonal Abs and polyclonal sera from patients. In addition, we have profiled a large cohort of HIV+ patients and some vaccinees to learn how the overall antibody response differs based on patient class or vaccine.

**Conclusion:** In the future we wish to further elucidate which ADEF are important in driving protective clinical responses. Such knowledge would be important for our understanding of the overall humoral response to HIV infection. On a more practical note, we would also be able to screen for such correlates in future vaccine trials using our robust and high throughput system.

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**P03.06**

**Improved Definition of Memory B Cells by the CD45RB Marker Allows the Identification of New Altered B Cell Subsets in HIV-1 Infected Patients**

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**Background:** HIV-1 infected individuals show an impaired B cell memory compartment. CD27 is widely used as surrogate marker for the identification of memory B cells in human. However, a CD27-memory B cell population, which is expanded in HIV-1 infected individuals, has been identified in humans. Recently, CD45RB has been suggested as a new marker for human memory B cells. Thus, we have explored whether use of both CD27 and CD45RB may be useful to study memory B cells in HIV-1 infection.

**Methods:** Viremic untreated HIV-1 patients (n=12), aviremic HIV-1-infected individuals under HAART (n=18) and uninfected healthy control (10) were analyzed. Memory B cell compartment was defined using anti-CD19, anti-IgD, anti-CD27 and anti-CD45RB antibodies.

**Results:** HIV-1 infected patients showed reduced CD27+CD45RB+ B cells, in both the IgD+ (marginal zone-like B cells) and IgD- (switched B cells) subsets. Interestingly, when the switched B cell subset was analyzed, the percentage of CD27+CD45RB- was higher in untreated HIV-1 infected individuals than in healthy controls (p< 0.05) and its normalization by HAART correlated with the extent of CD4 T cell recovery. Despite this, patients that recover CD4 T cells still harbored increased frequencies of CD27-CD45RB+IgD- B cells compared with uninfected controls (p<0.05).

**Conclusion:** The combination of CD27 and CD45RB allows a better identification of the memory B cell compartment. Whereas the CD27+CD45RB+ memory B cell compartment is reduced in HIV-1 infected patients, the CD27+CD45RB-IgD- is increased in untreated infected individuals and remains overrepresented in patient with a poor immunological responses to HAART, suggesting that the activation of the immune system may play a major role in the expansion of this B cell population.
**P03.07**

3D Map of Positional Variability in V1V2

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**Background:** A molecular correlate of risk for HIV-1 infection is associated with the V1V2 domain of gp120, suggesting that an epitope targeted by protective antibodies (Abs) is located in this region. In our study, we combined the most recent structural and epidemiological data to map the potential sites of broadly neutralizing antibody epitopes in V1V2.

**Methods:** First, each position of V1V2 was scored according to its amino acid variability as previously described by Almond et al. (V1V2 sequence information was obtained from the LANL HIV Database). Specifically, we calculated the variability score as \(1 - \frac{p}{n}\) (p is the number of occurrences of the most common amino acid at a given position; n is the total number of V1V2 sequences), and normalized it in a way that the most variable position in the loop had the score of 100%. Then, the resulting positional variability score was mapped onto the 3D structures of V1V2 bound to anti-V1V2 Abs PG9, PG16, CH58, and CH59.

**Results:** Our map of positional variability demonstrates that the most variable positions of V1V2 are clustered to three linear regions: one of which is located in V1 (positions 133-152) and two in V2 (169-172; 185-190). For example, the Abs CH58 and CH59 target one of these highly variable sites that accounts for their relatively narrow cross-reactivity. Nevertheless, the majority of the remaining, less variable positions in V1V2 are not likely to be accessible by Abs as they are masked by the glycans and/or the V1V2 fold, leaving only three relatively conserved prospective epitopes exposed: one linear epitope located on the tip of the V2 loop (162-168) and two discontinuous epitopes located in proximity to the integrin binding site.

**Conclusion:** Our 3D map suggests three conserved unmasked sites on V1V2, which could be utilized as leads for the detection of protective anti-V1V2 Abs.

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**P03.08**

Microplate-Formatted Mammalian Protein Expression for Dissection and Improvement of Antibodies and Immunogens

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**Background:** Mammalian protein expression is a critical step in the functional assessment of antibodies and immunogens. Recent technologies have enhanced the identification of antibody variants (e.g. from 454-based sequencing of B cell transcripts) as well as the design of immunogens. When hundreds of antibody variants or thousands of immunogen candidates need to be expressed and characterized, a bottleneck arises with current conventional expression and purification methods, which are often an inefficient, time-consuming and resource-intensive process.

**Methods:** Presented here is a 96-well microplate-formatted method of transfection, expression, and screening. The method has been optimized for secretion from mammalian cells, to improve the efficiency of a high-throughput protein expression, and to facilitate functional dissection of broad neutralization antibodies and immunogens.

**Results:** Compared to standard protein expression (e.g. from individual 100-ml expression), the reduction to 0.1-ml expression represents a 1000-fold reduction in volume. Despite this reduction, the expression of secreted antibodies such as VRC01 is generally higher. Throughput, however, is dramatically enhanced, with a single plate able to produce 96 different immunogens, at levels of expression for secreted immunogens which are suitable for direct assessment of function by ELISA.

**Conclusion:** The 96-well expression platform presented here offers ease of use and efficiencies in time and cost effectiveness, while maintaining – and even in many instances improving – relative higher quality and yield. The method provides a potential tool for high throughput characterization of antibody variants and secreted immunogens, which should help to facilitate discovery and development of HIV-1 neutralizing antibodies and immunogens.
**P03.09**

Infection Enhancement in Sieve Analysis of AIDSVAX Clinical Trials

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**Background:** Elicitation of a protective antibody (Ab) immune response by vaccination could prevent infection with HIV-1. However, some vaccines increase susceptibility to infection by the target pathogen. Despite the fact that the antibody-dependent enhancement (ADE) of HIV-1 infection in vitro was first described in 1980s, no indication of vaccine-induced enhancement was observed in clinical trials until recently, when the increased HIV-1 acquisition risk was detected in selected subgroups of vaccinees in the STEP study. More recently, a case-control analysis of RV144 revealed that binding of IgA to Env was directly correlated with infection risk.

**Methods:** We conducted a sieve analysis for ADE effects in the AIDSVAX clinical trials. The distributions of epitopes targeted by several anti-V3 monoclonal Abs (mAbs) among the breakthrough HIV-1 sequences were profiled, and the epitope-specific infection counts in vaccinated and placebo cohorts were compared. Distributions of the same epitopes in the breakthrough sequences from the STEP study were studied as a negative control.

**Results:** Using two epitope-prediction methods, Signature Motif Method and Method of Dynamic Epitopes, we detected a significant difference in VAX004 ‘vaccine’ cohort in the occurrence of overlapping epitopes targeted by mAbs 447-52D, 537-10D, and 268-D. For all three mAbs, the number of vaccinees infected with viruses bearing a cognate epitope was significantly greater than the corresponding number in placebo recipients, suggesting possible ADE effects. No significant difference was detected in STEP study.

**Conclusion:** From a purely molecular epidemiological point of view, certain mAb-defined V3 epitopes may have been associated with ADE in the VAX004 trial. Notably, in vitro enhancement of HIV-1 infection by mAb 268-D has previously been reported. The epitopes targeted by 447-52D and 537-10D are molecular subsets of the 268-D epitope. Our results raise the hypothesis that vaccine-elicited Abs similar in specificity to 268-D may have enhanced HIV-infection in subjects in the VAX004 clinical trial.

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**P03.10**

Characterization of Highly Neutralizing Antibody Sensitive HIV-1 gp120 Induced Under High Concentrations of Maraviroc (MVC) In Vitro

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**Background:** The small-molecule CCR5 antagonist maraviroc (MVC) is the first CCR5 inhibitor licensed for clinical use. Recently, we selected HIV-1 variants resistant to MVC in vitro, and found that the resistant variants became highly sensitive to anti-Env neutralizing monoclonal antibodies (nMAbs). In this study, to determine the key mutations for the accessibility of nMAbs to the epitopes in Env, we constructed the MVC-resistant clones and investigated the susceptibility to anti-Env nMAbs and autologous plasma IgGs.

**Methods:** We exposed high CCR5-expressing PM1/CCR5 cells to a clinical isolate (HIV-1KP-5) with MVC for induction of MVC-bound CCR5-adapted variants at 48 passages. We also passaged HIV-1KP-5 in low CCR5-expressing parental PM1 cells for low CCR5-adapted variant. We constructed three infectious clones with each 48-passaged Env.

**Results:** The MVC-bound CCR5-adapted variants acquired four mutations, T297I (V3), M434I (C4), V200I (C2), and K305R (V3) in the gp120. The MVC-bound CCR5-adapted clones containing the four mutations and low CCR5-adapted clones showed higher sensitivity to MAbs b12 (anti-CD4bs) and 4E9C (anti-CD4i) than the passage control clone. Interestingly, the clone with the MVC-selected Env was highly sensitive to anti-V3 MAb KD-247, while the low CCR5-adapted and the passage control clones were not. We also examined whether the infectious clones were neutralized by autologous plasma IgGs. The plasma IgGs were able to completely neutralize the clone with the MVC-selected Env (IC50: 2.6–37 µg/ml), but not the low CCR5-adapted and the passage control clones.

**Conclusion:** These findings indicated that such MVC-bound CCR5-adapted mutations might contribute to the expression of neutralizing epitopes for not only CD4bs and CD4i, but also the anti-V3 MAb. The data also suggest that the four mutations modulate exposure of V3 epitope. Further elucidation of this mechanism using a number of mutant clones with single mutation is underway.

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**Topic 03: B Cell Immunology and Antibody Functions**

Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L1, Room 111 – 112. Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L1, Room 111 – 112.
P03.11

Elicitation of Broadly Neutralizing HIV-1 Antibodies by Guiding the Immune Responses Using Primary and Secondary Immunogens

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Background: Broadly neutralizing HIV-1 monoclonal antibodies (bnmAbs) are potential key components of protective immunity. Such antibodies have not been induced by any vaccine immunogens so far. HIV-1 envelope proteins lack detectable binding to putative germline form of bnmAbs, which may partly explain the poor performance of envelop-based vaccine. We hypothesized that B cell-lineage immunogens targeting the germline B cell receptors of bnmAbs may help prime the immune system and guide the immune response.

Methods: Using b12 as a model antibody, we screened various cDNA libraries against b12 germline and intermediate antibodies. Isolated (poly) peptides were either synthesized or expressed as human Fc fusion proteins. Rabbit immunization was carried out by priming with the (poly) peptides and boosting with SF162 gp140 trimer. Immunization with SF162 trimer alone was used as a control. Rabbit IgGs were tested by competition ELISA with mature IgG1 b12, and by TZM-bl neutralization assay.

Results: Prime with b12 germline antibody specific peptides or peptide-Fc fusions followed by boost with envelop trimer induced rabbit antibodies that competed with mature IgG1 b12, while immunization with SF162 envelop trimer alone did not induce b12-like antibodies. The estimated EC50s were 675nM and 133.33nM for peptide and fusion proteins, respectively. Rabbit immunization was carried out by priming with the (poly) peptides and boosting with SF162 gp140 trimer. Immunization with SF162 trimer alone was used as a control. Rabbit IgGs were tested by competition ELISA with mature IgG1 b12, and by TZM-bl neutralization assay. Preliminary result indicated that immune rabbit IgGs did not neutralize four tier 2 HIV-1 isolates.

Conclusion: Our results indicate that “two immunogens” (primary and secondary) strategy can direct the immune response and elicit antibodies that mimic the target bnmAb(s) in binding, but eliciting antibodies that mimic the target bnmAb(s) in neutralization requires further investigation. Boost with HIV-1 envelop trimer may distract the targeted immune responses. Our study suggests that non-HIV-derived (poly) peptides may be used as primary immunogens to guide HIV-1-specific immune responses.

P03.12

Evidence for a Continuous Drift of the HIV-1 Species Towards Higher Resistance to Neutralizing Antibodies over the Course of the Epidemic

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Background: Most of the patients develop autologous neutralizing antibodies (NAbs) during HIV-1 infection. These NAbs drive the viral evolution and lead to the selection of escape variants at the individual level. The aim of our study was to check if, subsequently to the selective pressure exerted by the individual NAbs responses, the HIV-1 species evolved at the population level towards an enhanced resistance to antibody neutralization.

Methods: We compared the neutralization sensitivity of early/transmitted HIV-1 variants from patients infected by subtype B viruses at three calendar periods (1987-1991/1996-2000/2006-2010). Infectious pseudotyped viruses expressing envelope glycoproteins representative of the viral quasi-species infected each patient were generated. Their sensitivity to neutralization was compared by pools of human neutralizing sera and by an updated panel of 13 human monoclonal (HuMoNAbs) broadly NABS. In addition, to verify whether the viral evolution coincided with a poorer capability to induce NAbs, the neutralizing activity of sera collected at least 3 years after seroconversion from 60 (2x30) subtype B infected patients enrolled at the two extreme periods of the study (1987-1991/2003-2007) was tested towards reference tier 2 heterologous viruses.

Results: A significantly progressive enhanced resistance to neutralization was observed over time, both by human sera and by most of the HuMoNAbs tested (b12, VRC01, VRC03, NIH45-46G54W, PG9, PG16, PGT121, PGT128, PGT145). Despite this evolution, one combination of two HuMoNAbs (NIH45-46G54W and PGT128) still was able to neutralize the most contemporary HIV-1 variants. Moreover, we observed a significant reduction of the heterologous neutralizing activity of sera from individuals infected most recently (2003-2007) compared to patients infected earlier (1987-1991), suggesting that the increasing resistance of the HIV species to neutralization coincided with a decreased immunogenicity.

Conclusion: These data provide evidence for an ongoing adaptation of the HIV-1 species to the humoral immunity of the human population over the course of the epidemic.
P03.13

Improving HIV-1-Neutralizing Antibodies Through Use of Somatic Variants Identified by Next Generation Sequencing

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Background: The discovery of HIV-1 neutralizing antibodies with high potency and breadth makes the passive immunization with these antibodies increasingly promising. Improving of these antibodies against a diverse of target viruses should facilitate the passive immunization development. Efforts have been made to improve antibody parameters such as neutralizing potency, breadth, solubility, autoreactivity, and half-life. In each donor, millions of somatic variant of broadly neutralizing antibodies can be found, and some of them may have desired properties. We hypothesized somatic variants of existing VRC01-like HIV-1 neutralizing antibody identified by next generation sequencing can improve neutralizing potency and breadth.

Methods: We determined sequences of somatic variants of HIV-1 neutralizing antibody heavy and light chain genes by next generation sequencing from the same patient where VRC01 was isolated. When they were paired with VRC01 heavy and light respectively, selected antibodies show high neutralizing activity. We then use the heavy chain and light chain genes to pair with optimized versions of VRC01 heavy and light chains to make antibody variants. Neutralizing activity of the antibody variants was assessed on a panel of HIV-1 viruses.

Results: Results showed a few antibodies among these pairs increased neutralizing potency for up to three times. Two antibodies had a geometric mean IC 50 under 0.1ug/ml. These antibodies are under construction and functional analysis. Mutants derived from the geometric mean IC 50 under 0.1ug/ml. These antibodies are under evaluation for autoreactivity and solubility. Mutants derived from the autoreactivity, and half-life. In each donor, millions of somatic variant of broadly neutralizing antibodies can be found, and some of them may have desired properties. We hypothesized somatic variants of existing VRC01-like HIV-1 neutralizing antibody identified by next generation sequencing can improve neutralizing potency and breadth.

Conclusion: We conclude that exploring of somatic variants is a useful tool to improve breath and potency for such therapeutic antibody that targets multiple virus variants. Human immune system provides a natural mechanism to optimize antibodies, and identify antibody variants through next generation sequencing could facilitate the development of the passive therapeutic approach.

P03.14

Isolation and Characterization of an Autoreactive CD4bs Broad Neutralizing Antibody from a Chronic HIV-1 Controller with Systemic Lupus Erythematosus

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Background: The HIV-1 broadly neutralizing antibodies’ (bNabs) frequent polyreactivity, long HCDR3s and/or high levels of somatic mutations suggest that their development might be limited by immune tolerance controls. We hypothesized that concomitant autoimmune diseases may facilitate the development of bNabs in HIV-1 infected subjects. We have identified a HIV-1 infected patient with Systemic Lupus Erythematosus (SLE) who controlled viral load despite the absence of protective HLA types and whose plasma broadly neutralized HIV-1, mostly via CD4bs antibodies. We sought to identify these antibodies and isolated bNab CH98.

Methods: Memory B cells were cultured at near-clonal dilution. CH98 autoreactivity was tested by Hep-2 cell indirect IF staining, ANA assay and a ProtoArray5 microchip for 9600 human host cellular antigens. CH98 epitope was mapped on a panel of 31 YU2 gp120 core mutants. CH98 threading onto known gp120-complexed CD4bs bNAb structures was modeled using the Rosetta 3.3 software.

Results: CH98 was retrieved from a memory B cell culture with RSC3/RSC3Δ3711 differential binding and neutralized 63% of 43 isolates in a multi-clade panel of tier-1 and 2 viruses (median IC50 ~4.2ug/ml [range <0.02-49ug/ml]). CH98 heavy (VH 3-30) and light (VL 2-23) chains displayed high levels of somatic mutations (25% and 16%, respectively), long CDR3s (21 and 12 aa, respectively), an apparent 24-nt n-region in LCDR3, and a ProtoArray5 microchip for 9600 human host cellular antigens. CH98 reacted with numerous human proteins and bound strongly to the E3 ubiquitin ligase STUB-1. Remarkably, CH98 bound to dsDNA, an SLE-related specificity. Epitope mapping and structural modeling studies suggest that CH98 binds to the CD4bs through the HCDR3 with an orientation similar to that of CD4bs bNAb CH103.

Conclusion: These data are consistent with the hypothesis that tolerance dysregulation induced by SLE may have played a role in CH98 bNAb development and maturation.
Dynamic Profiling of Antibody Responses Against HIV-1 Throughout Acute and Chronic Infection

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Background: Antibody response plays a crucial role in controlling HIV-1 infection. However, our comprehensive understanding of this intricate process is far from complete.

Methods: We report here a novel and robust technique to characterize polyclonal antibody responses using a large combinatorial antigen library displayed on the surface of the yeast. By positive selection of viral antigens recognized by patient plasma, a comprehensive profile of immune response can be qualitatively and quantitatively measured.

Results: We characterized anti-HIV antibody responses in chronic and acute patients. In the former, we found that antibodies primarily (~70%) targeted gp41, while a smaller proportion targeted gp120 (~30%). Within gp41 and gp120, major antigenic regions were identified in the V1-V2, the V3-V5 domain, N-terminal gp41 and C-terminal gp41 domains. For acute patients, we profiled the responses of an epidemiologically-linked pair for two years post-infection. In both cases, we found that the majority of antibodies were directed against N-terminal gp41 during the first three months of infection. An anti-V3-V5 response was detected soon afterwards, followed by emergence of an anti-V1-V2 response about six months post-infection. Over the course of infection, the proportion of antibodies targeting N-terminal gp41 decreased, while that against V3-V5 and V1-V2 increased, indicating that the in vivo polyclonal response is dynamic and evolving nature. We are currently studying the contribution of each antigenic domain to overall neutralization activity.

Conclusion: These results demonstrate that our yeast library system can comprehensively assess HIV-specific antibodies generated in vivo, and for the first time offers an efficient means of qualitatively and quantitatively mapping polyclonal immune responses. We believe that applying this technique to a cohort of HIV-infected individuals with distinct virological and immunological features, as well as recipients of candidate vaccines, will improve our understanding of protective immunity and guide the development of effective vaccines and therapeutics.

A Unique Inflammatory Signature Tracks with the Development of bNAb in the Absence of High Viremia

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Background: Over the past decade, there has been an exponential increase in the discovery of a number of new broadly neutralizing Abs (bNAb). However, the mechanism by which bNAb are induced is unclear. While previous studies have implicated high viremia and associated immune activation as potential drivers for the development of bNAb, it is unclear whether the virus and/or inflammation drive this potentiation of the antibody response, or whether particular inflammatory profiles may tune B cell immunity effectively. Here we sought to dissect the inflammatory signals associated with bNAb evolution in a large cohort of subjects that spontaneously control viral replication (Controllers), and to define whether viremia and inflammation can be unlinked as predictors of the induction of bNAb.

Methods: Neutralizing activity, 19-plex cytokine analyses, and antibody subclass titers were performed in 103 Controllers. A decision tree was used to define the inflammatory profile associated with the evolution of bNAb activity.

Results: Among the 19 cytokines/chemokines tested, we observed that high plasma levels of CXCL13, sCD40L, TNFa, IP10, IL8 and MIP1b were enriched in Controllers that evolved bNAb compared to those that did not. While none of the cytokines could independently predict bNAb evolution, we found that a specific pattern of cytokine production was associated with the evolution of bNAb including high levels of TNFa and sCD40L but low levels of IL6. Conversely, subjects unable to induce bNAb elicited a different cytokine profile marked by the absence of TNFa and IL8 but high levels of IFNg.

Conclusion: Overall, these data suggest particular inflammatory profiles; in the absence of active high level viral replication and diversification are associated with the evolution of protective humoral immune responses. Therefore vaccine adjuvanting approaches potentially able to elicit these profiles may play a key role in driving bNAb that may provide sterilizing protection from infection.
Background: HIV-1 infection induces a specific loss of CD27+ memory B cells, which decrease in parallel with disease progression. Moreover, these cells are not properly recovered after HAART. Increased apoptosis of memory B cells could explain this phenomenon. Therefore, we have evaluated the absolute number and frequency of several B cell subsets and their sensitivity to spontaneous-death in HIV-1 infected patients.

Methods: Naïve and memory B cells from 40 HAART-treated virologically-suppressed HIV-1+ patients and 27 uninfected individuals were evaluated by flow cytometry using antibodies against CD19, IgD and CD27. Absolute numbers were analyzed in fresh samples, while spontaneous cell-death of the different B cell subsets was determined using DiOC6 and Sytox Blue after 24-hours of ex-vivo culture.

Results: Selected HIV infected individuals showed lower CD4 T cells than uninfected individuals (648±235 cells/µL vs 812±342 cells/µL, p=0.046). However, the number of B cells was equivalent (151±91 cells/µL vs 129±73 cells/µL). The analysis of B cells subsets confirmed the reduced proportion of CD27+ memory B cells in HAART-treated patients, in both, the IgD+ (p=0.0012) and the switched compartment (p=0.012). However, the analysis of spontaneous cell-death in B cell subsets did not show significant differences between HAART-treated HIV-1+ patients and uninfected individuals. In both groups, the CD27+ memory B cell subsets were more resistant to cell death than naïve B cells or CD27- switched B cells (p<0.0001), strongly suggesting that the expression of CD27 is associated with the survival of B cells in both HIV+1 patients and uninfected individuals.

Conclusion: Our results indicate that HIV-1+ patients showing a similar number of B cells and a lower frequency of CD27+ memory B cells exhibit similar levels of spontaneous memory B cell death suggesting that mechanisms other than apoptosis may be the major contributors to incomplete B cell subsets recovery in these patients.

Background: Structural changes in the N-linked glycan of the IgG Fc region are known to significantly alter antibody effector functions, including antibody dependent cellular cytotoxicity (ADCC), complement deposition, and antibody dependent phagocytosis. To date, little is known about how these glycans are regulated in vivo and whether vaccination can effectively elicit particular glycan structures.

Methods: In this study, we investigated the glycosylation structures of bulk and HIV-specific antibodies elicited in multiple HIV vaccine trials including RV144 (canarypox-Env/Gag/Pro & rgp120), VAX003 (rgp120), Y001 (DNA-Gag/Pol/Env & Ad5-Gag/Pol/Env), IPCAVD (Ad26-Env), and P002 (MVA-Env). We used capillary electrophoresis to separate and quantify the glycan structures released from IgGs to determine whether the vaccine groups elicited different glycosylation patterns.

Results: We found that the trials that utilized multiple doses of recombinant gp120, VAX003 and RV144, significantly changed the glycosylation patterns on bulk IgGs in vaccinees compared to the placebo groups. However, none of the other trials induced any significant changes in IgG glycans. Changes in RV144-induced IgG glycans were significantly correlated with antibody effector function. In particular, ADCC activity was negatively correlated with fucose content and sialic acid while galactose content correlated with FcR binding. Furthermore, we observed that the antibody glycan structures in RV144 vaccinees reverted to those observed in pre-vaccination samples between six and twelve months after the final immunization, which correlates with the previously reported waning of the vaccine-induced immune response.

Conclusion: In this study, we observed that significant changes in IgG glycosylation occur only in vaccines that contain multiple doses of recombinant protein, and these changes do not correlate with vaccine-elicited antibody titer. Additionally, these data support using IgG glycosylation as marker of antibody functionality and vaccine-induced protection in the humoral immune response that should be investigated further along with other, better-studied humoral functions such as titier, ADCC activity, or FcR binding.
P03.19

Analysis of Interaction Between Gp120 and CD4 Mimic Small Compounds that Enhance the Activity of Anti-HIV Neutralizing Antibodies

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Background: CD4 mimic small compounds (CD4MCs), NBD-556 and its analogues bind to the Phe43 cavity of gp120 and inhibit the gp120-CD4 interaction. NBD-556 can also induce conformational changes in the gp120 architecture thereby exposing masked epitopes on the Env protein. In this study, we investigated the evolution of HIV-1 under the CD4MCs pressure to elucidate the interactions between gp120 and CD4MCs.

Methods: To select HIV-1 variants resistant to CD4MCs in vitro, we exposed PM1 cells to not only the bulked primary KP-5P virus but also the cloned KP-5P virus. The viruses were serially passaged in the presence of CD4MCs.

Results: Initial in vitro selection experiments were carried out with the bulked primary KP-5P virus. Resistance against CD4MCs was associated with V255M, T375N/I, and M426I substitutions. For exclusion of the selection of preexisting variants within the KP-5P population, we constructed the molecular clone KP-5P virus. Using the cloned virus for induction of resistant variants against the CD4MCs, we also observed same mutations, V255M, T375N/I, and M426I. The finding indicates that these substitutions were acquired by de novo mutation. We observed F217L, V255M, G321D, T375N/I, I424T, N425K, M426I and I439V substitutions in vitro passages using the bulked and cloned viruses. The resistant profiles of the bulked and cloned viruses to CD4MCs were almost same, but not all. However, V255M, T375N/I and M426I substitutions might be key positions because the mutations appeared in different CD4MC-escaped variants.

Conclusion: In summary, the three mutated positions (V255, T375, and M426) are found in the Phy43-cavity, and are closely interact to the surface of NBD-556 molecule on the crystal structure (PDB: 3TG5). Elucidation of the molecular details governing the interactions between another gp120 and CD4MCs will assist in synthesizing novel CD4MCs to search for drugs with more potent power to change the tertiary structure of Env.

P03.20

A Family of Broad and Highly Potent V1V2-Directed HIV-1 Neutralizing Antibodies with Long CDRH3s from a South African Seroconverter

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Background: Understanding the natural development of broad and potent HIV-1 neutralizing antibodies (NAbs) provides critical clues for rational vaccine design. The V1V2 region of HIV gp120 is a major site of vulnerability to NAbs. We therefore studied longitudinal samples from an HIV-1 seroconverter, CAP256, whose serum breadth and specificity had been mapped to V1V2.

Methods: Using PBMC samples collected from donor CAP256 at 1, 2, and 4 years post-infection, we performed single-B cell cultures, followed by screening of culture supernatants by microneutralization assay. Positive wells were subjected to RT-PCR to recover heavy and light chain immunoglobulin genes, which were subcloned, paired, and expressed. The resulting monoclonal antibodies (mAbs) were characterized by TZM-bl neutralization assay and ELISA.

Results: We isolated twelve V1V2-directed neutralizing mAbs from one somatic family from donor CAP256. The mAbs are 8-15% mutated in the VH gene, with extremely long CDRH3s of 37-39 amino acids. The broadest mAb neutralized 47% of a panel of 200 global HIV isolates, and 60% of isolates from Africa, with a highly potent geometric mean IC50 of 0.008 ug/ml. The mAbs are dependent on quaternary structure of the viral spike, binding to intact virus-like particles but not to monomeric Env proteins. Their neutralization activity maps to amino acid contacts within strand C of V1V2. Unlike the other broad V1V2 mAbs such as PG9, the CAP256 mAbs are only like particles but not to monomeric Env proteins. Their neutralization activity maps to amino acid contacts within strand C of V1V2. Unlike the other broad V1V2 mAbs such as PG9, the CAP256 mAbs are only partially and variably dependent on the glycan at position N160 for neutralization. Despite this difference, neutralization fingerprint analysis shows a strong correlation between these mAbs and the PG9 class.

Conclusion: The broad and potent neutralizing activity of donor CAP256 can be attributed to a family of V1V2-directed mAbs. The discovery of mAbs with properties similar to the PG9 class yet different epitope requirements suggest a multiplicity of modes of recognition for this site of vulnerability.
P03.21

Characterizing the Functional Autoreactivity and Polyspecificity of the MPER-Specific bNAb 4E10 and an Ensemble of Its Germline-Encoded Precursors

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Background: The bNAb 4E10 recognizes an epitope in the HIV gp41 MPER. Previous attempts at eliciting 4E10, by vaccination with envelope-derived or reverse-engineered immunogens, have failed, but have yielded tight-binding, computationally-designed 4E10 ‘epitope-scaffolds’ that can elicit epitope-specific responses. The ontogeny of functional 4E10-equivalent responses has been presumed to be blocked by inherent autoreactivity. Previously identified candidate 4E10 autoantigens include the mitochondrial lipid cardiolipin and a nuclear splicing factor, 3B3.

Methods: Heavy-chain knock-in mice were generated to confirm functional autoreactivity. A large peptide library was used to identify candidate protein autoantigens. Carefully-controlled SPR binding assays were performed to characterize 4E10, substructure, and germline precursor recognition of candidate autoantigens and epitope-scaffolds. Immunohistochemistry was used to validate autoantigens. Crystal structures of a panel of 4E10 germline precursors were determined, alone and in complexes.

Results: 4E10 knock-in mice displayed significant B cell compartment dysregulation, consistent with autorecognition. However, 4E10 bound only weakly and non-specifically to cardiolipin-containing liposomes, through interactions dominated by electrostatic rather than hydrophobic interactions. Based on peptide library binding patterns, 4E10 displays limited, focused polyspecificity and recognizes a novel epitope shared by three inositol trisphosphate receptors (ITPRs). Tissue staining demonstrated reactivity consistent with ITPR1 as the likeliest candidate autoantigen and precluded nuclear antigens, such as 3B3.

Conclusion: These results argue that 4E10 recognition of lipids in general, or cardiolipin specifically, is unlikely to affect selection or neutralization mechanisms and that 4E10 MPER and ITPR1 recognition are distinct enough to permit eliciting 4E10-like responses while evading autoimmunity. Functional, structural and binding studies of an ensemble of the twelve likeliest 4E10 germline-encoded precursors show no neutralizing potency, extremely weak binding to Env, but strong affinities for epitope-scaffolds, and remarkable conservation of recognition mechanisms. These results, in combination with the observed variation in the human 4E10 precursor repertoire, suggest pathways to achieve 4E10-equivalent responses by vaccination.

P03.22

Engineering a Next Generation VRC01-Like Antibody with Improved In Vitro and In Vivo Function

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Background: VRC01, which targets the conserved CD4 binding site on HIV-1 Env, is able to neutralize diverse strains of HIV-1 in vitro and can protect non-human primates (NHP) from SHIV challenge. Phase 1 clinical trials of VRC01 are scheduled to begin this year. Here, we present a new, engineered, second-generation VRC01-like monoclonal antibody (mAb) with increased potency, breadth, and half-life.

Methods: Using deep sequencing and bioinformatics, we identified VRC07, a clonal relative of VRC01 with increased neutralization potency and breadth. Structure guided designs were used to further optimize the potency and breadth of VRC07 while maintaining minimal auto-reactivity. Pharmakokinetics of top candidates were determined in Rhesus macaques.

Results: Both in vivo safety and efficacy were primary concerns; thus, we screened structure-based, rationally designed variants of this antibody for both neutralization potential and auto-reactivity. Through this process, we identified four optimized VRC07 candidates that had at least a 10-fold increase in potency, increased breadth, and minimal auto-reactivity. An FcRn binding site mutation was added to the optimized mAbs to increase their half-life. Pharmacokinetic analyses in non-human primates confirmed both the increased half-life and minimal auto-reactivity.

Conclusion: mAbs represent a promising intervention for the prevention and treatment of HIV/AIDS whose efficacy in humans has yet to be determined. For optimal activity, mAbs should be optimized for potency, breadth, and in vivo stability and safety. Here, we have successfully used a rational, structure-based optimization strategy to engineer a next generation HIV-1 mAb. Clinical manufacturing of our top candidate has begun. Additionally, we are initiating NHP passive transfer studies to determine the plasma mAb concentration necessary for protection from a high dose SHIV challenge.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L1, Room 111 – 112.
Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L1, Room 111 – 112.

**P03.23**

**Nature of Synergistic ADCC Activity Mediated by Human Monoclonal Antibodies (mAbs) Against HIV-1 Env**


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**Background:** Antibody-dependent cellular cytotoxicity (ADCC) appears to play a key role in the protection against HIV-1 infection. It inversely correlates with progression in natural infection and has emerged as a correlate of protection in non-human primate vaccine protocols and human clinical studies. It is known that mAbs can yield synergistic neutralization of HIV-1 but it is not known whether this occurs for ADCC. Accordingly, we developed an approach to address this question.

**Methods:** Our studies used an ADCC assay format based on gp120 sensitized target cells and pairs of human IgG1 mAbs specific for highly conserved epitopes of gp120 that are exposed during viral entry and are potent ADCC targets. Synergy versus antagonism between pairs of these mAbs was quantified by employing the Chou-Talalay method.

**Results:** Analysis of dose-response curves revealed optimal regions of synergy for each mAb pair. Interestingly, regions of additivity and antagonism were also observed with patterns unique to each mAb pair indicating that they are epitope dependent. Notably, strong synergy was maintained or enhanced when one mAb was paired with a variant partner engineered to abrogate Fc receptor binding (LALA mutation). These findings indicate that synergistic ADCC activity does not demand that all contributing antibodies engage Fc receptor. Accordingly, we hypothesize that ADCC synergy arises because certain combinations of antibodies 1) yield stabilized antigen crosslinking on target cells and/or 2) position FcR binding domains in a manner that optimizes Fc receptor engagement. Cases where synergy is enhanced with LALA variants could be further explained by reduced local competition for Fc receptor binding. These possibilities are being explored in ongoing studies.

**Conclusion:** These results provide the basis for future studies to increase the potencies of mAb cocktails for in vitro ADCC assays and also for passive immunization in non-human primates.

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**P03.24**

**CD4-Induced Epitopes Are Exposed on Cell-Bound HIV-1: Potential Targets for Fc Receptor Mediated Humoral Immunity**

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**Background:** The partially successful RV144 vaccine trial produced non-neutralizing antibodies that mediate antibody-dependent cellular cytotoxicity (ADCC) against the Human Immunodeficiency Virus type 1 (HIV-1). These antibodies recognize the CD4-induced (CD4i) C1 region of gp120. However, such findings are enigmatic in view of previous arguments that CD4i epitopes are hidden on viral trimers before, and during interaction with host cell. It is therefore critical to understand the antigenicity of conserved epitopes on the HIV envelope that become exposed during productive viral replication.

**Methods:** We studied the antigenicity of CD4i epitopes on cell-bound virions, by visualizing epitope exposure on single virus particles as they interact with target cells using confocal microscopy. CD4i antibodies are thought to be sterically occluded from the virus – cell interface, but they have been linked with potent ADCC activity. So we examined the location of CD4i epitopes recognized by A32, 17b, and C11 with ~ 20nm precision using stochastic optical reconstruction microscopy (STORM).

**Results:** We find that some CD4i epitopes are poorly represented on free HIV-1 triggered with soluble CD4, but became exposed upon attachment to CD4+ cells. Patterns of exposure varied with antibody; however, in general the level of CD4i exposure was similar to neutralizing epitopes b12 and 2G12. CD4i epitopes appear distal to the virus – cell contact site, where they can be accessed by antibodies involved in ADCC.

**Conclusion:** The patterns of CD4i epitope exposure are consistent with the ADCC activities of cognate antibodies against bound virions. CD4i antibodies were able to gain access to their targets due to the unexpected epitope exposure on gp120, distal to the site of contact with cell surface CD4. These findings indicate that HIV-1 exhibits a diversity of epitope exposure upon attachment that may provide unique insights for understanding how humoral immunity impacts HIV infection.
**P03.25**

Antibody-Dependent Cellular Cytotoxicity Mediated by Passively Acquired Antibodies Is Negatively Correlated with Mortality in HIV-Infected Infants

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**Background:** Rational design of an effective vaccine against HIV-1 requires understanding the functional characteristics of antibodies capable of preventing disease transmission or providing a therapeutic benefit. Antibody-dependent cellular cytotoxicity (ADCC) has been described as a potential correlate of protection in both human and macaque vaccine studies. Mother-to-child transmission provides another setting in which to examine the role of ADCC in infection as infants acquire passive antibodies from their mothers while in utero. These antibodies are present prior to HIV-1 exposure through breastfeeding and may protect from infection or progression.

**Methods:** We evaluated the ADCC activity of passively acquired antibodies from 72 infants of HIV-1 positive mothers. The infants were HIV RNA-negative at birth but continually exposed to the virus via breastfeeding and followed for up to two years during which time 21 infants became infected. Inclusion criteria included: breastfed >/= 3 months, >/= 6 months follow-up of negative infants, and availability of a plasma sample from the first week of life. These plasmas were tested against an infant-derived, subtype A envelope variant using a rapid and fluorometric ADCC assay.

**Results:** Infants who remained uninfected had higher ADCC activity than those who became infected, however this correlation did not achieve statistical significance. The results were similar when the analysis was restricted to infants infected in the first six weeks of life, at which time passively acquired antibody levels are the highest. In those infants who became infected, ADCC activity was negatively correlated with risk of mortality after infection. In a cox proportional hazards model, a one-unit increase in ADCC activity was associated with a 5% decrease in risk of death (p=0.041).

**Conclusion:** These data suggest that passively acquired antibodies that act through ADCC may impact disease progression in infants, with higher HIV-specific ADCC activity at the time of exposure providing a protective effect.

**P03.26**

A Distinct Nef-Specific CTL Escape Selection Preceding Chronic Phase Neutralizing Antibody Induction Against Highly Resistant SIVmac239

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**Background:** Neutralizing antibodies (NAbs) against human immunodeficiency virus type 1 (HIV-1) are promising immunological effectors but are absent in primary infections. Moreover, how NAbs against especially resistant HIV strains eventually develop in vivo remains unknown. To address this, we examined mechanisms of chronic phase NAb induction against the maximally NAb-resistant simian immunodeficiency virus (SIV) strain SIVmac239.

**Methods:** Through continuous screening, we have newly identified a cohort of Burmese rhesus macaques (n = 9) inducing autologous SIVmac239-NAbs in the chronic phase of infection. Temporal course of plasma viral loads, NAb titers, viral sequence variations, T cell responses and in vitro properties of selected mutant SIVs were examined.

**Results:** The SIVmac239-NAb inducers were all conventional progressors. Seven out of nine animals selected for a mutation Nef G63E, preceding NAb induction at around 1 year post-challenge. The frequency of selection was significantly high (p = 0.0008 by Chi-square test) when compared with a previously characterized NAb non-inducer cohort (n = 17). This was viral escape from CD8+ T cell responses specific for a Nef62-70 QW9 (QGQYMNTPW) epitope restricted redundantly by Mamu-B*039:01 and Mamu-B*004:01. SIV with the Nef G63E CTL escape mutation exhibited accelerated apoptosis with attenuation of Akt Ser473 phosphorylation in infected cells. The difference in Akt was preserved in Nef-invaded bystander B cells in coculture, in which Akt signaling negatively regulates cellular maturation.

**Conclusion:** This is the first study to report in vivo events preceding NAb induction against highly resistant AIDS virus and implicates that B cell signal modulation in vivo may be crucial for antibody-based AIDS vaccine design.
P03.27

Antibody Responses to Recombinant gp120, gp70 V1/V2 Proteins and Cyclic V2 Peptide in Thai Phase I/II Vaccine Trials Using Different Vaccine Regimens

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Background: RV132 and RV135 were phase I/II Thai clinical vaccine trials preceding RV144. Both trials used canarypox vCP1521 as a prime. Bivalent HIV-1 gp120 subtypes B/CRF01_AE (SF2/CM235) in MF59 and bivalent gp120 subtypes B/CRF01_AE (MN/A244) in alum was used as boosts in RV132 and RV135 (RV144 equivalence), respectively.

Methods: IgG binding antibody responses induced by RV132 and RV135 regimens were compared using ELISA with a panel of antigens: recombinant gp120 CRF01_AE (A244 and TH023) and subtype B (MN); gp70 V1/V2 (CaseA2 and TH023) scaffolds (in RV144 these inversely correlated with infection); and cyclic V2 peptide TH023. Responses were further characterized into IgG1 and IgG3 subtypes.

Results: IgG responses were absent in placebo and post second immunization with ALVAC in both regimens. Antibody responses to gp120 were detected post first boost increasing at post second boost with geometric mean titers (GMT) of 606 and 2425 (A244), 419 and 3676 (TH023), and 174 and 1270 (MN) in RV132, and of 4422 and 9051 (A244), 230 and 4422 (TH023), and 4222 and 19855 (MN) in RV135. IgG responses to gp70TH023 were detected in both RV132 (GMT, 481) and RV135 (GMT, 1131) but to gp70CaseA2 were only detected in RV135 (GMT, 191). GMT to cyclic V2 were similar for both regimens (GMT ~300). IgG1 and IgG3 responses to gp120A244 and gp70TH023 were lower in RV132 (GMT, 209 and 87, and 52 and 22) than in RV135 (GMT, 746 and 325, and 102 and 29), respectively. IgG1 and IgG3 responses to gp70CaseA2 were only found in RV135 (GMT=13).

Conclusion: IgG responses against recombinant gp120 induced in RV135 were more robust than in RV132. However, both vaccine regimens induced similar responses to the cyclic V2 peptide. Antibodies to gp70CaseA2 were only detected in RV135. These data support the need for head-to-head clinical studies for future comparison and down-selection of vaccine regimens.

P03.28

HIV Antibodies Catch Infectious Particles Independently of Their Neutralising Profiles

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Background: Formation of antibody/virus immune complexes has been proposed to decrease the number of virus accessible to target cells, limiting virus infectivity. Therefore, anti-HIV specific antibodies (Abs) able to bind HIV infectious particles may be particularly relevant at the mucosal site, to participate in HIV protection.

Methods: Following incubation of HIVSF162 with increasing concentrations (from 1 to 50 µg/mL) of antibodies, HIV/IgG complexes were purified onto protein A columns. Bound and unbound fractions were submitted to anti-HIV p24 and anti-IgG ELISA to determine quantity of virus and immunoglobulin in each fraction. Infectivity of captured and non-captured virus was further assessed by titration on TZM-bl cells.

Results: We observed that neutralizing IgGs 2G12 and 2F5 dose-dependently bind p24-containing particles, with 2G12 catching more HIV particles than 2F5. The capacity of the non-neutralizing inhibitory IgG 4B3 to bind the p24-containing particles is not modified by incubation with increasing IgG concentrations, suggesting that 4B3 retention capacity is already maximal at 1 µg/mL. Interestingly, this virus capture resulted in decreased infectivity whatever the neutralizing or non-neutralizing profiles of IgG, indicating that these antibodies are bound to infectious HIV-1 particles, even if such catching capacity was weak. This assay is currently adapted to define capture of infectious particles by antibodies of lgA subtype. Differential capacities of IgG versus lgA isotypes to catch HIV infectious particles will be investigated.

Conclusion: We demonstrated that anti-HIV antibodies were able to catch HIV infectious particles, independently of their neutralization profile. This capture activity may be particularly relevant at the mucosal site, since it may limit the quantity of accessible infectious particles. Immunisation strategies inducing HIV specific antibodies with high capture potency should therefore be considered as an added-value in development of an effective HIV vaccine.
**P03.29**

**Improving the Potency of Neutralizing Anti-HIV-1 Antibodies with a CD4 Mimetic Compound**

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**Background:** The pursuit of a prophylactic vaccine against HIV-1 and the development of novel therapeutic strategies are primordial. Recently, broad and potent neutralizing monoclonal antibodies (MAbs) against HIV-1 had been reported; however, these antibodies are difficult to elicit during infection and vaccination. We aim to find strategies able to improve the modest and narrow neutralizing activity of antibodies commonly elicited by HIV-1 infected patients.

**Methods:** MAbs were obtained by immortalization of a patient’s B cells with EBV and molecular cloning of IgG. Binding to monomeric gp120 and Env was determined by ELISA and flow cytometry, respectively. Neutralization activity was evaluated with the single-round infection assay using TZM-bl cells and pseudovirus.

**Results:** YYA-021 has anti-viral activity; low cytotoxicity, and enhances the neutralization activity of MAbs against JR-FL. We observed that YYA-021 also enhances the binding of CD4i MAbs to monomeric gp120 of subtype B and to Env of subtype B and C; and when we evaluated the neutralization activity of five anti-V3 MAbs, two CD4bs MAbs and three CD4i MAbs against 12 viruses of the Standard Panel B; we observed significant enhancement in the neutralization activity of all the anti-V3 and CD4i antibodies over a range between 2 to 9 of the evaluated viruses. In a series of 12 viruses of the Standard Panel C, YYA-021 was able to enhance neutralization of 3 viruses for two CD4i MAbs and one virus for an anti-V3 MAb. Moreover, when we evaluated the effect of YYA-021 over six transmitted/founder virus, we found that three out of six were sensitive to YYA-021 and that the neutralization activity of the anti-V3 and CD4i antibodies was also enhanced in the presence of YYA-021.

**Conclusion:** CD4 mimetic compounds, such as YYA-021, could improve the performance of classical neutralizing antibodies with limited reactivity against viruses of subtype B, C and transmitted/founder viruses.

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**P03.30**

**Evidence for Conservation of the V1/V2 but Not V3- Glycan-Dependent Neutralizing Epitopes Within Divergent Groups of HIV-1**

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**Background:** HIV-1 has been classified into 4 groups: M, N, O and P. Cross-group broadly neutralizing antibodies have been detected in some HIV-1 infected individuals. The aim of this study was to analyze the sensitivity to neutralization of a panel of non-M primary isolates (PI) to a series of highly potent human monoclonal broadly neutralizing antibodies (HuMoNabs).

**Methods:** The sensitivity to neutralization of 14 non-M HIV-1 PIs (12 O, 1 N, and 1 P) was analyzed in neutralization assays using TZM-bl cells. The panel of HuMoNabs was composed of PG9, PG16, PGT121, PGT128, PGT145, VRC01 and VRC03. These HuMoNabs were isolated from patients infected by HIV-1 group M variants. PG9, PG16 and PGT145 target a glycan-dependent epitope located in the V1-V2 region of gp120 whereas PGT121 and PGT128 target a glycan-dependent epitope located in the V3 region. VRC01 and VRC03 target the CD4 binding site (CD4bs) of gp120.

**Results:** Three group O PIs were neutralized by PG9 and PG16 (IC50 range = 0.23-7.62 µg/mL) and a fourth group O PI was neutralized by PG9 only (IC50 = 9.39 µg/mL). The group N PI (YBF30) was highly sensitive to neutralization by both PG9 (IC50 = 0.28 µg/mL) and PG16 (IC50 < 0.12 µg/mL), but resistant to other HuMoNabs. The group P PI (RBF168) was neutralized by PGT145 only (IC50 = 0.13 µg/mL). None of the non-M PIs was neutralized at the highest concentration tested (10 µg/mL) by the HuMoNabs targeting the V3 glycan-dependent epitopes, PGT121 and PGT128. Similarly the HuMoNabs VRC01 and VRC03 that target the CD4bs did not neutralize any of the non-M isolates at the concentration tested.

**Conclusion:** Our data suggest that the V1-V2 glycan-dependent neutralizing epitopes targeted by the HuMoNabs PG9/PG16 and PGT145 are conserved within the divergent groups of HIV-1. It makes them potentially interesting targets for vaccine strategies.
**Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L1, Room 111 – 112.**

**Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L1, Room 111 – 112.**

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**P03.31**

**A V2 Conformational Immunodominant Epitope Recognized by Human Monoclonal Antibodies**


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**Background:** The level of V1V2-specific plasma antibodies (Abs) was shown to be an inverse correlate of risk in the RV144 clinical vaccine trial. V2-specific Abs of two types appear to have been induced by the vaccine regimen: Abs that target a continuous helical region in the C-strand of V2 and Abs that target a discontinuous surface on the opposite side of V2 which overlaps and surrounds the putative α4β7 binding site. Four mAbs from RV144 vaccinees specific for the C-strand helix of V2 have been produced (Liao et al., 2013). Here we describe the reactivity of four new conformational V2 mAbs along with seven similar mAbs which were previously characterized (Gorny et al., 2012).

**Methods:** These 11 conformational V2 mAbs were derived from six clade B-infected individuals using molecular methods (n=4) or EBV transformation (n=7) and were selected using trimeric or monomeric gp140, monomeric gp120, or a V1V2-gp70 fusion protein. Reactivities were assessed by ELISA and standardized TZM.bl neutralization.

**Results:** Nine of the 11 conformational V2 mAbs neutralize 20-80% of Tier 1 pseudoviruses from clades A, B and C with a median IC50 value of 8.6 µg/ml; however, none neutralizes Tier 2 pseudoviruses. Five of the 11 conformational V2 mAbs are encoded by VH1-69, a finding consistent with the biased usage of the VH1 gene family by HIV Abs. The conformational V2 mAbs display a VH mutation frequency of 2.4% to 13.0% (mean 7.0%) compared to germline. These mAbs display similar patterns of ELISA reactivity with fusion proteins carrying the V1V2 regions of clade A, B, C and AE strains.

**Conclusion:** The epitope targeted by these conformational V2 mAbs appears to be an immunodominant region of V2 which elicits Abs that are highly cross-reactive, mediating neutralization of Tier 1 viruses.

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**P03.32**

**Crystal Structure of the 2F5-like Antibody m66 in Complex with Its HIV-1 gp41 Epitope**

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**Background:** Neutralizing antibodies that target the membrane-proximal external region (MPER) of the HIV-1 gp41 transmembrane glycoprotein have been shown to target either the N-terminus of the MPER, such as 2F5 and m66, or the C-terminus of the MPER, such as z13e1, 4E10, and 10E8. Antibody m66, originally isolated by phage display from a donor with 2F5-like serum activity, targets an epitope within the N-terminus of the gp41 MPER that overlaps the 2F5 epitope.

**Methods:** We determined the crystal structure of m66, both free and in complex with its gp41 epitope. The structure allowed us to compare its mode of gp41 recognition with that of other MPER-specific antibodies. Elements of m66 critical for recognition of the epitope in lipid and soluble contexts, and for virus neutralization, were delineated through accompanying biochemical analyses.

**Results:** When bound by m66, the gp41 MPER adopts a conformation that has both similarities and differences from the conformation bound by 2F5, with similarities occurring largely within the core of the epitope. The angle at which m66 approaches gp41 is similar to the angle of 2F5 approach, but appears distinct from the angles of approach of non-neutralizing MPER-specific antibodies. While the heavy chain third complementarity determining region (CDR H3) of m66 is largely occupied by contacts with the epitope, mutagenesis of the CDR H3 delineates residues critical for recognition of the epitope in a lipid context and for virus neutralization.

**Conclusion:** The structure of m66 reported here reveals a novel antibody-bound conformation of the gp41 MPER, with select structural and functional elements shared in common with other neutralizing antibodies that target this region. The structure of m66 in complex with its epitope thus helps define a conserved site of vulnerability within the N-terminus of the HIV-1 gp41 MPER.
P03.33

Decreased HIV-Specific Antibody-Dependent Cellular Cytotoxicity in HIV-Infected Subjects on cART: Implications for Therapeutic Vaccines

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**Background:** cART cannot eliminate HIV and life-long treatment is required. HIV-specific CTL responses decline rapidly following cART but alterations in other HIV-specific immune functions that may assist in clearing latent HIV infection, specifically antibody-dependent cellular cytotoxicity (ADCC), are unknown. We studied ADCC immunity in subjects on cART to understand the implications for therapeutic vaccines to control HIV infection.

**Methods:** Forty-nine cART-naive HIV-infected subjects from Thailand started cART at baseline. Median baseline CD4 count was 186 cells/µl (range: 10-350 cells/µl) and viral load 4.9 log10 copies/ml (range: 2.9-6.3 log10 copies/ml). Longitudinal blood samples were collected up to 96 weeks (median CD4 count: 338 cells/µl range: 113-990 cells/µl; median viral load: <40 copies/ml range: 40-137 copies/ml). Serum was analyzed for HIV-specific antibody binding titers against subtype AE envelope (Env/AE) by ELISA. ADCC-mediated killing against Env/AE targets was determined using the RFADCC assay. ADCC responses to Env/AE and Rev/Tat/Vpu peptide pools were measured using the NK cell activation assay.

**Results:** A significant reduction in Env-specific ADCC-mediated killing (p<0.0002) was observed between baseline (median: 11.8%, IQR: 7.7-14.1%) and week 96 (median: 7.7%, IQR: 3.9-10.3%). A reduction in ADCC-mediated NK cell activation to Env was also detected in 26/49 subjects but this difference was not significant. However, ADCC responses to Rev/Tat/Vpu were significantly reduced (baseline: median 0.8% NK cell activation; IQR: 0-1.7%; week 96: 0%; IQR: 0-1.3%; p=0.0082). A 10-fold reduction in ADCC endpoint titers between baseline and week 96 was detected. Similarly, serum Env-specific antibody binding titers significantly decreased 10-fold after 96 weeks of cART (baseline: median: 5 log10, IQR: 4.5-5; week 96: median: 4 log10, IQR: 3.5-4.5).

**Conclusion:** A significant reduction in HIV-specific ADCC occurs in subjects following prolonged cART. ADCC-boosting therapeutic vaccines and/or modulation of ADCC effector functions could assist in the control of HIV and the ability to clear reactivated latently infected cells.

P03.34

A Novel Methodology for Isolating Broadly Neutralizing HIV-1 Human Monoclonal Antibodies

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**Background:** Identification of broadly neutralizing HIV-1 human monoclonal antibodies (bnmAbs) may aid in development of an effective HIV-1/AIDS vaccine and antibody drugs for prevention and treatment of HIV-1 infection. Many HIV-1 bnmAbs have been identified from HIV-1-infected ‘elite controllers’, especially in the past three years. Most of known bnmAbs were isolated based on their binding to HIV-1 envelope glycoprotein (Env) or engineered Envs, thus, large-scale expression and purification of isolated clones and characterization of purified soluble antibodies for neutralization breadth and potency were required, which was costly and time-consuming.

**Methods:** Here, we developed a novel methodology for isolating HIV-1 bnmAbs based on antibody neutralization activity by displaying immune antibody libraries on target cell surface followed by sorting the cells by antibody neutralization ability to specific pseudotype virus.

**Results:** After several rounds of sorting, a panel of human mAbs has been isolated from two ‘elite controllers’ that can neutralize various isolates from clade B and clade C.

**Conclusion:** We are currently measuring the neutralizing capability of these mAbs against different clades of virus, and expressing these mAbs as soluble form and will test them in a standardized TZM-bl neutralization assay to confirm the neutralization breadth and potency of isolated mAbs.
P03.35

Characterization of Broadly Neutralizing Antibodies (bNAbs) to HIV-1 Present in a Cohort of Long Term Non-Progressors (LTNPs)

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Background: One major obstacle to induce bNAbs resides in the high variability of the viral envelope and structural mechanisms hiding crucial epitopes for neutralization. Besides, maturation of bNAbs represents a major challenge for the immune system that can be impaired by the immune deficiency associated with HIV infection. We have explored the hypothesis that preserved B cell function in LTNPs could result in the production of a broad humoral response.

Methods: Samples (129) from the cohort of LTNPs were kindly provided by the HIV BioBank integrated in the Spanish AIDS Research Network (RIS). A population of 191 untreated typical progressors (TPs) from Hospital Clinic, Barcelona, was analyzed as control. Sera were preincubated with Env recombinant viruses harboring a luciferase gene and viral infection assessed by luciferase activity. bNAbs specificities were studied by ELISA using mutated gp120 that abrogates antibody binding; neutralization assays with mutated viruses and peptide competition neutralization assays. Epitope specificities were also elucidated from the serum pattern of neutralization against a panel of diverse HIV-1 isolates.

Results: Elite neutralizers (9.3%) were found among LTNPs. Broadly neutralizing sera were screened for the presence of epitope-specific antibodies. By ELISA techniques CD4 binding site antibodies were detected in 33% of LTNP and 57% of TPs. To determine whether these antibodies were responsible for the broad neutralization, competition neutralization assays by using RSC3 (antigenically resurfaced glycoprotein containing the CD4bs) were performed. RSC3 addition inhibited neutralization mediated by 2 sera from LTNPs (17%) and no serum of normal progressors. Anti-MPER antibodies were detected in 50% of LTNPs and 57% of TPs, including several sera with 4E10 and Z13-like antibodies. Glycan-dependent HIV-1 neutralizing antibodies were more abundant in LTNPs (75%) as compared to control population (43%).

Conclusion: LTNPs showed broad humoral immune responses against HIV-1 suggesting that other factors than antigenemia could drive the development of bNAbs.

P03.36

p24-Specific Antibody Dependent Cellular Phagocytosis Is Associated with Antiviral Control in Chronic HIV-1 Subtype C Infection

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Background: There are limited data describing the functional characteristics of non-neutralizing antibodies (Abs) in chronic HIV-1 subtype C (HIV-1C) infection. However, mounting evidence suggests that functions such as antibody dependent cellular phagocytosis (ADCP) may have a critical role in control and prevention of infection.

Methods: The capacity of plasma Abs to mediate ADCP in the presence of fluorescent beads conjugated to HIV-1C p24 protein was examined in a THP-1 cell based-in-vitro assay. Correlations between p24-specific IgG subclass titers, IgA titers, ADCP responses and markers of disease progression were determined.

Results: In an initial exploratory analysis involving 340 HIV-1C chronically infected antiretroviral-naïve participants, Gag-specific (p17, p24 and p55) but not envelope (gp120 and gp140) IgG titers correlated with lower viral loads (VL) and higher CD4 counts. Based on these results, a subset of 122 patients with p24 antibody titers higher (n=56) or lower (n=66) than the cohort median were selected to further dissect the functional profile of p24-specific Abs. Plasma p24 specific IgG1 titers but not others were highly correlated with the ADCP responses to both gp120 and p24 (p<0.0001). We found no correlation between plasma gp120 IgA titers and gp120 ADCP responses (p=0.96) nor p24 IgA titers and p24 ADCP responses (p=0.61). Plasma gp120-specific ADCP responses did not correlate with VL (p = 0.29) and CD4 count (p = 0.17). In contrast, p24-specific ADCP responses were associated with lower VL (p=0.02) and showed a trend towards association with higher CD4 count (p = 0.08).

Conclusion: Our data suggest that p24-specific IgG1 Abs, in chronic HIV-1C infection, mediate ADCP responses that may contribute to viral control. There is need to investigate mechanisms by which Gag-specific Abs may contribute to viral control and how Ab-mediated phagocytosis may be harnessed in the context of vaccine design.
P03.37

TNFα Impairs Peripheral T Follicular Helper (pTfh) Cell Function In Vitro and Is Associated with Decreased Ab Response to Influenza Vaccine In Vivo

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Background: Peripheral T follicular helper cells (pTfh) have been shown to provide critical help to B cells in producing Ab in response to influenza vaccines. We investigated whether generalized inflammation and immune activation are associated with impaired antibody response to seasonal 2011/2012 influenza vaccine in a cohort of elderly HIV+ women, and examined the role of the pro-inflammatory cytokine TNFα for its influence on B cells.

Methods: Fifteen HIV+ virally suppressed post-menopausal women on ART (median CD4 count, 519 cells/mm3) and 12 age-matched HIV– controls, all recipients of 2011/2012 influenza vaccine (Fluvarix®, GSK), were investigated for Influenza Ab titers 4 weeks after vaccination, plasma TNFα and IL-21 levels and activation markers (CD38, HLA-DR) on pTfh (CD3+CD4+CD45RO+CXCR5+) cells. B cells and memory CD4 T cells were isolated, co-cultured at a 1:1 ratio and stimulated with SEB in the presence of TNFα, IL-21, anti-TNFα or anti-IL-21R Abs. IL-21 and immunoglobulin levels were analysed in culture supernatants by ELISA. Statistical methods included Student t-test and Pearson analysis, with P values <0.05 being considered significant.

Results: Protective Ab titers to influenza developed after vaccination in 75% of the HIV+ and 91% of the HIV– women. Plasma TNFα levels were inversely correlated with the post-vaccination Ab titers (r=−0.4686, p=0.0158), with the frequency of activated pTfh cells (r=−0.4831, p=0.0198), and with the post-vaccination plasma IL-21 (r=−0.4511, p=0.0207). In vitro TNFα blockade improved the ability of CD4 T cells to produce IL-21 (p=0.0203) and of B cells to secrete immunoglobulins (p=0.0399).

Conclusion: Excessive immune activation of pTfh and elevated pro-inflammatory cytokine TNFα can impair influenza vaccine-induced Ab responses. Chronically activated pTfh cells fail to help vaccine-induced B cell Ab production. Interventions aimed at reducing chronic inflammation and immune activation in HIV+ patients, especially in the elderly, may improve their response to vaccination.

P03.38

Long CDR H3 of a Broadly-Neutralizing Antibody Is Present at Recombination


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Background: A family of highly-potent, broadly-neutralizing V1/V2-directed antibodies has been isolated from an African donor. As with other known antibodies that target epitopes in this region, such as PG9 and PG16, the interaction depends on an unusually long antibody CDR H3 region. Up to this point it has been unclear whether such long CDR H3 loops are generated at recombination or whether they elongate over time by somatic hypermutation, beginning from shorter CDR H3 recombinants.

Methods: A longitudinal analysis was carried out using B cells isolated from the donor at several different time points. We used 454 next-generation sequencing (NGS) to sequence cDNAs corresponding to the antibody variable regions from the cells. After processing with a standard antibodyomics pipeline to identify somatic variants of the isolated antibodies, a maximum-likelihood phylogenetic tree was constructed to infer the original unmutated common ancestor (UCA) and trace the development of the clonal lineage.

Results: The UCA sequence calculated from the maximum-likelihood tree contains a full-length (37 amino acid) CDR H3 loop. We also find several extremely-low divergence sequences in the 454 data with 37 amino acid CDR H3 loops, supporting the conclusion that the long CDR H3 loop is formed whole by the recombination process, rather than elongating from a shorter recombinant through somatic hypermutation.

Conclusion: Broadly-neutralizing antibodies with low rates of somatic hypermutation are of particular interest in the search for an effective HIV-1 vaccine, as they should be easier to elicit. Here we have shown that long CDR H3 loops can be generated by the initial recombination event, indicating that V1/V2-directed antibodies may represent a promising pathway for vaccine development.
P03.39  V2 Peptide Binding to HIV-1 Variants and Interaction of HIV-1 with α4β7 Integrin Receptor

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Background: The mucosal homing integrin receptor α4β7 interacts with the HIV-1 gp120 second variable loop (V2). Analysis of the RV144 samples showed that antibodies against the Env gp120 V1V2-region, which contained the α4β7-binding site (aa 179-181) were associated with lower risk of infection. Sequence analysis showed that breakthrough viruses in vaccinees and placebo differed at two sites in V2, with an increased proportion of K169 and a lower proportion of I181X in vaccinees. A critical unanswered question is the role of α4β7 in transmission efficiency.

Methods: Primary viruses and infectious molecular clones (IMC) were pre-incubated with α4β7-expressing RPMI8866 cells and then added to plates coated with either MadCAM-1 or streptavidin-biotinylated linear-V2 peptide. In separate experiments, RV144 plasma from infected vaccinees and placebos were added to peptide-coated plates followed by cells and binding/inhibition was determined. Seventy-eight infected samples were analyzed by Biacore for binding to four linear V2-92TH023-peptides (consensus, I181L, K169Q/Q170R, K169Q/I181X).

Results: Primary viruses SF162 and BaL inhibited α4β7 binding (30%) to MadCAM-1 in a dose-dependent manner (p<0.02), while US1 and JRFL did not. Acute IMCs A, C, and chronic IMC subtype C also inhibited binding (p<0.02), while acute/chronic CRF_01 AE IMCs showed a trend towards inhibition. None of the RV144 plasma showed significant differences in binding to the four peptides. Individuals infected with K169-containing viruses showed a 5-fold higher binding to V2-consensus peptides compared to individuals infected with K169X-variants (p=0.04). There was no difference among placebos (p=0.81). Peptide binding did not differ between subjects who carried I181 or I181X-variants. Preliminary results with 16 samples showed no correlation between V2-length and α4β7-inhibition.

Conclusion: V2-binding is affected by the viral sequence rather than the linear peptide sequence, suggesting a conformational determinant. Inhibition of α4β7-binding by certain viruses warrants further study to understand if there is a relationship between transmission efficiency and HIV-1 sequence.

P03.40  Eliminating Autoreactivity of Variants of Antibody VRC07 by Glycan Masking

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Background: Antibody VRC07, a clonal relative of VRC01 identified from next-generation-sequencing analysis of PBMC’s from Donor 45, shows improved HIV-1 neutralization breadth and potency compared to VRC01. Efforts are being made to further improve the potency of VRC07, and a number of mutations improve the potency of VRC07. Some of the more potent mutations, however, induce autoreactivity, which in turn negatively impacts effectiveness and safety. Here we investigate the ability of introduced N-linked glycans to shield potency-enhancing mutations from auto-antigen and to eliminate the induced autoreactivity.

Methods: For three different autoreactive VRC07 antibody variants, we designed a total of five single glycan antibody mutants by computationally designing N-linked glycosylation sequons proximal to the antibody residues responsible for autoreactivity. The NglycPred algorithm, a Random Forest-based classifier utilizing structural properties and patterns as attributes, was used to predict the glycan occupancy of the N-linked glycosylation sequons. Three autoreactivity assays, Anti-cardiolipin ELISA, Luminex AtheNA Multi-Lyte ANA test, and HEp-2 ANA test were used to assess the autoreactivity of these glycan mutants.

Results: Gel analysis of intact and PNGaseF treated VRC07 glycan mutants demonstrated that N-linked glycans were successfully added for all five antibodies as predicted from the computational algorithm. Addition of a single N-linked glycan eliminated the autoreactivity generated from each of the potency-enhancing mutations, as assessed with the three autoreactivity assays.

Conclusion: We have demonstrated that adding an N-linked glycan proximal to autoreactive residues eliminated autoreactivity of VRC07 variants. When potential therapeutic antibodies show autoreactivity, our protocol of N-linked glycan masking may be used to eliminate this autoreactivity and to improve the properties of promising therapeutic candidates for AIDS and other diseases.
**P03.41**

**Broad Neutralizing Antibody Responses in HIV-1 Superinfected Women Do Not Target Known Epitope Specificities**

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**Background:** We have shown that superinfection leads to a broader and more potent neutralizing antibody response when compared to single infection in a cohort of high-risk women. This suggests that exposure to antigenically diverse HIV-1 variants may contribute to the elicitation of broadly neutralizing antibodies. However, the antigenic targets of these broad responses observed after superinfection are not known. Here, we examined whether superinfection focuses the antibody response on epitopes that are recognized by recently characterized HIV-1 specific broadly neutralizing monoclonal antibodies.

**Methods:** Plasma samples obtained ~5 years after initial infection from 21 superinfected women identified from a seroincident cohort in Mombasa, Kenya were tested for antibodies directed to the CD4-binding site by ELISA and neutralization assays using RSC3 and ∆RSC371I mutant proteins. The effect of glycosylation residues at positions N160 and N323 were examined by comparing the neutralization profiles of wildtype virus to mutant viruses with these residues removed.

**Results:** One out of the 21 women’s antibody responses (5%) displayed differential binding affinity to RSC3 and ∆RSC371I mutant proteins. However, competition with these proteins in a neutralization assay did not indicate the presence of CD4-binding site neutralizing antibodies, suggesting that this woman has binding, but not neutralizing antibodies to this site. Neutralization assays using wildtype and mutants for N160K (Q461d1, Q23-17, DU156) or N323A (Q23-17) also did not show differential IC50s, demonstrating that these women did not develop responses to either of these residues, which would be indicative of PG and PGT-directed antibody specificities.

**Conclusion:** Overall, this study suggests that the broad and potent neutralizing antibody responses elicited following superinfection in these women is focused on epitopes that have yet to be identified.

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**P03.42**

**Biochemical, Immunological and Structural Characterization of a Panel of Novel Rabbit Monoclonal Antibodies Elicited by HIV-1 Env Vaccination**

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**Background:** Until now, the monoclonal antibodies (mAbs) widely studied in the AIDS vaccine field have been generated from HIV-1 infected individuals. Given the vast difference between infection and prophylactic vaccination, study of the antibody profiles elicited by candidate AIDS vaccines is urgently needed.

**Methods:** Here we report the development of a panel of novel rabbit mAbs (RmAbs) produced by HIV-1 Env-based DNA prime-protein immunization. Extensive analysis was conducted on the specificity and binding characteristics of these RmAbs. Furthermore, structural analyses were carried out by crystallization of three RmAbs in complex with their corresponding epitope peptides.

**Results:** These RmAbs revealed a broad diversity of Env epitopes, including not only the highly immunogenic V3 loop, but also several previously underappreciated epitopes in the C1, C4, and C5 regions. Overall, these RmAbs showed high binding affinities, a majority of them could cross-react with gp120 antigens from more than one subtype, and several had neutralizing activities. Furthermore, one of these RmAb, R20, targeting at the glycan rich V3 base, shares the same peptide region as that of the highly potent broadly neutralizing human mAb, PGT128.

**Conclusion:** Overall, this study suggests that the broad and potent neutralizing antibody responses elicited following superinfection in these women is focused on epitopes that have yet to be identified.
P03.43

Inhibition of B Cell Function by HIV-1 Envelope: Role of Binding to Integrin α4β7

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Background: During the early stages of HIV infection the immune system of the infected individual is already somewhat impaired. Among the defects described is the impairment of normal B cell function that includes a significant delay in the development of the anti-HIV humoral immune response. The mechanisms underlying this delay are not fully understood. In the present study, we asked whether gp120-induced signaling through α4β7 on B cells could disrupt their function.

Methods: Peripheral blood B cells were treated in vitro with gp120s derived from viral isolates that exhibited different affinity for α4β7. Treated B cells were tested for alterations in their transcriptional program as determined by RNA microarray analysis. CFSE proliferation assay and FACS analysis were also employed.

Results: gp120 signaling through α4β7 modified the expression of genes involved in chemotaxis, regulation of apoptosis, regulation of lymphocyte proliferation, immune/inflammatory response. Several of these genes have previously been associated with B cell dysfunction in HIV-infected patients. B cells were also tested for proliferative capacity and changes in the expression of surface receptors. gp120s with high affinity for α4β7 mediated a strong inhibition of B cell proliferation. Among the genes upregulated by gp120 was FCRL4, an inhibitory receptor. Increased FcRL4 expression is observed in HIV-infected individuals, but its expression normalizes upon initiation of antiretroviral therapy. We observed induction of FcRL4 surface expression by treatment with soluble HIV-1 gp120 and by exposure of B cells to CD4+ T cells that had been infected in vitro.

Conclusion: Taken together these results suggest that gp120s with a high affinity for α4β7 have the potential to disrupt B cell function. These studies have implications for understanding the immunopathogenic mechanisms of HIV-1 infection, particularly the ability of high levels of viremia observed during acute HIV infection to blunt an appropriate antibody response to the virus.

P03.44

Threshold Mutation Rate Is Required for V3 Antibodies Displaying Cross-Neutralization of HIV-1 Tier 2 Viruses

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Background: The RV144 clinical vaccine trial showed that reduced HIV-1 infection in vaccine recipients was inversely correlated with higher levels of plasma anti-V2 antibodies (Abs). Anti-V2, along with anti-CD4-binding site (CD4bs) and anti-V3, Abs are present in almost all HIV-infected subjects and can be relatively easily induced by vaccines; however, they exhibit rather weak neutralization. We have analyzed human monoclonal Abs (mAbs) with the same specificities to compare their neutralizing activities and determine a threshold of the mutation rate in Abs required for cross-neutralization of HIV-1.

Methods: Sixty-six human mAbs specific for CD4bs, V3 or V2 were produced by cellular and molecular methods from individuals infected with various subtypes of HIV-1. The percentage of mutations in the heavy chain variable fragment of each mAbs was analyzed and compared with neutralizing activity against a standard panel of 41 pseudoviruses (psVs).

Results: All 66 mAbs neutralized, with varying frequencies, a panel of sensitive tier 1 psVs, while the more resistant tier 2 psVs were neutralized sporadically and only by V3 mAbs. Mutation frequencies ranging from 2.4% to 18.1% in VH genes were comparable for CD4bs, V3 and V2 mAbs and had no correlation with the neutralization of tier 1 viruses. However, neutralization of tier 2 viruses by anti-V3 mAbs correlated with the percentage of mutations suggesting they can be related to a higher affinity. Some mAbs had mutation rates comparable to the level observed in Abs induced by the HIV vaccine used in the RV144 trial and these mAbs displayed cross-neutralizing activity.

Conclusion: Taken together these results suggest that gp120s with a high affinity for α4β7 have the potential to disrupt B cell function. These studies have implications for understanding the immunopathogenic mechanisms of HIV-1 infection, particularly the ability of high levels of viremia observed during acute HIV infection to blunt an appropriate antibody response to the virus.
P03.45

New Insight on DNA Vaccination to Prime High Quality B Cell and Antibody Responses

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Background: The use of DNA immunization to elicit high quality antibody responses is receiving more attention in HIV vaccine development. If viral vector priming was effective in the RV144 trial, there is no scientific reason to suggest that DNA vaccine cannot be as effective, if not more so. While we, along with others, have shown that DNA prime-protein boost is highly effective in eliciting cross-subtype binding and neutralizing antibodies in both animal and human studies, the uncertainty on DNA immunization’s mechanisms has prevented DNA vaccines from moving to more advanced studies.

Methods: Two independent mice studies were conducted. First, using HIV-1 gp120 as model immunogen, wild type (WT) C57/BL6 mice were immunized with DNA prime-protein boost, DNA alone, and protein alone. T follicular helper (Tfh) and germinal center (GC) B cells responses were analyzed. Second, antigen specific IgG responses in Aim2 knockout and WT mice were studied.

Results: Here out data indicated that DNA immunization can take advantage of recently identified novel intracellular mechanisms involved in both acquired and innate immune response pathways. First, DNA priming is able to effectively re-orchestrate germinal center responses. At early phase (day 3) post final immunization, increased numbers of Tfh cells and GC B cells are observed in the DNA prime-protein boost group compared to the group that did not receive DNA priming. Second, we discovered that Aim2, an important member of the inflammasome family responsible for IL-1β and IL-18 release, is involved in DNA immunization as Aim2 knockout mice demonstrated decreased antibody titers when compared to WT mice.

Conclusion: Findings from these two independent studies not only link DNA immunization to critical intracellular immune pathways but also bridge the antigen-specific (acquired immunity) and non-antigen-specific (innate immunity) arms of immune system, which are important for the development of effective HIV vaccines.

P03.46

Analysis of Env Regions Important for Binding and Resistance to B404, a Potent Neutralizing Antibody Against SIV

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Background: Inducing neutralizing antibodies (NAb) is the key to developing a protective vaccine against HIV-1. Although non-human primate models of SIV infection are commonly used to develop vaccines against HIV-1, epitopes for potent and broad neutralization of SIV remain unclear because few monoclonal NAbs have been available. Recently, we isolated NAbs from rhesus macaques infected with SIVsmH635FC, which causes a vigorous and potent antibody response in the infected macaques. To clarify the neutralization mechanism of SIV, we analyzed Env regions important for binding and resistance to B404, a representative NAb with potent and broad neutralizing activity.

Methods: Binding site for B404 was analyzed by competition ELISA with monoclonal antibodies and by examining reactivities to Env mutants by flow cytometry. B404-resistant variants were induced by passages of SIVmac316 in PM1/CCR5 cells in the presence of increasing concentrations of B404. The Env residues associated with the resistance to B404 were identified by genetic analysis of the env gene, molecular dynamics simulations of variants, and neutralization assay using mutant viruses.

Results: The V3 and V4 loops were critical for B404 binding. The reactivity to the B404 epitope on trimeric, but not monomeric, Env was enhanced by CD4 ligation. The B404-resistant variant accumulated amino acid substitutions in the C2 region of gp120, which could effectively alter the structural dynamics of the V3/V4 loops and their neighboring regions. The Q733stop mutation, which truncates the cytoplasmic tail of gp41, was also associated with resistance to B404, and increased infectivity in human cells.

Conclusion: A conformational epitope consisting of the V3 and V4 loops is the target for B404 that potently and broadly neutralize various SIV strains. Conformational change of Env trimer conferred by the mutations in C2 and gp41 is important for the resistant to B404.
**P03.47**

**Rapid Screening of Human Sera for the Presence of Neutralizing Anti-MPER Antibodies**

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**Background:** The definition of the mechanism(s) of elicitation of neutralizing antibodies against the membrane proximal extracellular region (MPER) of gp41 will benefit from the analysis of new antibodies isolated from individuals with high titers of these antibodies. To identify these individuals, we have developed a fast screening method to measure neutralizing anti MPER antibodies.

**Methods:** Sera from 78 HIV+ individuals with detectable viremia and 10 uninfected individuals were analyzed. The presence of anti-MPER antibodies was screened by ELISA and by using a flow cytometric assay that measures IgG binding to 293 cells stably transfected with two different truncated forms of gp41 covering the HR2, the MPER and the transmembrane domains, differing in the N-terminal sequence. Control cells and competition assays with 2F5 antibody were performed to confirm specificity.

**Results:** Both cell-surface expressed truncated gp41 molecules properly exposed the MPER epitope, as assessed by staining with antibodies 4E10 and 2F5, and were recognized by most sera from HIV infected individuals. While the binding to both cell surface expressed gp41 truncated proteins strongly correlated ($p<0.0001$) it was unrelated to standard ELISA assays for MPER linear epitopes. In competition assays with labeled 2F5 antibody, sera from HIV infected individuals that strongly recognized gp41 truncated proteins showed significantly higher inhibitory activity than samples from weakly reactive HIV-1 infected patients or uninfected individuals ($p<0.0001$). Therefore, the level of recognition of cell surface expressed gp41 proteins, but not data from ELISA assay, showed a correlation with the ability of sera to compete with the 2F5 antibody.

**Conclusion:** Small proteins exposing the MPER on the cell membrane are useful tools for the analysis of 2F5-like neutralizing anti HIV gp41 responses. This improved tool compared to ELISA assays, may facilitate large screening of serum samples to identify and characterize new anti MPER antibodies and to monitor neutralizing immune responses in vaccine trials.

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**P03.48**

**Steric Access to the HIV-1 Co-Receptor Binding Site Is Manifest on the Virus Prior to CD4 Engagement at the Target Cell Surface**

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**Background:** It is well known that classical co-receptor binding site (CoRbs)-directed antibodies (eg 17b IgG) do not neutralize HIV-1 primary isolates. However, they can neutralize the lab-adapted virus HXBc2, suggesting that a more open conformation allows access on this virus that is not permitted on primary isolates. In contrast, single chains (sFvs) derived from CoRbs monoclonal antibodies (Mabs) can neutralize primary isolates, suggesting steric restriction. Restriction to the CoRbs was suggested to be steric occlusion to the viral Env once engaged with target cell CD4. Such engagement would form or expose the CoRbs.

**Methods:** To investigate neutralization dynamics mediated by the co-receptor-directed antibodies, 17b and 17b sFv (and/or X5) we performed antibody-virus “washout experiments” using viruses containing envelope glycoproteins derived from both lab-adapted and primary viruses.

**Results:** We performed washout experiments on the lab-adapted virus, HXBc2 with CoRbs mAbs. As expected, the full length IgG, 17b could neutralize HXBc2 and this neutralization activity was not significantly reduced by antibody-virus washing, indicating direct access of 17b epitope on the virus. We performed similar experiments on primary isolates and observed that, also as expected, 17b IgG, is unable to neutralize primary viruses both in “no-wash” and “wash-out” conditions, presumably due to lack of access to its epitope. Next we performed wash-out analysis with the neutralizing 17b sFv and we observed that the sFv resisted washout from primary viruses, suggesting direct access to the CoRbs prior to target cell CD4. Such engagement would form or expose the CoRbs.

**Conclusion:** The data indicate that for lab-adapted isolates, the CoRbs is exposed prior to CD4 engagement and that the induced conformation recognized by 17b is at least sampled prior to CD4 binding. The sFv “washout” data suggest that prior to target cell engagement, the co-receptor binding site is sterically occluded for full IgG on the virus but the smaller sFv can access this region.
P03.49

Genetic and Functional Characterization of Somatic Variants of Vaccine-Induced HIV-1 CD4bs-Directed Monoclonal Antibodies

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Background: Recent studies of broadly neutralizing antibodies targeting the primary receptor binding site (CD4bs) of HIV-1 isolated from chronically infected individuals suggest that extensive affinity maturation is required to achieve broad neutralization. By comparison, CD4bs-directed antibodies elicited by vaccination are far less driven by somatic hypermutation (SHM), a feature that likely contributes to the comparably narrow range of neutralization these antibodies display. We recently isolated a set of CD4bs-specific monoclonal antibodies (MAbs) from rhesus macaques immunized with envelope glycoprotein (Env) trimers and described their genetic and functional properties.

Methods: Here, we isolated somatic variants of selected MAbs using two complementary approaches for a more comprehensive analysis of these lineages. In one approach, we designed clone-specific primers that align to the DH and JH junctions to selectively amplify somatic variants of the respective heavy chain (HC) sequences. The somatic variants were cloned and expressed together with the original light chains and evaluated for CD4bs specificity by ELISA and for their neutralization activity. In the other approach, we subjected peripheral blood mononuclear cells to 454 sequencing to create a database of HC VDJ sequences from the same rhesus macaque. Sequences representing proposed somatic variants of the specific MAbs were identified and their frequencies and degree of SHM were determined.

Results: We identified HC sequences that displayed a higher degree of SHM (up to 18% at the aa level) compared to their germline counterparts. Furthermore, in at least one case we found that increased SHM translated to improved neutralizing capacity. Further, our results suggest that the size of each clonal family is relatively small.

Conclusion: This approach allows us to characterize clonal families of vaccine-elicited antibodies archived in the peripheral memory B cell compartment for an improved understanding of vaccine-induced B cell responses targeting the HIV-1 CD4bs.

P03.50

Copy Number Variants (CNV) in the Immunoglobulin Heavy Chain Locus (IGH) and Tests for Association with Progression to AIDS

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Background: The immunoglobulin heavy-chain locus (IGH) encodes variable (IGHV), diversity (IGHD), joining (IGHJ), and constant (IGHC) genes and is responsible for antibody heavy-chain biosynthesis, which is vital to the adaptive immune response. Programmed V(D)J somatic rearrangement and the complex duplicated nature of the locus have impeded attempts to reconcile its genomic organization based on traditional B-lymphocyte derived genetic material. As a result, sequence descriptions of germline variation within IGH are lacking, haplotype inference using traditional linkage disequilibrium methods has been difficult, and the human genome reference assembly is missing several expressed IGHV genes.

Methods: We obtained high-quality finished Sanger sequence from the IGH region of large insert clones of a haploid human hydatidiform mole library and from a panel of nine diploid genomes of diverse ethnic origin from the HapMap collection. We characterized 11 large germline copy-number variants, several previously unmapped IGHV genes, and an additional 221 Kbp of insertion sequence. Based on this new haplotype information, we genotyped four CNVs by PCR in 425 individuals from nine human populations. We found that all four are highly polymorphic and show considerable stratification (Fst 0.3–0.5), with the greatest differences observed between African and Asian populations. We are now testing for an increase in copy number of IGHV1-69 alleles associated with broadly-neutralizing anti-HIV antibodies, comparing spontaneous controllers of HIV infection to normal progressors.

Conclusion: Much of the CNV in the IGH locus and other immune loci remain uncharacterized, and high-throughput genomic assays (GWAS and NextGen Sequencing) do not properly genotype these regions, invalidating association studies searching for hidden heritability of susceptibility to autoimmune and infectious diseases, such as HIV/AIDS.
P03.51

What Can Be Learnt About Broad ADCC Immunity to HIV from Influenza?

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Background: HIV and Influenza are variable viruses and it is difficult for infected or vaccinated subjects to generate antibodies that neutralize the majority of field strains for both viruses. However, non-neutralizing antibodies with effector functions such as Antibody-Dependent Cellular Cytotoxicity (ADCC) may be able to recognize a broader array of viral strains and help reduce infection or control disease. We studied the breadth of recognition of HIV and Influenza strains by ADCC antibodies.

Methods: Serum samples from cART-naïve HIV+ subjects (n=79) and influenza-seropositive healthy adults (n= 182) were studied for ADCC to multiple strains of HIV or Influenza using both killing-based ADCC assays (RFADCC for HIV and ADCVI for influenza) and antibody-dependent NK cell activation assays.

Results: Influenza-specific ADCC antibodies were found in serum from over 90% of healthy adults and typically recognize multiple divergent strains including the hemaglutinin of seasonal H1N1 and H3N2 strains. Over half of adults had ADCC antibodies to the swine-origin H1N1 strain PRIOR to the onset of that epidemic. Pandemic H1N1-specific ADCC antibodies pre-2009 were most common in subjects >45 years of age (<0.01), an age group that was relatively protected from disease. ADCC recognition of HIV is also common and broad in HIV+ subjects, with ADCC recognition of HIV exceeding 90% of subjects and the majority of infected subjects recognizing multiple HIV-1 subtypes. ADCC recognition of the HIV protein Vpu was rarer (11%) and only found in subjects with slow HIV progression.

Conclusion: Striking parallels exist in the breadth of ADCC recognition of HIV and Influenza. Increased breadth of influenza ADCC antibodies likely represents stimulation from multiple prior infections. HIV vaccination strategies may be able to mimic this. The association of ADCC with reduced infection (for influenza) and disease progression (for HIV) suggests that ADCC-based vaccines or immunotherapies may hold promise for these infections.

P03.52

Escape from HIV-1 Neutralising Antibodies Drives an Increase in Plasma Neutralisation Breadth Through Recognition of Multiple Epitopes and Immunotypes

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Background: Identifying the targets of broadly cross-neutralising (BCN) antibodies and understanding how they develop is critical to the rational design of an HIV-1 vaccine. Four sites of vulnerability on the envelope are known: V2; V3/ the co-receptor binding site; the CD4 binding site (CD4bs); and the membrane proximal external region of gp41. Here we mapped the BCN antibody targets and escape mutations in one individual who developed exceptional breadth (>90% neutralisation breadth) over 4.5 years of infection.

Methods: Site-directed mutagenesis was used to map the targets of BCN antibodies. Single genome amplification of autologous envelope sequences from twelve time points (7 to 240 weeks post-infection) identified potential escape mutations, which were introduced into heterologous viruses to characterise their effects on BCN antibody kinetics.

Results: After 6 months of infection early type-specific V2 neutralising antibodies targeted a PG9-like epitope. Escape from these antibodies through an N167D mutation was associated with the development of BCN antibodies targeting the same epitope. Subsequent escape mutations that deleted the N160 glycan one year later exposed a second vulnerability for BCN antibodies targeting a glycan-dependent epitope in the CD4bs. Early escape from this BCN specificity (eg: N279D) resulted in the emergence of CD4bs antibodies with reduced dependence on the mutated amino acids, and this correlated with an increase in neutralisation breadth.

Conclusion: These data suggest that rare V2 immunotypes selected for by anti-V2 antibodies exposed the CD4bs to antibodies with a novel glycan-dependent mechanism of neutralisation, representing a major sub-epitope of the CD4bs that may be important for vaccine design. Additionally we identified the escape pathways from V2 and CD4bs antibodies that drove the development of neutralisation breadth. This information on the interactions between HIV-1 and its host identity templates for sequential immunisation strategies aimed at inducing BCN antibodies to both V2 and the CD4bs.
**P03.53**

**Arginine-Scanning of PG16 Paratope Defines Quaternary Epitope**

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**Background:** PG9 and PG16 are broad and potent neutralizing antibodies that target HIV-1, neutralizing 72-78% of viral strains with an average IC50 of 0.2 µg/ml. These somatic variants preferentially bind a quaternary epitope on the HIV-1 gp120 envelope glycoprotein, contained in V2 and possibly V3. Crystal structures of PG9 and PG16 bound to scaffolded V1/V2 show they recognize a site of vulnerability comprising two glycans and a strand. The glycan at position Asn160, which is required for neutralization, is Man5GlcNAc2, whereas some diversity is allowed at a second site, located at Asn156 or Asn173.

**Methods:** To define the PG16 paratope, we developed a method that bioinformatically identifies surface residues capable of being mutated to arginine without disturbing the PG16 structure, and used this method to define a collection of PG16 arginine mutants, each separated by ~5 Å. We individually mutated 34 residues from the PG16 combining site to arginine, produced the mutated antibodies, and performed neutralization assays on 9 HIV-1 strains.

**Results:** Strain-dependent effects were observed, though the average arginine-defined paratope was contained in the long hammerhead-shaped CDR H3 and in the CDR L2 and L3. This arginine-defined paratope closely matched the crystal structure of PG16 bound to a monomeric scaffolded V1/V2. However, there were a few differences at residues Tyr32, Ala96 and Tyr100s of the heavy chain and Val51 of the light chain. Remarkably, these residues were those predicted to interact with a neighboring Asn160 glycan, based on the recent cryoEM structure of PG9 bound to a trimeric BG505 gp140 protein.

**Conclusion:** Our data provide a new method, arginine “pin-cushioning”, to determine the interactive surface of a protein. We show here how this method can be used to guide the definition of an antibody interacting with the HIV-1 viral spike, even if the epitope is quaternary in nature.

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**P03.54**

**A Mechanistic Understanding of Immune Escape Pathways in the HIV-1 Envelope Glycoprotein**

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**Background:** The extraordinary genetic diversity and the conformational plasticity of HIV-1 Env proteins, gp120 and gp41, present a formidable obstacle for effective immune control and vaccine design. Mutations that are distant from the antibody binding site can lead to escape, probably by changing the conformation or dynamics of Env; however, these changes are difficult to identify and define mechanistically. Here we develop a network analysis-based approach to identify potential long-distance immune evasion mechanisms using three known HIV-1 Env gp120 protein structures from two different clades, B and C.

**Methods:** First, correlation and principal component analyses of molecular dynamics simulations identified a high degree of long-distance coupled motions that exist between functionally distant regions within the intrinsic dynamics of the gp120 core, supporting the presence of long-distance communication in gp120. Then, by integrating MD simulations with network theory, we identified the optimal and suboptimal communication pathways and modules within the gp120 core.

**Results:** We show that many different pathways exist for communication between spatially distant sites in gp120 and a suboptimal pathway in one strain can serve as the optimal pathway in another strain. While the long distance coupled motions are highly conserved across the three gp120 cores considered, the shortest route for communication between spatially distant sites in gp120 varied with the sequence. Our analysis indicates that HIV-1 gp120 could retain its function and escape from antibody through mutations that allow it to utilize one of the suboptimal paths if the optimal pathway becomes blocked. Hence, blocking or altering dominant and suboptimal pathways in gp120 should also be considered in vaccination strategies to increase exposure and/or immunogenicity of conserved epitopes.

**Conclusion:** The results unveil both strain-dependent and -independent characteristics of communication pathways in gp120. The identification of conserved elements within these communication pathways, termed inter-modular hotspots, presents a new opportunity for immunogen design.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L1, Room 111 – 112.

Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L1, Room 111 – 112.

**Posters**

**P03.55**

**Association Between HIV-1 Coreceptor Usage and Resistance to Some Broadly Neutralizing Antibodies**

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**Background:** The recent discovery of broadly neutralizing antibodies against many different HIV-1 strains has revitalized hopes of developing a universal vaccine against HIV-1. HIV-1 variants that can only use the CCR5 coreceptor for cell entry are mainly responsible for new infections, while CXCR4-using variants can become dominant in later infection stages.

**Methods:** We performed a statistical analysis on two different previously published data sets. The first data set was a panel of 199 diverse HIV-1 isolates for which IC50 neutralization titers were determined for the broadly neutralizing antibodies VRC01, VRC-PG04, PG9, and PG16. The second data set contained env sequences of viral variants extracted from HIV-1 infected humanized mice treated with the antibody PGT128 as well as from untreated control mice.

**Results:** We found a statistically significant association between viral resistance to the broadly neutralizing antibodies PG9 and PG16 and CXCR4 coreceptor usage (p-value = 0.0011 and p-value = 0.0010, respectively) for the 199-isolate panel. This association was not present for antibodies VRC01 and VRC-PG04 targeting the CD4 binding site. Furthermore, certain amino acids at specific positions of the Env protein sequence were associated significantly with viral sensitivity/resistance to PG9, overlapping partly with amino acids at certain positions significantly associated with coreceptor usage.

Additionally, our analysis on viral variants from HIV-1 infected humanized mice under treatment with the broadly neutralizing antibody PGT128 indicated that certain antibodies might drive a viral population towards developing CXCR4-coreceptor usage capability (p-value = 0.0011 for the comparison between PGT128 and control measurement).

**Conclusion:** This analysis highlights the importance of accounting for a possible coreceptor usage bias for an HIV vaccine as well as for passive antibody transfer treatment. For a vaccine one would want antibodies that are best against CCR5-using viruses and for treatment of salvage patients one might exclude antibodies with a CCR5-bias to avoid a coreceptor switch.

**P03.56**

**Computational and Mutagenesis Analyses of the Epitope of Human V2 mAb 2158**

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**Background:** The RV144 correlates analysis indicated that antibodies targeting the V2 region of gp120 were associated with a lower risk of infection. Thus, structural mapping of all V2 epitopes will be crucial to the design of an effective V2 vaccine. A panel of human mAbs, including mAb 2158, target a conformational V2 region encompassing the α4β7 integrin binding site, and provide a unique opportunity for characterizing this V2 region which was not visualized in the V1V2/PG9, V2/CH58, or V2/CH59 crystal structures.

**Methods:** We constructed a full-length V1V2 model based on the V1V2/PG9 crystal structure using Rosetta 3.4, and systematically computed the in silico stability energy for each residue. The computational analysis identified a group of V2 residues that can either stabilize the epitope region or directly mediate mAb 2158 binding. We then cloned and expressed a set of mutants based on the computational data, and tested these mutants for mAb 2158 binding by an ELISA.

**Results:** Our extensive computational Monte Carlo simulation has led to a structural model of the missing V2 region in the V1V2/PG9 crystal structure, providing a complete V1V2 model with the integrin-binding site. Comparison of our energy stability data with our ELISA results allows us to group V2 residues into two sets which play key roles in the epitope region or directly mediate mAb 2158 binding. We then cloned and expressed a set of mutants based on the computational data, and tested these mutants for mAb 2158 binding by an ELISA.

**Conclusion:** These data provide a model of the binding surface recognized by mAb 2158 and insights for understanding the antigen binding of related V2 antibodies. Our results may contribute to the design of V2-targeted immunogens.
P03.57

HIV-1 Infection and Systematic Lupus Erythematosus Display Distinct IgG VDJ Profiles Defined by Pyrosequencing that Differ from Healthy Individuals

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Background: IgM heavy chain (H) CDR3 transcriptome repertoire has reduced biodiversity in the peripheral blood (PB) in HIV-infection or systematic lupus erythematosus (SLE) reflecting restricted VDJ recombinants compared to healthy controls (HC). We hypothesized that HIV-infection and SLE will have distinct IgG profiles.

Methods: 454-deep sequencing of full-length IgG H VDJ transcriptome (~500 bp) in PB from 4 therapy-naïve or 4 treated HIV-infected young adults (HIV) was compared with 8 age-balanced HC or 3 counterparts with SLE. A customized bioinformatics software, IgSEQ, was developed to automate integrated analysis of somatic hypermutation (SHM) and VDJ allele plus reading frame (RF) use by IMGT system, metagenomics of antibody biodiversity by ESPRIT, and predicted response to antigen. Comparisons among groups were evaluated by Multivariate Analysis of Variance with p<0.05 as significant.

Results: IgG H VDJ transcriptome repertoire in HIV and SLE compared to HC showed a trend toward decreased biodiversity, diminished SHM, particularly nonsilent mutations in CDRs, and reduced proportion of sequences estimated to reflect antigen selection. Repertoire expression of VH3, DH3 with RF2, and JH4 predominated in HC and HIV, whereas SLE had increased use of VH4, DH5 and DH6 with RF3, and JH3. Longer CDR3 lengths (15 to 17 aa) predominated in SLE, in contrast to shorter CDR3 lengths (13 or 14 aa) in HIV, while predominant CDR3 length varied from 13 to 19 aa among healthy individuals. Overall profiles of the IgG repertoire differed between HIV and SLE and were distinct from HC.

Conclusion: Decreased VDJ diversity in both SLE and HIV infection likely reflects polyclonal B cell activation. Mechanisms of VDJ recombination and generation of SHM are intact. Alterations in the IgG repertoire are clearly apparent using deep sequencing, which provides a unique means to monitor the B cell repertoire in health and disease, or following immunization.

P03.58

Structural Characterization of Two Monoclonal Antibodies Targeting the Fourth Constant Domain in the Bridging Sheet of HIV-1 gp120

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Background: The fourth constant domain (C4) of HIV-1 gp120 is a structural element important for the function of HIV-1 envelope complex; it involves in the bindings of the receptor CD4 and co-receptor CCR5/CXCR4. This region is immunogenic to both T cell and B cell, and a number of monoclonal antibodies (Mabs) against this region, named CD4-blockers, have been isolated since 1990s and characterized biologically. However, there has not been any study on their antigen-antibody interactions at the atomic level.

Methods: We determined Fab/epitope complex crystal structures of two neutralizing anti-C4 antibodies, a rabbit Mab R53 and a mouse Mab 11a8. R53 was raised by immunization with recombinant gp120 using a DNA prime-protein boost regimen, while 11a8 with multiple identical copies of covalently linked C4 peptides (MCPs).

Results: Our data show that R53 and 11a8 target at the same epitope region in C4 with slightly different binding modes. The epitope of R53 (431GKAMYAPPIRG441) is shaped like a stretched spring, lying across on top of the light and heavy chains. Its N-terminal part lies in a groove on the heavy chain side while the C-terminal part straddles on a saddle formed by the light chain. The antigen binding site surface of R53 is negatively charged, complementing the epitope with a positively charge residue at each terminus. The epitope of 11a8 (AMYAPPIE) is shaped like a hook, lying between the light chain CDRs and heavy chain CDRs. Although the 11a8 epitope has one negatively charged residue at the C-terminus, its side chain does not involve antibody binding.

Conclusion: This is the first structural characterization of any anti-C4 Mabs whose epitopes consist of a conserved C4 sequence motif AMYAPP, which overlaps with known gp120 T cell epitopes T1 and PS05, thus a potential target for HIV vaccine development.
P03.59

**Fitness Cost of Viral Escape from Broadly Neutralizing CD4-Binding Site Antibodies**

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**Background:** The receptor-binding site on the HIV glycoprotein gp120 is a highly conserved epitope, and certain antibodies directed against this CD4 binding site (CD4bs) such as VRC01 can potently neutralize the majority of HIV-1 isolates. Previous studies demonstrated that HIV can escape from an anti-CD4bs response by accruing mutations in the Loop D and V5 domains of gp120. Although conservation of the CD4bs renders it an ideal target for broad neutralization, it also suggests that viral escape from CD4bs antibodies may reduce affinity for CD4 and lower viral replication.

**Methods:** Envs-pseudoviruses and infectious molecular clones (IMC) were constructed using the near-full length gp160 env gene of YU2 with 4 mutations at residues associated with VRC01 resistance made singly and in combination as well as from autologous env genes from the VRC01 donor (both sensitive and resistant to VRC01). Replication kinetics of IMCs were assessed by in vitro infection of primary CD4 T cells.

**Results:** Two mutations in LoopD and two in V5, have been associated with resistance to VRC01. Introduction of these mutations into YU2 Env resulted in complete resistance to six additional antibodies of the VRC01 class and this IMC displayed severely impaired replication. Singly, the G458D mutation in V5, which conferred resistance to some CD4bs antibodies, also lowered replication. An N279K mutation in LoopD did not affect replication though it conferred partial resistance to the VRC01 class mAbs. Analysis of VRC01-escaped autologous Envs from the donor revealed non-consensus residues at 279 and no replication deficit.

**Conclusion:** These data demonstrate escape from neutralization by the VRC01 class of antibodies can impair replicative fitness of HIV. Viral Envs from the VRC01 donor displayed no reduction in replicative fitness, suggesting that compensatory mutations can restore viral replication over time. The conserved CD4bs is a potentially important target for vaccine-induced antibodies aimed at preventing HIV infection.

P03.60

**Systemic IgG and IgA Responses to gp70V1V2 and C1 in HIV-Infected Elite Controllers Versus Controllers and Noncontrollers on HAART**


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**Background:** Identification of the immune mechanisms which enable HIV-infected elite controllers (EC) to inhibit viral replication will contribute to our understanding of protective immunity and vaccine development for HIV. Based on RV144 trial results, we hypothesized that EC may have greater systemic IgG responses to V1V2 and reduced serum IgA against the C1 domain of gp120 when compared to infected individuals who require highly-active antiretroviral therapy (HAART) to manage disease.

**Methods:** Serum samples collected at a two year interval from age- and race-matched HIV negative women (n=20) and HIV-infected women were obtained from the Women's Interagency HIV Study. Infected subjects were in one of the following groups: 1) EC (n=10) or 2) HAART controllers (HC; n=20) both with viral loads ≤ 80 RNA copies/ml and CD4 cells > 350/µl, and 3) HAART progressors (HP; n=19) with viral loads > 1000 and CD4 < 250/µl. ELISA was used to measure Ig and specific antibodies to gp120, gp41, p24, gp70V1V2 and C1 peptides.

**Results:** Many infected women, including EC, had IgG (but not IgA) hypergammaglobulinemia. EC had greater anti-p24 IgG and IgA responses when compared to HC and HP. Though IgG and IgA responses to gp120 or gp41 were similar in all infection groups, EC more frequently demonstrated anti-gp70V1V2 IgG than HC, and they had higher magnitude responses than HP. Anti-C1 IgA (but not IgG) was detected at low levels and was increased roughly 10-fold in all HIV groups after 2 years. In contrast, systemic IgG and IgA responses to other antigens did not change significantly during this time.

**Conclusion:** These data support a beneficial role of anti-V1V2 IgG in control of HIV infection, but not a detrimental effect of anti-C1 IgA in disease. The results further suggest that B cell abnormalities and undetected viral replication may be occurring in many EC.
P03.61 LB

Exploring the Human Genomic DNA Antibody Repertoire for Development of an Effective HIV-1 Vaccine

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Background: Broadly neutralizing HIV-1 antibodies (bnAbs) are expected to be a key component of immune protection conferred by an effective HIV-1 vaccine. All known HIV-1 bnAbs were isolated from the human cDNA antibody repertoires of “elite controllers” and they exhibited uncommon characteristics. Vaccine immunogens designed based on some of the known bnAbs did not induce the same and similar bnAbs in vivo. The human genomic DNA (gDNA) antibody repertoire has not been investigated for HIV-1 vaccine development.

Methods: Nonimmune (300 uninfected individuals) and three immune human gDNA antibody libraries and the corresponding cDNA antibody libraries were constructed and deep sequenced. Sequence results were analyzed and compared. The human gDNA and cDNA antibody libraries were furthered panned against an engineered envelope glycoprotein RSC3 by phage display.

Results: Human gDNA antibody repertoire was more diverse than the cDNA antibody repertoire in using IGHV lineages. The frequencies of heavy and light chain variable regions that had exactly the same V(D) J recombinations and the same lengths of CDR3s as known HIV-1 bnAbs were extremely low. We isolated a panel of RSC3-specific mAbs from the human gDNA antibody libraries, but did not isolate the same or similar mAbs from the corresponding cDNA libraries. The isolated mAbs competed with bnAbs VRC01 and b12 for binding to RSC3. But unlike VRC01 and b12, these mAbs used various IGHV lineages and exhibited uncommon characteristics. Vaccine immunogens designed based on known bnAbs may not elicit the same or similar bnAbs in vivo.

Conclusion: Our results suggest that antibody diversity in the human antibody gene repertoire is not infinite, and immunogens designed based on known bnAbs may not elicit the same or similar bnAbs in human population for lack of the putative germline antibodies. The gDNA-derived RSC3-specific mAbs may have the potential to mature to VRC01-like bnAbs, but there may be obstacles to their further maturation in vivo.

P03.62 LB D

Analysis of Vaccine-Elicited Antibodies to the HIV-1 gp120 CD4 Binding Site Reveals Distinct Modes of Env Recognition to Guide Immunogen Re-design

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Background: Structure-function studies of broadly and non-broadly neutralizing antibodies directed toward the HIV-1 CD4 binding site (CD4bs) indicate that accessibility to the viral spike is a critical neutralization determinant. We cloned CD4bs-directed antibodies from non-human primates immunized with YU2 gp140-foldon trimers, which neutralize Tier 1, but not Tier 2 isolates.

Methods: To elucidate their limited functional capacity, the Fab fragments of two antibodies, GE136 and GE148, were crystallized, yielding the first high-resolution structures of vaccine-elicited CD4bs-directed antibodies.

Results: Interaction of the antibodies with gp120 and the functional spike were further defined through paratope alanine scanning of their complementarity determining regions. These data, coupled with previous gp120 epitope scanning, were used to generate docking models of the Fabs in complex with gp120, revealing testable high-resolution interactions. Targeted substitution in the mAb paratopes both decreased and enhanced neutralizing activity as predicted by the modeling. EM reconstructions of GE148 and GE136 in complex with gp120 and PGT122, compared to PGV04-gp120-PGT122 EM densities, revealed different angles of binding to the gp120 CD4bs. Fitting the complexes into both cryo-EM and SOSIP EM densities suggested that the vaccine-elicited NHP antibodies attempt to approach the trimeric Env from the “top of the spike” thereby engendering clashes with the glycosylated variable region cap of primary isolates. This angle of approach contrasts with the more “side approach” taken by the bnAbs VRC01 and PGV04. Subsequent EM analysis confirmed that the YU2 gp140-foldon trimers are too open in conformation, suggesting the design of V loop-capped trimers to restrict access of B cells to the CD4bs by a “lateral only” mode of engagement. We have generated well-ordered 2nd generation soluble trimers as defined by EM and antigenicity (Guenaga et al Abstract).

Conclusion: We conclude that well-ordered trimers provide reagents to test the hypothesis whether faithful mimics of the native spike will elicit neutralizing antibodies.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L1, Room 111 – 112.

Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L1, Room 111 – 112.

**P03.63 LB**

**Antibodies VRC01 and 10E8 Require Minimal Somatic Framework Mutations to Achieve High Neutralization Breadth and Potency**

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**Background:** The majority of broadly neutralizing antibodies (bNAbs) against HIV-1 are characterized by substantially increased frequencies of somatic mutations. Moreover, mutations within the antibody framework regions, which are typically less associated with direct antigen interactions, have been shown to be generally required for broad and potent neutralization.

**Methods:** We utilized a partial framework reversion approach for two bNAbs targeting two different epitopes on the HIV-1 envelope: CD4-binding-site antibody VRC01 with 42% heavy/28% light and MPER antibody 10E8 with 22% heavy/17% light chain framework mutations. First, the mature CDR regions were transplanted onto a putative human germline gene. Next, a set of germline framework residues were selected for mutation to their mature counterparts based on structure and sequence considerations. Three constructs with different numbers of framework mutations were created for each of the heavy and light chains of VRC01 and 10E8. Partial revertants were tested for neutralization on a panel of 21 diverse HIV-1 strains.

**Results:** A total of ten constructs combining partially reverted and/or mature heavy and light chains were created for each of VRC01 and 10E8. The VRC01 variant with no framework mutations (100% framework reversion) showed complete loss of neutralization activity, whereas the analogous 10E8 variant had a ~10-fold loss in potency. Interestingly, variants with ~80% (VRC01) and ~90% (10E8) framework reversion had a ~1.5-fold and ~3-fold loss in potency, respectively. For both VRC01 and 10E8, partial revertants with breadth and potency similar to the mature antibodies were identified. For VRC01, a light chain with ~50% reversion of framework mutations showed a ~2-fold improvement in potency over the mature antibody.

**Conclusion:** Our results indicate that many antibody mutations found in mature anti-HIV-1 antibodies may not be necessary for broad and potent neutralization. BNAbs with lower numbers of mutations can be of utility as antibody therapeutics and as targets for immunogen design.

**P03.64 LB**

**Neutralizing Antibody to HIV-1 gp41 MPER Alters Recognition Properties of the Infectious Spike**

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**Background:** Human antibody 10E8 exhibits potent cross-clade neutralization against HIV-1 that has been linked to a helix in the membrane proximal external region (MPER) of envelope glycoprotein (Env) gp41. However, the affinity of antibodies for MPER antigens does not correlate with neutralization suggesting that some feature is unaccounted for on native, trimeric spikes.

**Methods:** Mutations that have been shown to diminish 10E8 binding to MPER peptides were introduced into different HIV-1 Envs and tested in thermostability assays and neutralization assays in different formats. Various antibodies and inhibitors were also tested against HIV-1 in the presence or absence of 10E8. Binding of 10E8 to native Env was examined using gel mobility shift native PAGE and Western blot.

**Results:** Several MPER mutants showed complex behavior in neutralization assays with 10E8 in which low concentrations of 10E8 partially neutralized viral infectivity but saturating concentrations did not fully block infection. Surprisingly, we find that when saturated with 10E8, such mutant viruses become less sensitive to other MPER antibodies and to soluble CD4 while becoming more sensitive to antibodies and inhibitors against distal sites on gp41. At saturation, 10E8 binds to wild-type and MPER mutant spikes with 3:1 and 1:1 stoichiometry, respectively. Hence, at partial occupancy by 10E8 mutant spikes can be infectious but have altered sensitivity to ligands. Moreover, 10E8 accelerates functional decay and physical disruption of wild-type HIV-1 Env but can stabilize MPER mutant Envs. Partial neutralization by 10E8 is also shown to be modulated by Env glycosylation.

**Conclusion:** We describe a novel ability of an antibody to stabilize, partially neutralize and alter neutralization sensitivity of a virion spike. Our results identify quaternary structure-dependent features of HIV-1 Env that are crucial to MPER recognition by antibody 10E8, which has implications for immunoprophylaxis and vaccine design.
**P03.65 LB**

**High Proportion of Simian Immunodeficiency Virus gp120-Reactive Antibodies in B Cell Repertoire of Chronically Infected African Green Monkeys**

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**Background:** Natural hosts of Simian Immunodeficiency Virus (SIV), such as African green monkeys (AGMs), rarely transmit the virus to their infant. Moreover, SIV-infected AGMs have strong autologous virus neutralizing antibody responses in milk, unlike humans and rhesus monkeys. Therefore, defining the SIV Envelope (Env)-specific B cell repertoire of AGMs could guide development of vaccines that elicit protective mucosal antibody responses.

**Methods:** The constant and variable gene segments of immunoglobulin (Ig) heavy- and light-chain \((V_H\) and \(V_L)\) were identified in the AGM genome using human and rhesus Ig gene sequences. The Ig genes were amplified by RT/PCR from single memory B cells sorted by flow cytometry from blood and milk of four SIV-infected lactating AGMs and used to produce recombinant monoclonal antibodies (mAbs) for characterizing their specificities.

**Results:** We localized the constant and variable gene segments of \(V_{H}\), \(V_{K}\) and \(V_{L}\) to AGM chromosomes 24, 14, and 19 respectively. Numbers of AGM \(V_{H}\), \(V_{K}\) and \(V_{L}\) gene segments were comparable to that of humans. We found a significantly higher proportion of memory B cells isolated from blood (18.3%) and milk (29%) of SIV-infected AGMs \((n=4)\) produced Env-specific mAbs than that of memory B cells populations isolated from blood (0.6%); and milk (7.3%) of chronically HIV-infected individuals \((n=7)\) \((p<0.0001\) and \(p=0.004\), respectively, Fisher’s exact). Moreover, a higher proportion of the Env-specific AGM blood and milk antibodies were directed against gp120 (11.0% and 10.0% respectively) than gp41 (3.7% and 5.0% respectively). Usage of the \(V_{H}\)\(^{<4}_{4}--59\) human ortholog was overrepresented in both blood and milk Env-reactive (46.7% and 24.1% respectively) and non-reactive (17.9% and 23.9% respectively) AGM antibodies.

**Conclusion:** A considerably larger proportion of the SIV-infected AGM B cell repertoire is committed to responding to the SIV Env than that of HIV-infected humans. Isolation of these AGM antibodies will facilitate exploration of their transmission-blocking properties.

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**P03.66 LB**

**Crystal Structure of a Cleaved, Stabilized HIV-1 Env Trimer in Complex with a Glycan-Dependent Broadly Neutralizing Antibody at 4.7 Å**

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**Background:** The functional, cleaved HIV-1 Env trimer is central to vaccine design strategies due to its role in HIV-1 entry, the first step of the viral infection causative of AIDS. Although structures of portions of its individual core subunits, gp120 and gp41, has been achieved, structural characterization of an intact, soluble HIV-1 Env trimer has thus far remained elusive.

**Methods:** Here, we describe the crystal structure at 4.7 Å of an antigenically near-native, cleaved, stabilized recombinant HIV-1 Env construct of isolate BG505 (termed SOSIP.664) in complex with a glycan-dependent, broad and potent neutralizing antibody (bnAb), PGT122.

**Results:** In addition to corroborating previously described high-resolution crystal structures of core gp120 and gp120 V1/V2, the cleaved, stabilized HIV-1 Env trimer crystal structure reveals unique features and organization of the gp120 V1/V2/V3 loops and insights into gp41 structure in the context of gp120. Furthermore, the co-crystal structure delineates the full epitope of PGT122, that includes gp120 V1 and V3, in addition to several surrounding glycans. The cleaved, stabilized HIV-1 Env trimer structure also provides insights into the delineation of the complete epitopes for other bnAbs, where only partial information was previously available.

**Conclusion:** Together, structural details of the antigenically near-native HIV-1 Env SOSIP.664 provide a blueprint for structure-based vaccine design strategies looking to re-elicit bnAbs with unusual features, as well as provide key insights into Env function.
Distinct Changes in B Cell Subsets in Rhesus Macaques in Blood and Lymph Node During Chronic Viral Infections and Acute SIV Infection

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Background: Studies in humans and rhesus macaques (RM) suggest that general B cell dysfunction including hypergammaglobulinemia, polyclonal B cell activation, and delayed antibody maturation are associated with HIV/SIV infection. Mechanisms for B cell dysfunction during HIV/SIV infection have not been thoroughly determined.

Methods: B cell subset profiles in blood were first compared between healthy RM and humans and between healthy SPF and non-SPF RM. Next changes in B cell compartments in blood were examined during acute SIV infection in the RM model for HIV. Adult female SPF RMs were challenged weekly intravaginally with a low dose of SIVmac251 until animals demonstrated persistent plasma viremia. Peripheral blood samples were assessed longitudinally by flow cytometric analysis and ELISA. Dynamics of SIV antibody development and avidity were compared to antibody responses to other antigens including rhesus cytomegalovirus (RhCMV) during acute and early chronic SIV infection.

The composition of peripheral B cells, including naïve B, resting memory B, activated memory B, and plasmablast subsets during the acute phase of infection was compared to the pre-SIV-infected state.

Results: Firstly, significant differences in B cell subsets were noted between healthy RM and humans and between healthy SPF and non-SPF RM. Regarding SIV infection, peripheral CD20+ B cell counts exhibited a substantial drop corresponding with a peak in viral titer within the first two weeks of acute infection and rebounded subsequently. Acute SIV infection induced significant changes in distribution of B cell memory subsets and defects in SIV-specific antibody maturation. Additional findings regarding B cell and T follicular helper cell subsets in lymph nodes during early chronic infection will be presented.

Conclusion: Our findings demonstrate distinct patterns of B cell subset alterations in circulation caused by chronic infections in general and by SIV infection. These findings will contribute to understanding mechanisms of B-cell dysfunction in HIV-associated disease.

Investigation of Antibody Responses Induced in RV305 a Late Boost Vaccination of HIV-1 Uninfected Volunteers that Participated in RV44, a Thai Trial

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Background: RV305 is a randomized, double-blinded, placebo controlled study designed to investigate immune responses generated after a late boost in RV44 volunteers who completed the full vaccine series given 6-8 years earlier. Healthy, HIV uninfected volunteers from RV44 were enrolled and received 2 injections of ALVAC-HIV or AIDSVAX-B/E or a combination of ALVAC-HIV/AIDSVAX-B/E at day 0 and 6 months.

Methods: IgG and IgA responses at baseline and 2 weeks post first and second vaccination were tested by ELISA against HIV-1 envelope protein, gp120 CRF01_AE (A244), and gp70V1V2 scaffolds subtype E (92TH023) and subtype B (CaseA2). Antibody responses were compared to those induced in RV44 at 2 weeks post last vaccination in the primary vaccination series.

Results: At baseline, antibodies to gp120 were similar in all vaccination groups (geometric mean titers (GMT) ranging 148-183). After the first vaccination, gp120 GMT in ALVAC-HIV/AIDSVAX-B/E group increased to 23,387 followed by AIDSVAX-B/E to 12,068 and ALVAC-HIV to 279. Responses dropped 6 months post first vaccination in all groups, but increased again following the second injection, although lower than those following the first injection. After the first injection, ALVAC-HIV/AIDSVAX-B/E group also provided higher responses against gp70V1V2 subtype E (GMT=7,903) and subtype B (GMT=610) compared to AIDSVAX-B/E group, subtype E: GMT=3,294 and subtype B: GMT=81 and subtype E: GMT=50. Weak gp120 IgA responses were induced in ALVAC-HIV/ AIDSVAX-B/E (GMT=70) and AIDSVAX-B/E (GMT=69) but not in the ALVAC-HIV group.

Conclusion: Residual antibody responses against gp120 were detected 6-8 years post-vaccination in RV44. Late boosted IgG responses were highest following ALVAC-HIV/AIDSVAX-B/E vaccination followed by AIDSVAX-B/E and ALVAC-HIV alone. Although ALVAC-HIV alone generated weak responses, it potentiated antibody responses when co-administered with AIDSVAX-B/E. IgA responses to all antigens tested were similar in ALVAC-HIV/AIDSVAX-B/E and AIDSVAX-B/E groups suggesting that ALVAC-HIV does not substantially contribute to IgA.
P03.69 LB

Genetic Assessment of B Cell Responses Following Immunization with HIV Env Protein and Adjuvants or Pathogenic SHIV Infection in Non-human Primates

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Background: A highly effective preventive vaccine against HIV-1 will require the generation of potent neutralizing antibody responses against the HIV envelope (Env). Recent evidence shows that broadly-neutralizing antibodies from HIV+ individuals often have unique characteristics such as extensive somatic hypermutation (SHM), long CDRH3 regions and V\_H family restriction.

Methods: To prospectively study these functional characteristics, we characterized Env-specific B cells using sample barcoding and 454 deep sequencing in nonhuman primates (NHP) following a highly pathogenic SHIV-AD8 infection or vaccination with HIV Env clade C protein with a variety of adjuvants.

Results: The initial analysis from a group of 8 NHP infected with SHIV shows progressively increasing levels of SHM after ~100 weeks of infection in only certain animals; this correlated with greater neutralization against a broad panel of HIV isolates. Indeed, SHM increased as high as 66% over basal levels. In contrast, animals with weak neutralization responses maintained basal levels of SHM of only ~8% divergence from their putative germlines. Furthermore, greater SHM and neutralization correlated with higher viral loads and trended toward lower CD4\(^+\) T cell counts. To compare the genetic attributes of Env-specific B cells responding to vaccination, NHP were immunized four times with a Clade C trimeric Env protein alone, with alum or MF59 with or without a TLR4 or TLR7 agonist, polyICLC or ISCOMs. Robust Env-specific B cell responses- as high as 20% of IgG cells—were detected across multiple adjuvant groups. However, preliminary analysis suggests that the adjuvant formulations tested here do not significantly affect SHM. Differences in neutralization between adjuvant groups instead correlate with overall Env binding titers. Further studies assessing CDRH3 length and gene family usage are underway.

Conclusion: Taken together, we have developed NHP vaccine and infection models that utilize next-generation B cell sequencing to understand the development of broadly neutralizing antibody responses.

P03.70 LB

HIV-1 Antibody Epitope Mapping of Individuals Treated with Antiretroviral Therapy During Acute/Early HIV-1-Infection

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Background: One vaccine strategy to overcome the challenge of HIV diversity is to elicit antibodies specific to multiple HIV sequence variants. To better characterize the potential diversity of HIV-specific antibodies in humans, we performed antibody epitope mapping in individuals treated with antiretroviral therapy during acute/early HIV-infection both before and after treatment interruption.

Methods: Plasma from North American individuals treated during acute/early HIV-infection (N=20, undetectable viral load >6 months) and at 20 weeks following treatment interruption (N=8, mean viral load 12,183 copies/ml) were incubated on peptide-microarrays containing 6564 peptides covering 46-72% of all clades for Env, Gag, Pol, Rev, Tat, and Vif. Microarrays were incubated with labeled anti-IgG and imaged by GenePix scanner. Threshold for positivity was >5x noise signal distribution. Positive peptides overlapping >5 amino acids were considered one binding site.

Results: Antibodies from individuals treated during acute/early HIV-infection bound primarily to Env, Pol, and Gag (27, 12, and 9 average sites, respectively). Of all bound gp140 sites, 21% were shared by >85% of subjects (predominantly V3 and gp41 C-C loop). Following treatment interruption, there was significant expansion in average Env breadth (27 vs. 37 sites, P=0.0036) and depth (6 vs. 12 peptides/site, P<0.0001). Binding to sequences that circulate outside North America increased (38 vs. 44% non-B peptides among all bound gp140 peptides, P=0.157), as did the proportion of bound gp140 peptides shared by >85% of subjects (45%, predominantly V2 and gp41 HR2). Overall, there was a strong positive correlation between breadth and depth of antibody binding (R=0.65, P<0.0002).

Conclusion: Individuals treated during acute/early HIV-infection had antibodies specific to dozens of sites along the HIV proteome, with similar binding patterns and substantial cross-clade binding. After HIV antigen levels increased, the average breadth and depth of Env binding expanded significantly. These observations suggest that vaccines designed to elicit diverse HIV-specific antibodies are feasible.
**P03.71 LB**

**Structural Analysis of the Unmutated Common Ancestor Antibodies of the HIV Envelope V2 Antibodies CH58 and CH59 Derived from RV144 Vaccinees**


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**Background:** CH58 and CH59 are antibodies isolated from RV144 vaccinees and bind at lysine (K) 169 of the HIV-1 Env gp120 V2 region. Both mAbs neutralize select tier 1 HIV-1 AE strains and mediate ADCC against virus-infected CD4 T cell targets. Previous crystal structures show that CH58 and CH59 recognize V2 in helical conformations (Immunity 38:176, 2013). The inferred clonal lineages leading to the CH58 and CH59 antibodies show that the mature antibodies are mutated ~2-3% from germline.

**Methods:** To study structural correlates of affinity maturation, crystal structures of the unmutated common ancestor (UCA) antibodies of CH58 and CH59 were determined and compared to published structures of the mature antibodies, respectively.

**Results:** Alanine-substituted peptide analysis demonstrated that the footprints of the CH58 and CH59 UCAs were broader than those of the corresponding mature mAbs. Structural analysis of the mature and UCA antibodies in their unliganded and liganded forms found conformational shifts associated with maturation in the cases of CDRL2 in the CH58 lineage and CDRH1 in the CH59 lineage. A significant conformational shift occurs in CDR-H3 between the unliganded CH58UCA/mature and liganded CH58UCA/mature structures. Both CH58 and CH59 UCA epitopes included the V2 amino acid K169; however the CH58 lineage displayed additional contacts being made with K169 during maturation, whereas the CH59 lineage showed a pre-configured binding site for K169. The CH58 lineage also showed mutations during maturation that served to establish specific polar contacts with ligand.

**Conclusion:** The CH58 and CH59 epitopes narrowed with affinity maturation. Both the CH58 and CH59 lineages showed heavy and light chain CDR conformational changes associated with maturation. The CH58 lineage additionally displayed a CDRH3 conformational ordering associated with binding. Thus, several structural features observed in the mature antibodies are already present in the UCAs, while other features develop with maturation.

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**P03.72 LB**

**Phylogenetic Conservation of a Dominant Antibody Light Chain HIV Env V2 Binding Motif in Human and rhesus Macaque Antibodies**


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**Background:** Antibodies against the first and second variable loops (V1V2) of HIV-1 gp120 were a correlate of transmission risk in the RV144 HIV-1 vaccine trial. One dominant antibody induced in the RV144 trial was centered on a region around lysine (K) at V2 position 169. Four anti-V2 antibodies (CH58, CH59, HG107, HG120) from two RV144 vaccinees were isolated that utilized different VA segments (V6-57 by CH58 and VA3-10 by CH59, HG107 and HG120). All four antibody light chains shared a Glu-Asp (ED) binding motif in the CDRL2 encoded in their germline VLS that for CH58 and CH59 were shown to form salt bridges with two lysines in the V2 epitope. Env V2 K169 has been implicated in a viral genetic study as a site of immune pressure in the RV144 trial.

**Methods:** To determine if the restricted ED motif usage for dominant V2 antibody K169 binding is conserved in rhesus macaques, we isolated antibodies from blood memory B-cells using antigen-specific flow cytometry single cell sorts from rhesus macaques immunized with RV144 vaccine Env proteins.

**Results:** We isolated 19 antibodies in V2-reactive clonal antibody lineages from rhesus macaques immunized with RV144 A244 gp120 Env. Remarkably all 19 V2 antibodies use VA3-17, the rhesus ortholog of human VA3-10 that contains the CDRL2 ED motif.

**Conclusion:** The CDRL2 ED antibody light chain motif represents a restricted sequence required for antibody binding the K169 epitope of the HIV-1 V2 loop that is conserved between man and rhesus macaques. This motif is shared in all human and rhesus K169-targeted V2 antibodies isolated to date. Thus, the dominant antibody response to K169 V2 in both humans and rhesus macaques is highly restricted and limited to usage of two light chains in man and one light chain in rhesus macaques.
**P03.73 LB**

**Genetic and Immunological Evidence for a Role of Env-V3 Antibodies in the RV144 Trial**


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**Background:** The RV144 vaccine trial showed an estimated vaccine efficacy of 31%. The impact of vaccine-induced immune responses can be investigated through studying cases and controls (vaccinees who became infected or not) and breakthrough infections (infected vaccine and placebo recipients).

**Methods:** Studies in vaccinees (case-controls) included: 1) Detection of Env linear epitopes with PepStar peptide microarrays (JPT Peptide Technologies GmbH); 2) Assays of antibody functions such as neutralization (TZM-BL, A3R5 cells), binding, and capture; 3) Isolation of V3-specific mAbs from peripheral B. Cases-only studies included several sieve analysis methods aimed at detecting differences between proteome sequences from breakthrough infections (vaccine vs placebo).

**Results:** Among RV144 vaccinees, peptide microarrays identified a very frequently targeted epitope in V3 (AA304-AA320). When vaccine recipients had low gp120-specific plasma IgA, high responses to this V3 peptide were associated with a decreased risk of HIV infection (OR=0.49, p=0.007). Neutralization from vaccinees’s samples was weak, but due mostly to V3-specific antibodies. Two V3 mAbs were isolated: they bound to epitopes that included site 307 and mediated in vitro ADCC, infectious virion capture, and Tier 1 neutralization.

Comparison of CRF01_AE breakthrough viruses from 43 vaccine and 66 placebo recipients showed that viruses differed significantly between the vaccine and placebo group at V3 site 317 (p=0.040), while site 307 was borderline significant (p=0.065). Vaccine efficacy was estimated at 52% against viruses with the consensus residue matching all the vaccine inserts at 307(I) (CI: 20-72%; p=0.003) and 85% against viruses mismatching the vaccines at 317 (F317X) (CI: 32-97%; p=0.004). Interestingly, sites 307 and 317 are part of the hydrophobic core of V3, which is recognized by multiple neutralizing V3-specific monoclonal antibodies.

**Conclusion:** Evidence of vaccine-specific genetic signatures and antibody targeting in V3 demonstrate how the combined analysis of viral genetics and immune responses can contribute to understanding potential mechanisms of vaccine protection.

**P03.74 LB**

**Comparative Evaluation of Assays for Fc Receptor-Mediated Antibody Effector Functions Targeting HIV-1**

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**Background:** The multiplicity of antibody-effector functions mediated through different receptors and divergent effector cell types has resulted in the development of numerous functional assays. These include antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular viral inhibition (ADCVI), and antibody-dependent cellular phagocytosis (ADCP) assays, as well as multi-parameter effector function arrays. In this study, ten such assays developed in seven different labs were evaluated on a common set of samples.

**Methods:** Labs were provided uniform reagents (when applicable) and performed assays on a total of 140 blinded IgG samples purified from chronic treated (n=31), chronic untreated (n=28), elite controllers (n=31), VAX004 vaccinees (n=30), seronegatives (n=20), 10 matching unfractionated plasma samples, and 12 IVIG, HIVIG, and HIVIG-C control samples. Descriptive and inferential statistical analyses were performed on individual assays and across assays.

**Results:** Most assays incurred a low false positive rate based on the 24 seronegative and IVIG samples. While different summary measures showed moderate-to-high correlations within each assay, readouts amongst different assay types showed weak-to-moderate correlations indicating that they may capture unique functional aspects of the antibody response. Good dynamic ranges were observed, with the ADCV response rate varying from 17% among vaccinees to 90% among chronic untreated subjects, ADCVI response rate from 3% among vaccinees to 29% among chronic treated subjects, and ADC response rate of 3% among vaccinees and 17% among elite controllers. HIV-specific antibodies exhibited potent binding to Fcy receptors IIa, IIb, IIIa and IIIb. The levels of various antibody effector functions were similar among all infected subject groups, but considerably weaker among vaccinees. Contrastingl, IgG4 responses among Vax004 vaccinees were substantially higher than those of IVIG infected subjects.

**Conclusion:** Comparisons among the assays tested here may be used to identify those that capture distinct antibody activities, facilitating assessment of functionally unique humoral responses in future HIV vaccine research efforts.
P03.75 LB

The N276 Glycosylation Site Is Required for HIV-1 Neutralization by the CD4 Binding Site specific HJ16 Monoclonal Antibody

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Background: Immune design for HIV-1 vaccines can be based on epitope identification of naturally occurring neutralizing antibodies in patients. A tier 2 neutralizing monoclonal antibody (mAb), HJ16 has been described by us (Corti D et al. 2010 PloS one 5: e8805). We already established that it recognizes a new epitope in or near the CD4 binding site (CD4bs) that only partially overlaps with the b12 epitope. Here, we identified the critical binding site by resistance induction in V1090, a sensitive CRF02_AG HIV-1

Methods: A 2000-fold resistance was induced by culturing V1090 in activated human mononuclear cells with escalating doses of HJ16. Resistance-associated mutations were revealed by sequencing and confirmed by site-directed mutagenesis. Possible cross-resistance with other gp120 inhibitors was analyzed in the TZMbl neutralization assay.

Results: In 4 dose-escalation studies, the N276D mutation was the only alteration found and was confirmed to be responsible for resistance by site-directed mutagenesis in the homologous CRF02_AG HIV-1 subtype A and a subtype C isolate. This mutation removes an N-linked glycosylation site. The effect of N276D was selective: it failed to confer resistance to several other entry inhibitors. Remarkably, sensitivity to the potent and broad CD4bs VRC01 mAb was increased in the N276D mutated viruses.

Conclusion: The N276 glycan is essential for neutralization by the CD4bs-blocking HJ16, suggesting that its full epitope is a glycopeptide on gp120. This is similar to the PG9 and PGT128 mAbs, which also recognize glycopeptides near the CD4bs. Interestingly, N276 glycosylation hinders recognition and maturation of VRC01-like antibodies, while our data suggest that N276 glycan may be required for HJ16-like antibody induction. Therefore, our observations are of interest to design new Env immunogens aiming at induction of tier 2-type CD4bs specific neutralizing antibodies.

P03.76 LB

Distinct VH Gene Usage and HIV Envelope Specificity of Colostrum and Peripheral B Cell Monoclonal Antibodies of HIV-Infected, Lactating African Women

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Background: A successful HIV vaccine must elicit immune responses that impede mucosal virus transmission, though functional roles of protective HIV Envelope (Env)-specific mucosal antibodies have yet to be established. Isolation and characterization of HIV Env-specific antibodies produced by colostrum B cells could help define mucosal antibody responses that are protective against mucosal virus transmission via breastfeeding.

Methods: To examine the HIV Env-specific B cell repertoire in colostrum, single memory or HIV Env-specific B cells were sorted from colostrum and blood of 17 chronically HIV-1-infected, lactating women. Immunoglobulin heavy and light chain variable region genes (VH and VL) were amplified by nested RT-PCR to produce 49 blood and 35 colostrum HIV Env-specific B cell monoclonal antibodies (mAbs).

Results: All HIV Env-specific colostrum B cell mAbs were IgG1 isotype, while those in blood were IgG1, IgG3, IgD, IgA, and IgM. There was no significant difference in CDR3 length or mutation frequency of VH genes derived from the HIV Env-reactive blood and colostrum B cells. However, Vv gene subfamily 1–69 usage was more frequent among colostrum-derived (51.4%) than blood-derived (20.4%) HIV Env-reactive antibodies (p = 0.005, Fisher’s exact test). In addition, colostrum contained a higher percentage of gp120-directed antibodies and a lower percentage of gp41-directed antibodies than blood (65.7 and 14.3% gp120-specific, and 34.3 and 77.6% gp41-specific, respectively; p < 0.0001, Fisher’s exact test). One cross-compartment HIV Env-specific clonal B cell lineage was identified.

Conclusion: The IgG1 isotype predominance, more restricted Vv gene usage, and higher percentage of gp120-specificity of HIV Env-specific antibodies isolated from colostrum B cells compared to peripheral B cells of HIV-1-infected women suggest selective homing of restricted populations of IgG-secreting memory B cells to the lactating mammary gland. Thus, effective maternal vaccination to eliminate postnatal virus transmission may require specific targeting of this distinct population of mucosal B cells.
**P03.77 LB**

**Vaccine-Elicited B Cell Responses Against the HIV-1 Primary Receptor Binding Site**


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**Background:** Due to the high level of structural variability of HIV-1 Env, elicitation of neutralizing antibody (NAb) responses to conserved neutralizing determinants, such as the CD4 binding site (CD4bs), is a major focus of HIV-1 vaccine development. Accordingly, a detailed understanding of how trimeric Env immunogens activate the naïve primate B cell repertoire and how Env-elicited antibody responses evolve during the course of the immunization schedule may indicate how to direct the response toward desired neutralizing targets.

**Methods:** Recently, we established methodology to characterize NAb responses elicited by soluble Env immunogens in rhesus macaques by antigen/epitope-specific single B cell sorting, RT-PCR to recover immunoglobulin G (IgG) heavy/light chain genes, in vitro expression of IgG, and epitope fine-mapping to the conserved CD4bs. Here, we extend the use of this methodology to conduct a comprehensive analysis of the evolving vaccine-elicited CD4bs-specific B cell response at the clonal level.

**Results:** We interrogated the Ab repertoires from two rhesus macaques following a total of five inoculations with trimeric Env. We observed similar VH gene usage in the CD4bs- and Env-specific memory B cell repertoires while the level of somatic hypermutation was higher following the 5th immunization compared to following the 2nd immunization in both compartments. Interestingly, the heavy chain CDR3 regions from the CD4bs-specific B cells were on average longer than those from the total Env-specific cells and the CD4bs-specific B cell subset has higher level of clonality than that of the Env-specific subset. However, only a small portion of the CD4bs-specific Ig repertoire following the 2nd immunization is maintained following the 5th immunization, which suggests that clonality is only partially maintained and the repertoire is dynamically recruiting new B cells to generate diversity.

**Conclusion:** These data provide an improved understanding of the evolving B cell response following Env immunization.

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**P03.78 LB**

**Computational Analysis of Anti-HIV-1 Antibody Neutralization Panel Data to Identify Potential Functional Epitope Residues**

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**Background:** Advances in single cell antibody cloning methods have led to the identification of a variety of broadly neutralizing anti-HIV-1 antibodies. Initial characterization of these antibodies often involves measurement of their neutralization activity against a panel of viruses, but such experiments do not generally lead to conclusive identification of an antibody’s epitope.

**Methods:** We developed a computational tool, Antibody Database, to help identify critical residues on Env whose natural variation affects antibody activity. Our simplifying assumption was that for a given antibody, a significant portion of the dispersion of neutralization activity across a panel of HIV-1 strains is due to the amino acid identity or glycosylation state at a small number of specific sites, each acting independently. A model of an antibody’s neutralization IC50 was developed in which each site contributes a term to the logarithm of the modeled IC50. The analysis program attempts to determine the set of rules that minimizes the sum of the residuals between observed and modeled IC50s.

**Results:** As a test case, we analyzed antibody 8ANC195, an anti-gp120 antibody of unknown specificity. The model for this antibody indicated that glycosylation sites at Env positions 234 and 276 were critical for neutralization. We evaluated this prediction by measuring neutralization potencies of 8ANC195 against HIV-1 in vitro and in an antibody therapy experiment in humanized mice.

**Conclusion:** These experiments confirmed that 8ANC195 represents a distinct class of glycans-dependent anti-HIV-1 antibody and validated the utility of computational analysis of neutralization panel data. The Antibody Database program implements this analysis as well as providing an environment where sequence, neutralization, and structural data can be examined together.
Background: DNA vaccines have typically been safe in clinical trials, but weakly immunogenic. Strategies to enhance their immunogenicity include i) co-administration of adjuvants and ii) intramuscular administration by in vivo electroporation (IM/EP).

Methods: Seventy-five HIV-uninfected healthy adults were enrolled into a randomized, double blind, placebo controlled trial. Multi-antigenic HIV plasmid DNA (HIVMAG) vaccine encoding clade B gag-pol, env, nef-tat-vif (3mg/dose), alone or co-administered with pDNA IL12 given IM/EP using TriGridTM Delivery System, and recombinant Ad35 vaccine containing HIV-1 subtype A gag, RT, int, nef and gp140 env genes (Ad35-GRIN/ENV; 2x10^11 vp) given IM were tested in different prime-boost regimens (M0,1,2 + M6 or M0+M4). Group 1: HIVMAG (x3)-Ad35 (x1), Group 2: HIVMAG+100ug IL12 (x3)-Ad35 (x1), Group 3: HIVMAG+1000ug IL12 (x3)-Ad35 (x1), Group 4: HIVMAG+1000ug IL12 (x1)-Ad35 (x1), Group 5: Ad35 (x1)-HIVMAG+1000ug IL12 (x1).

All IM/EP vaccinations required bilateral administrations, one into each deltoid. Safety, tolerability and immunogenicity were assessed at predetermined time points.

Results: Both vaccines were safe and well-tolerated. All but one local and all systemic reactivity endpoints were mild or moderate in severity. No SAEs were reported. Preliminary group un-blinded IFNy ELISPOT results show that 2 weeks post 3rd dose DNA prime the proportions of vaccine responders, and measures of magnitude and breadth of vaccine responders, and measures of magnitude and breadth of CD8+ T cell responses to six peptide pools staining were 87%, 83%, and 82% of Groups 1, 2, and 3, respectively. All IM/EP vaccinations required bilateral administrations, one into each deltoid. Safety, tolerability and immunogenicity were assessed at predetermined time points.

Conclusion: Repeated administration of HIV MAG +/-IL12 by IM/EP was acceptable among African volunteers. Preliminary ELISPOT data showed that HIVMAG by IM/EP was immunogenic but currently there is no clear indication that pDNA IL12 enhanced the immune responses.

Background: HIV-specific CTL responses help control HIV replication. Immunodominance towards certain antigens may limit the breadth of responses. We evaluated whether polytopic vaccination – administration of immunogens in multiple anatomical sites – alters HIV-specific immunity.

Methods: We conducted a randomized, double-blind trial comparing three vaccine administration strategies at one time point: Group 1) VRC rAd5 Gag-Pol/Env A/B/C (1x10^10 PU) was administered in one deltoid; Group 2) the four vaccine antigenic components were administered separately in each deltoid or thigh; and Group 3) the vaccine was administered at one-quarter dose (0.25x10^10 PU) in each of four anatomic sites. T cell responses to six peptide pools matching the vaccine inserts were assessed by intracellular cytokine staining.

Results: 90 participants were enrolled, including 56 men and 34 women; primary immunogenicity data at week 4 post-immunization were available on 87. CD8+ T cell responses to any peptide pool were seen in 87%, 83%, and 82% of Groups 1, 2, and 3, respectively. However, there were more responders in Group 3 vs Group 1 to Pol (81% vs 45%, p=0.01) and EnvA (68% vs 40%, p=0.04), and more in Group 2 vs Group 1 to EnvB (66% vs 37%, p=0.04). The magnitude of CD8+ T cell responses was greater in Group 3 than Group 1 against any peptide pool and any Env pool (p=0.002 and 0.014). There were no differences in magnitude of CD8+ T cell responses between Groups 2 and 1. The breadth of CD8+ T cell responses was greater in Group 3 than Group 1 (responses to 3.5 vs 2.8 of 6 pools, p=0.03), but did not significantly differ between Groups 2 and 1.

Conclusion: Polytopic administration can modify the proportion of vaccine responders, and measures of magnitude and breadth of cellular immune responses. Epitope mapping and antibody binding studies are underway to characterize differences further.
Distinct HIV-Specific Antibody Fc-Profiles in RV144 and VAX003 Vaccinees

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Background: The RV144 ALVAC-HIV/AIDSVAX® B/E regimen (2 vaccinations prime and 2 protein boosts) showed an estimated 31.2% efficacy against HIV infection, whereas VAX003, consisting of only AIDSVAX® B/E (7 protein vaccinations), was not protective. As protection in RV144 was observed in the absence of neutralizing antibody (Ab) activity or cytotoxic T cell responses, but was associated with Ab binding to specific viral variants across the V2-loop, we speculated that qualitative differences in the specificity and/or Fc-effector profiles of Abs may have accounted for the observed differences in the two trials.

Methods: Abs were purified from 100 RV144 study subjects (80 vaccine and 20 placebo recipients taken weeks 0 and 2 weeks post final vaccination), 50 Vax003 vaccinees (taken 2 weeks post 2nd, 3rd and final vaccination) and 10 Vax003 placebo recipients (taken 2 weeks post final vaccination). IgGs were assayed for gp120-specific ADCC, AIDC, NK degranulation and cytokine production and Ab isotype selection to specific HIV-1 proteins and V2-loop breakthrough viral epitope sequences.

Results: Here we show that the RV144 regimen elicited highly coordinated Fc-mediated effector responses, with the selective induction of highly functional IgG1 and IgG3 Abs. By contrast, VAX003 elicited monofunctional Ab responses influenced by IgG4 selection. Significantly weaker IgG titers and Fc-mediated immune responses were observed from VAX003 subjects post 2nd vaccination. Additional VAX003 protein vaccinations did, however, increase Fc-mediated responses, and IgG1 and IgG4 subclass titers, but not IgG3 titers, which remained low despite additional protein vaccination. Moreover, only RV144 induced IgG1 and IgG3 Abs targeting the crown of the HIV envelope V2-loop, albeit with low coverage of breakthrough viral sequences.

Conclusion: These data suggest that subclass selection differences associated with coordinated humoral functional responses targeting strain-specific protective V2-loop epitopes may underlie differences in vaccine efficacy observed across the trials.
Background: To overcome the problems of HIV-1 diversity in the development of an effective vaccine, we have designed a universal immunogen HIVconsv utilizing the most conserved regions of the HIV-1 proteome across the major clades.

Methods: A gene coding for HIVconsv was inserted into plasmid (DNA; D), modified vaccinia virus Ankara (MVA; M) and non-replicating chimpanzee adenovirus (ChAdV-63; C) and used in heterologous prime-boost vaccine regimens in HIV-1-negative healthy adults in Oxford. Here, we determined the T cell response breadth at last bleed of the clinical trial (week 28) and employed fresh ex vivo responses at 1 year after the last vaccine administration to determine the longevity of these responses.

Results: Frozen PBMC from 17 participants (CM: 7 and DDDCM: 10) were used to map specificity using a panel of 199 overlapping peptides corresponding to the entire HIVconsv immunogen. Cells were expanded for 10 days using six peptide pools and then tested in IFN-γ ELISPOT assays against individual pool peptides. Participants responded to a median of 13 individual peptides (range: 4-30) and 10 (range 3-19) individual epitopes, of which approximately 10% and 59% were derived from Gag and Pol proteins. 20% were functional, spanning two adjacent conserved regions. 60% of the responses were to peptides that contained known T cell epitopes matching the participants HLA types. Follow up samples were obtained from 12 participants (CM: 5 and DDDCM: 7). Fresh, ex vivo IFN-γ ELISPOT responses measured against six peptide pools showed detectable responses in both groups with median 600 and 1535 total SFU/106 PBMC, respectively. In both groups the pattern of responses to individual pools was maintained with reference to peak responses.

Conclusion: HIVconsv vaccination induced T cell responses that lasted at least one year. Vaccinees’ T cell responses map to epitopes, including previously identified CD8+ CTL epitopes, across the length of HIVconsv.
P04.07
In Vivo Targeting of HIV Gag to Dendritic Cells given with Poly ICLC Is Safe and Induces Durable CD4 T Cell and B Cell Responses in Healthy Volunteers

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Background: In vivo delivery of HIV antigens within α-DEC 205 antibodies to maturing dendritic cells in combination with maturation stimuli is a potential new vaccine platform. This phase-I study evaluates the safety and immunogenicity of DEC-targeting of HIV gag p24 in combination with poly ICLC in healthy volunteers.

Methods: 45 volunteers aged 18-60 were enrolled. 9 volunteers per dosage group (low: 0.3mg; mid: 1.0mg; high: 3.0mg) received α-DEC205-HIVp24 mAb plus 1.6 mg of poly ICLC s.c., 3 received poly ICLC only, and 3 received saline. Volunteers were vaccinated at weeks 0, 4, 12 and followed for 12 months.

Results: Transient local and systemic reactogenicity occurred, without vaccine-related serious adverse events to date. Gag p24-specific IgG was induced in 9/15 (60%), 9 received vaccine plus adjuvant) volunteers in all groups. Responses persisted at least for 12 months after last dose in most responders. After last immunization, response rates by IFN-γ ELISPOT were 44%, 56% and 33% in the low, mid and high dose groups, respectively. Responses were detected in 10-25% of vaccines 12 months after last dose. Blinded intracellular cytokine staining data showed that IL-2 and TNF-α were the predominant cytokines. For CD4+ cells producing IL-2 or TNF-α, response rates ranged from 33 to 40% (5-6/15 volunteers, 9 received vaccine plus adjuvant), 20 to 27% (3-4/15 volunteers) and 43-50% (6-7/15 volunteers) in the low, mid and high dose groups respectively. Among positive responders, the median magnitude across dose groups were similar, with median magnitudes ranging from 0.06 to 0.16% for IFN-γ+, cells, 0.08 to 0.23% for IL2+, and 0.06 to 0.16% for TNF-α+ cells.

Conclusion: This novel DC-targeted protein HIV vaccine in combination with poly ICLC is safe and immunogenic in humans. Cellular and humoral immune responses are induced. Antibody responses remain detectable at 12 months after last immunization in most responders.

P04.08
HVTN Mentored Research Program for Black and Latino/a Medical Students Increases Intent to Pursue a Career in HIV/AIDS Vaccine Research

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Background: Blacks and Latino/as are disproportionately affected by the HIV epidemic, yet clinician scientists of color are severely underrepresented in the HIV/AIDS vaccine trial community.

Methods: The National Institute of Allergy and Infectious Diseases (NIAID)-funded HIV Vaccine Trials Network (HVTN) conducted formative research with Black and Latino/a senior HIV researcher, assembled an advisory group, developed a program logic model, and designed the Research and Mentorship Program (RAMP) for U.S. medical students. In September 2010, HVTN released the first RFA, with subsequent releases in 2011 and 2012. For all three releases combined, HVTN was contacted by 196 interested medical students of color, 59 competitive applications were received and reviewed, and 21 scholars were selected and awarded support from NIAID and the National Institute of Mental Health (NIMH) through the HVTN. Evaluation results presented here include pre- and post-program surveys, knowledge assessments, and hour-long semi-structured interviews with the 6 scholars and 9 of 11 mentors from the pilot year.

Results: Scholars conducted 3-12 month mentored research projects at global HVTN clinical research sites and presented their results at an HVTN conference. Training workshops included orientations for scholars and mentors, introduction to HIV vaccines, cultural responsiveness, and professional development. Compared to baseline, scholars’ self-reported skill in research methods increased 29%. Their knowledge of career opportunities in HIV vaccine research more than doubled and intent to pursue a career in HIV/AIDS vaccine research increased 64%. All mentors agreed or strongly agreed that they expected to maintain a relationship with their scholar in the future.

Conclusion: HVTN with NIMH strategically designed and implemented a mentored research program to increase the number of future Black and Latino/a HIV vaccine investigators by reaching early into the career pipeline and providing strong mentorship through a structured research project. Continued follow-up of scholars will provide additional data on HIV vaccine career outcomes.
Acceptability and Quality of New Mucosal Secretion Collection Procedures in an HIV Vaccine Trial in Thailand


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Background: The assessment of vaccine-induced mucosal immune responses may be critical for the development of a successful HIV-1 vaccine. RV305, conducted in previously vaccinated RV144 volunteers, aims at assessing the effect of late boosts on systemic and mucosal immunological responses. This study assessed the acceptability, tolerability, and quality of mucosal secretions in a population where use of intra-vaginal devices and rectal exams are uncommon.

Methods: RV305 enrolled 162 healthy volunteers who previously received the RV144 vaccine regimen. Women were asked to provide only cervico-vaginal secretions by insertion of a cervical soft cup and men to provide semen (masturbation) and rectal exams (sponge via anoscope). Female volunteers underwent Pap smears and all were tested for genital infections prior mucosal collection. Samples were tested for microscopic blood contamination. Counseling notes and source documents were reviewed to assess reasons for participation or refusal in these collection procedures.

Results: Acceptability rates were 89.7%, 86.1% and 23.4% for cervico-vaginal, semen and rectal secretions, respectively. Factors contributing to high acceptability rates included comprehensive counseling and education and prior relationship between the staff and RV144 volunteers enrolled in this trial. Up to 90% of cervico-vaginal and rectal specimens collected were suitable for immunological testing and were not contaminated with blood. Overall, acceptability for cervico-vaginal secretion collection using the soft cup method and for semen collection was high, but acceptability of rectal secretions was low.

Conclusion: These findings demonstrate that uncommon methods for mucosal secretion collection can be introduced to healthy volunteers with appropriate education and counseling. However, the acceptability of more uncomfortable procedures such as rectal secretions collection remains low.

Building Community Support for the NICCAV Project: Experience on the Field

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Background: The Nigerian Canadian Collaboration on AIDS Vaccine (NICCAV) community engagement programme developed a model to enable it achieve its four objectives of ensuring communities are actively engaged with the HIV Vaccine demonstration project. These are: to constitute a CAB that shall effectively engage with the research team; Build the capacity of identified CSOs to enable them address the research literacy needs of potential research volunteers; Build the capacity of the media to effectively educate the general public and other stakeholders on HIV vaccine research; and implement the GPP for the NICCAV project.

Methods: An independent Community Organisation was engaged on the project to facilitate the community engagement programme. Community mapping was conducted; identified community stakeholders and leaders were then reached through advocacy visits. Sixteen volunteer CSOs working within Jos metropolis had their capacity built to enable them conduct research literacy within their constituencies. Through community support, a CAB was constituted to play an advisory role to the project. Identified media persons were enrolled in a capacity building programme to enable them disseminate culturally and linguistically appropriate information on HIV vaccine research.

Results: The community mapping led to the zoning of the Jos metropolis, identification of populations, groups to be engaged with the project, locations where potential volunteers can be identified for educational and recruitment efforts. Advocacy visits to identified stakeholders was conducted. The structured capacity building efforts for CAB and CSO enables them to conduct community education and ensure research literacy. The strategic efforts to build the capacity of identified media partners led to more publications on new HIV prevention technology in the country. In 7 months, the project had reached over 10,000 persons in the community with HIV prevention research literacy information.

Conclusion: Investment in a structured community engagement programme results in sustainable project impact especially with respect to research literacy.
Strategy to Conduct Vaccine Trials: The GHESKIO Experience

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Background: GHESKIO has developed an integrated, holistic model of prevention and care for HIV and other infectious diseases based on research conducted in Haiti. GHESKIO is one of the largest sites working in the community to conduct HIV vaccine trials in the region. We describe the implementation strategy and outcomes of 10 vaccines studies conducted with support of HIV Vaccine Trials Network (HVTN) and the Haitian Ministry of Health.

Methods: The vaccine interventions included: 1) Preparation phase: Political support, community and staff training, development of educative materials, recruitment and retention plans; assessment of volunteers’ critical needs for incentives and services; laboratory capacity building and contingency plan development. 2) Implementation Phase: Multidisciplinary team approach comprised of physician, social worker, peer educators, nurses, data manager, laboratory, psychologist, site coordinator, and ethical review board and community advisory board (CAB) representatives. 3) Monitoring and Retention: Regular site monitoring and evaluation; contingency plan to enroll and retain participants during crisis; telephone cards, home visits and incentives to maintain high retention.

Results: From 2001 to 2012, 10 preventive HIV vaccine protocols were conducted at GHESKIO. Of those, 4 were HIV vaccine trials (HIVNET 026, HVTN 050, 204,502), 6 non-vaccine trials (HVTN 903,504,404,802,907, 910). A total of 1,082 subjects were enrolled (HIVNET 026, HVTN 050, 204,502), 6 non-vaccine trials (HVTN 903,504,404,802,907, 910). A total of 1,082 subjects were enrolled (HIVNET 026, HVTN 050, 204,502), 6 non-vaccine trials (HVTN 903,504,404,802,907, 910).

Conclusion: In collaboration with HVTN and support of the Ministry of Health and other partners, GHESKIO has been able to develop an effective, multidisciplinary model of vaccine implementation. GHESKIO’s ability to continue providing prevention and care services throughout periods of crisis reflects its capacity to respond and intervene in conducting large vaccine trials.

Dynamics of Viral Load (VL) Rebound After cART Interruption in Chronic HIV Infected Patients Receiving MVA-B plus Disulfiram

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Background: In vitro data suggest that stimulation of HIV-1-specific T lymphocytes with a therapeutic vaccine could facilitate elimination of latent viral reservoir after virus reactivation with drugs (i.e disulfiram). We present the results of a substudy of a phase I, double blind placebo-controlled trial of an MVA-B therapeutic trial combined with disulfiram virus-reactivation treatment in successful cART-treated HIV-infected patients.

Methods: Patients were randomly allocated to receive 3 intramuscular injections of MVA-B at 0, 4 and 16 weeks (n=20) or placebo (n=10). Twelve patients (9 MVA-B, 3 placebo vaccinated) received a 4th dose of MVA-B at week 36 followed by 2 months of disulfiram (250 mg qd). cART was discontinued in all 30 patients 8 weeks after the last dose of MVA-B and viral rebound dynamics were assessed during the first 12 weeks of cART interruption.

Results: VL was maintained below detectable levels in all patients while receiving MVA-B or MVA-B/disulfiram on ART but rebounded in all patients after cART interruption. The dynamics of VL rebound were not significantly different between the disulfiram treated/untreated groups. Proportion of patients with VL rebound at weeks 2 and 4 after cART interruption was similar between groups (w2: 7/12 (58%) and 8/16 (50%), w4: 11/12 (92%) and 15/17 (88%). In MVA-B/disulfiram vs MVA-B, P = 0.66 and 0.74), respectively. At week 12 after cART interruption, mean (SE) change VL (as compared with set-point VL before any cART) was −0.72 (0.4) vs −0.35 (0.3) in MVA-B/ disulfiram vs MVA-B, respectively (P = 0.46). CD4 T cell counts declined similarly between groups after cART interruption. Changes in immunogenicity and latent viral reservoir before and after MVA-B/disulfiram are being assessed.

Conclusion: A combination strategy of a therapeutic vaccine (MVA-B) plus disulfiram treatment neither prevented nor delayed viral load rebound after cART interruption as compared with MVA-B vaccination alone.
**P04.13**

**Transgender Participants in Phase 1-2a Trials of the HIV Vaccine Trials Network (HVTN): A Descriptive Analysis**

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**Background:** The US Institute of Medicine (2011) noted a dearth of information on transgender individuals and called for more data. We respond by describing transgender individuals in HVTN trials, a population of unique import as they stand to benefit greatly from an HIV vaccine. HIV prevalence in the US transgender population (2.64%) is five times the national average (0.45%).

**Methods:** Case report forms were analyzed for individuals who reported their gender identity differing from birth sex and who enrolled in 1-2a HVTN trials enrolling low-risk participants since 2009.

**Results:** Twelve transgender individuals (1.7% of 694 participants across six trials) were included, 9 (75%) male-to-female transgender participants (reporting male sex and female gender) and 3 (25%) female-to-male participants. The mean age was 25.8 years (SD=3.81). 25% identified as Hispanic, 83.3% as White, 8.3% as African-American, and 8.3% as “other.” The most common reasons for enrolling in an HIV vaccine trial were to: help find an effective vaccine (100%); help their community (100%); be informed about HIV research (88.3%); and because they know someone with HIV (66.7%). Fifty-seven pre-existing conditions were reported (mean 4.75, SD=3.02). 25% identified as Hispanic, 83.3% as White, 8.3% as African-American, and 8.3% as “other.” The most common reasons for enrolling in an HIV vaccine trial were to: help find an effective vaccine (100%); help their community (100%); be informed about HIV research (88.3%); and because they know someone with HIV (66.7%). Fifty-seven pre-existing conditions were reported (mean 4.75, SD=3.02). Fifty-seven adverse events were reported (mean 4.75, SD=4.09), two deemed related to vaccine. 83.3% reported hormone therapy for a gender identity indication; 50% reported medical care (16.7%).

**Conclusion:** Primary reasons for HIV vaccine trial participation were altruistic. Mental illness incidence approximated that reported from other surveys of transgender individuals and was not accompanied by social impacts or missed visits. HIV incidence was 12.7/100 person-years. Future analyses will include comparisons with non-transgender (cisgender) participants.

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**P04.14**

**Early and Continuous Engagement of the CAB Is Essential to the Successful Implementation of Invasive Procedures in Clinical Trials**


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**Background:** The Department of Retrovirology, AFRIMS, located in Bangkok, Thailand conducts acute cohort and HIV vaccine trials. As part of these studies, invasive procedures such as cervical and/or sigmoid biopsies, bone marrow aspirate, leukapharesis and lumbar puncture are performed to assess immune responses to vaccine or HIV infection. Efforts to assess volunteer acceptability of these novel procedures were initiated early in protocol development through engagement and education of the Retro Community Advisory Board (Retro CAB). Opinions, concerns, and advices were solicited to aid in the development of the protocol, informed consents, and educational materials.

**Methods:** Retro CAB membership represents and reflects the community and target population in which the trials are conducted. CAB members received GCP and GPP training and emphasis was placed developing research and social competency. Education on the protocols and invasive procedures was provided in advanced lay language. A variety of media and educational methods were employed. CAB members with more advanced research or medical training helped to educate peers with less scientific backgrounds.

**Results:** Six protocols employing invasive procedures were discussed in 9 of 13 CAB meetings. CAB members reviewed the Test of Understanding, Informed Consent, study recruitment and education materials, and volunteer risk, benefits and compensation. A key concern raised by the CAB was the provision of medical care for volunteers in the event of complications arising from invasive procedures, particularly in healthy volunteers. Researchers negotiated, developed, and purchased a novel Clinical Trials Insurance policy to provide coverage for serious complications arising from these procedures. Instituting CAB recommendations for consenting and volunteer education increased acceptability for cervical biopsy, sigmoid biopsy and leukapharesis from zero to just under enrollment goal.

**Conclusion:** Early, dedicated, and continual engagement of the CAB is essential to the successful implementation of invasive procedures that had previously never been performed in healthy volunteers in our protocols.
**P04.15**

**Recruitment, Enhanced Retention Strategies and Willingness to Participate Among Hard-to-Reach Female Sex Workers in Barcelona for HIV Vaccine Trials**

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**Background:** Studies assessing prevention interventions, including prophylactic vaccines, require recruitment, long-term follow-up and high retention rates of HIV high risk volunteers who are willing to participate in future vaccine trials. The potential for implementation of HIV vaccine trials in hard-to-reach female sex workers in an inner city area of Barcelona, Spain was assessed.

**Methods:** Baseline demographic, risk behavior, HIV testing and willingness to participate were recorded among 869 female sex workers, using an interviewer administered questionnaire. Moreover, we evaluated retention outcomes over 24 month’s period of follow-up between two groups in which different strategies were applied: enhanced retention (ER/n=130) and control retention (CR/n=121). In addition, risk behaviors over time, HIV seroincidence, and correlates of willingness to participate in future vaccine trials were assessed among the enhanced retention (ER) group.

**Results:** From 2005 to 2012 869 female sex workers who attended Ambit Prevenció center were enrolled. Overall, HIV baseline seroprevalence was 3% and 93% were willing to participate in HIV vaccine trials at baseline. At 6 months 76% of participants in the ER group and 16% in the CR group remain in follow-up; 69% and 13% at 12 months respectively. Willingness was significantly associated with higher HIV risk exposure, and higher education level. Overall, 5 new HIV-1 infections were found, yielding an estimated seroincidence rate of 1.1/100 PYO (95% CI: 0.3-4.3).

**Conclusion:** Our results indicate that recruitment, retention, and willingness to participate are feasible in vaccine preparedness studies among hard-to-reach populations. To ensure broad participation in HIV vaccine clinical trials and cohort studies including women at high risk for HIV infection, modifications of the retention strategies may also be required. Furthermore, the fact that retaining hard-to-reach populations is difficult should not exclude this target population for HIV vaccine and prevention trials.

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**P04.16**

**Mucosal Specimen Collection, Processing and Assay—Experience from a Resource-Limited Setting in Kenya**


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**Background:** HIV-1 infection occurs most commonly through sexual intercourse. Induction of mucosal immune responses will likely be necessary for an effective HIV vaccine. KAVI embarked on building capacity for mucosal immunological work and disseminating this to other African research sites.

**Methods:** With support from IAVI, KAVI clinical and laboratory teams designed SOPs for collection, processing and assay of cells and secretions from gastrointestinal, genital and upper respiratory mucosal surfaces over the course of 4 years and several studies. Specimens analyzed for humoral immune responses included saliva, nasal turbinates, nasopharyngeal, cervical-vaginal and rectal secretions. Cervical-vaginal and rectal cytobrush samples, and rectal and sigmoid biopsies were stained and analyzed by flow cytometry. Participants were free to opt out of any collection. Reasons for refusal and other acceptability/tolerability data were collected. Depending on acceptability/tolerability and assay results, collection methods were dropped or SOPs improved as needed. A curriculum was developed for training other African sites involved in HIV research on mucosal sample collection and processing.

**Results:** Four mucosal studies were conducted at KAVI, one involving participants from three HIV vaccine trials. Repeated mucosal sampling in both high and low risk participants was generally well accepted/tolerated (AIDS Vaccine 2010 and 2012, P10.07 and P122 respectively). Cellular and humoral immune responses to HIV were detectable in various mucosal compartments including relatively easier sampling sites like the mouth and nose. One site in Rwanda received training and subsequently conducted a mucosal study; training of more sites is being planned.

**Conclusion:** Mucosal sample collection and processing from various mucosal compartments and by various sampling techniques is possible in a resource-limited setting. HIV-relevant immunological responses are detectable in both genital and non-genital mucosal compartments. South-to-south collaborations for technology transfer in mucosal immunological studies is feasible and should be encouraged.
P04.17

Pride of Fulfillment and Self-Respect: A Follow-Up Study Among Volunteers Who Completed Phase I/II HIV Vaccine Trial in Dar es Salaam, Tanzania

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Background: Enrolment in HIV vaccine trials is important for subsequent development of an affordable and effective vaccine. In the HIVIS-03 HIV vaccine trial, a total of 60 volunteers were randomized to receive either placebo or active vaccine (DNA priming, followed by boosting with MVA and rgg-140/GLA). Following completion of all vaccinations and follow up, all volunteers were un-blinded. We hereby describe the experiences of volunteers after un-blinding.

Methods: A follow up study was conducted in Dar es Salaam, Tanzania about 3 years after un-blinding of volunteers who received the active vaccine. Of the 33 volunteers, 21 (63.6%) were available during this follow up study. A self-administered questionnaire with both open and closed ended questions was completed by the volunteers. Content analysis was used to analyze the data.

Results: The majority (18/21; 85.7%) reported that participation in a vaccine trial was a good experience in the sense that they were able to: accomplish their dreams as volunteers, experience comfort about their decision, and experience safety of the vaccine. However, minority (3/21) were doubtful about their future in terms of possible long term vaccine side effects. About two third (13/21; 61.9%) of the volunteers informed their spouses about the outcome of un-blinding; five (5/21) informed their parents, relatives and friends; three (3/21) did not inform anybody. The reactions of those who were informed ranged in severity; some volunteers’ and the community’s concerns.

Conclusion: Participation in phase I/II HIV vaccine trial in a Tanzanian context may be a rewarding experience. However, doubts from the surrounding community and some volunteers may persist. Therefore, follow up studies after un-blinding are important to address the volunteers’ and the community’s concerns.

P04.18

Immunogenicity of Homologous and Heterologous Regimens of Ad26-Enva.01 and Ad35-Enva HIV Vaccines in HIV-Uninfected Volunteers in the U.S. and Africa

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Background: Heterologous prime boost vaccine regimens offer a promising approach to improving T cell and antibody responses. Homologous and heterologous prime boost regimens were compared using two low seroprevalence Adenovirus vectors expressing two HIV-1 subtype A Env.

Methods: Ad26.EnvA.01 and/or Ad35-EnvA were administered at 5 x 10 10 viral particles intramuscularly in 217 volunteers (173 vaccine: 44 placebo) in three regions (US, East and South Africa) with two different intervals. US volunteers (Groups A-D) received homologous (Ad26. EnvA.01+Ad35-EnvA or Ad35-EnvA+Ad26.EnvA.01) regimens at 0/3- or 0/6-month intervals. African volunteers (Groups E-L) received the same heterologous regimens or homologous regimens consisting of two Ad26.EnvA.01 or two Ad35-EnvA at 0/3 months.

Results: Vaccine regimens were well tolerated. IFN-gamma ELISPOT responses were detected in 44-100% of subjects (A-D), 6-62% (E-L) at 2 weeks and 78-100% (A-D), 47-88% (E-L) at 4 weeks after second vaccination for Ad26. EnvA.01 matched peptide pools and 11-75% (A-D), 0-69% (E-L) at 2 weeks and 33-100% (A-D) and 0-87% (E-L) at 4 weeks for Ad35-EnvA matched peptide pools. Env-specific CD4 and CD8 response rates were up to 70% (CD4) and 80% (CD8) for IFN-gamma and/or IL2 expressing T cells in Groups H-L (South Africa) at 4 weeks post second vaccination. T cell response magnitudes by ELISPOT and flow cytometry were modest; differences between heterologous or homologous regimens were dependent on peptide sets. Env antibody responses were 93-100% in all groups with >10-fold boost in magnitude at 4 weeks post second vaccination. Baseline and vaccine-elicited Ad26 and Ad35 neutralizing antibodies were observed with marked regional differences; East Africa > South Africa > US and Ad26 > Ad35 titers.

Conclusion: Env antibody and T cell responses were detected in all groups and no correlation was detected between baseline vector-specific neutralizing antibodies and Env-specific humoral or cellular immune responses across all regions. Immunogenicity in heterologous and homologous regimens was comparable.
P04.19

Attracting, Equipping and Retaining Young Investigators in HIV Vaccine Research in South Africa

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Background: Upcoming HIV vaccine efficacy trials will require extensive research capacity, including at the investigator level. While senior level South African HIV vaccine researchers have developed tremendous expertise, junior clinician investigators are not being developed at the same rate. In 2010, the HIV Vaccine Trials Network (HVTN) with support from the National Institute of Allergy and Infectious Diseases (NIAID), collaborated with the Fogarty International Center to establish SHAPe (South African/HVTN AIDS Vaccine Early Stage Investigator Program), a peer reviewed MD/PhD program that recruits and supports young clinician investigators as they become independent investigators. Formative research was conducted to identify barriers and facilitators to attracting, equipping and retaining young investigators; develop recommendations to address challenges; and inform development of the SHAPe Program.

Methods: Qualitative data exploring the abovementioned issues were collected from interviews and focus groups with 5 senior and 6 junior HIV vaccine investigators, and 7 medical doctors not involved in research. Participants were recruited from clinical research sites and by referral throughout South Africa. Target participants comprised a small group of specialized individuals, and thus purposive sampling with specific criteria was used. Sessions were recorded and transcribed. Data were analyzed using ATLAS.ti software.

Results: Participants recommended: 1) greater focus on fostering interest and developing research skills during medical training; 2) a more clearly defined investigator career development pathway; 3) stable funding and support for additional mentored research programs; 4) better integration of research and academia; 5) targeted training; and 6) young investigator conference participation and networking.

Conclusion: Expanding programs that provide young investigators with funded independent research, mentoring, and professional development may contribute to building South Africa's next generation of HIV vaccine scientists. Researchers should consider creating a consortium to address challenges, and appeal to the local/national government for increased research support.

P04.20

Recruitment and Retention of Urban Population in Vaccine Trials in Uganda

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Background: Successful recruitment and retention of study participants is essential to the conduct of clinical trials. Makerere University Walter Reed Project has conducted three Phase I/II HIV vaccine trials and a Phase IIb Ebola/Marburg vaccine trial recruiting participants from Kampala and its suburbs. We describe the experience and lessons learnt in recruiting low risk HIV uninfected participants and strategies for their retention.

Methods: Participants responding to various recruitment methods to include radio announcements, newspaper adverts, posters, brochures and word of mouth were screened after obtaining informed consent. A computerised clinic appointments tracking system, telephone reminders, home visits and invitations to functions were methods utilized to improve retention.

Results: Approximately 1744 participants were screened to enrol 325 participants at a ratio of 5:4:1 (6.3: 1 for HIV vaccine studies and 3.5:1 for the Ebola/Marburg study). Screening to enrolment ratios were initially 7:1 and recently 5:1 for HIV vaccine studies with similar eligibility criteria. The majority (79.7%) of enrolled participants were males (259/325) while 20.3% (66/325) were females. All four studies included a greater number of men. While laboratory abnormalities were the most common cause of screen outs in the first trial as a complete set of local normal laboratory values was not available for use, other reasons such as health related exclusions and not returning for further screening visits were also common causes of screen outs overtime. High retention rates of 100% and 98% were achieved during one year of follow up for the first 2 HIV vaccine studies while the third is still ongoing. The overall retention for the two-year Ebola/ Marburg study was 92.6%.

Conclusion: The urban population surrounding Kampala is enthusiastic to participate in vaccine trials and can obtain high retention rates in Phase I/II studies. Emphasis should be placed on increasing female enrollment in vaccine trials.
**P04.21**

**Construction of an Optimized Multi-Arm Phase I-II Trial Design to Evaluate the Safety and Immunogenicity of Different HIV Vaccine Strategies**

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**Background:** To accelerate HIV vaccine development, optimized trial designs are needed. We propose an optimized early stage design to evaluate four preventive prime-boost HIV vaccine strategies in parallel, aiming at rapidly discarding those that are unsafe or non-immunogenic.

**Methods:** A multi-arm phase I/II trial is planned to study the safety and immunogenicity of four prime-boost strategies. One of the vaccines used as prime and boost in different strategies (vaccine 1) has yet to be tested in humans, thus requiring a phase I safety evaluation. However, its toxicity risk is considered minimal based on data from similar vaccines. We newly adapted a randomized phase II trial by integrating an early safety decision rule to emulate the phase I evaluation. Frequentist operating characteristics of the proposed design and scenarios for Bayesian analyses were evaluated in simulation studies. Timelines for the trial were projected.

**Results:** We propose a randomized four-arm phase I/II design with two independent binary endpoints for evaluating safety and immunogenicity. For immunogenicity screening in each arm at trial end, the observed proportion of interferon-γ ELISpot responders is compared to an unacceptably low proportion. No direct comparisons between arms will be made, but randomisation limits heterogeneity in volunteer characteristics between arms. To avoid exposure of additional participants to an unsafe vaccine during the vaccine boost phase, a stringent early stopping rule is imposed on the trial arm starting with injections of vaccine 1. In simulations of the frequentist operating characteristics of the design, the risk of erroneous conclusions was controlled at <15%. A 12-month gain in timelines is expected by this optimized design.

**Conclusion:** The proposed optimized design allows for accelerating early stage clinical development of HIV vaccine strategies by combining phase I and phase II evaluations in several arms in parallel. The set-up of a HIV vaccine trial using this design is currently ongoing.
P04.23

Benefits of a Comprehensive, Semi-Automated Quality Program for Cryopreserved PBMC Covering 29 Globally Distributed Clinical Trials Sites

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Background: The HVTN is a global network of clinical trial sites dedicated to identifying an effective HIV vaccine. Immunologic evaluation of experimental vaccines requires cryopreservation of peripheral blood mononuclear cells (PBMC) for the assessment of vaccine-induced cellular functions. The HVTN PBMC Quality Management Program is designed to ensure viable PBMCs are processed, stored and shipped for clinical trial assays from all HVTN clinical trial sites. The program has evolved by developing and incorporating best practices for laboratory and specimen quality and implementing automated, web-based tools. These tools allow both the site-affiliated processing laboratories and the central Laboratory Operations Unit to rapidly collect, analyze and report PBMC quality.

Methods: The HVTN PBMC Quality Management Program includes five key components: 1) Laboratory Assessment, 2) PBMC Training and Certification, 3) Internal Quality Control (IQC), 4) External Quality Control (EQC), and 5) Assay Specimen Quality Control (AQC). Fresh PBMC processing data is uploaded from each clinical site processing lab to a central database at the HVTN Statistical and Data Management Center (SDMC) for access on a web portal called Atlas. Samples are thawed at a central laboratory for assay or specimen quality control and sample quality data is uploaded directly to the Atlas database by the central.

Results: Two year cumulative data covering 13,853 blood draws reveals an average fresh PBMC yield of 1.43x10^6 ± 0.48 cells per milliliter of whole blood. Two year cumulative data covering 1818 randomly selected clinical trial specimens shows that 99.6% of specimens tested had acceptable viabilities (>75% live cells) with a mean viability of 89.3% ± 5.06%, and 97.0% of specimens tested had acceptable recoveries (70%-130%) with a mean of recovery of 87.3% ± 17.3% of the cryopreserved.

Conclusion: These data support the benefits of implementing a comprehensive, semi-automated, web-based PBMC quality program for large clinical trials networks.

P04.24

Low Rates of ARV Use and Uptake Among Discordant Couples Followed in Government Clinics in Zambia

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Background: Zambian National Guidelines include ARV for HIV+ individuals with CD4 counts less than 350 cells/mm³, and for discordant couples. Couples’ Voluntary HIV Counseling and Testing (CVCT) has also been endorsed by the Zambian government. Acceptability of “treatment as prevention” has not been evaluated among discordant couples. In addition, given the high HIV prevalence and declining budgets, access to ARV may be limited.

Methods: Couples are invited to take part in CVCT services at Zambia government clinics. Data regarding history of prior testing, pregnancy, and ARV use was collected anonymously from January 2008 to March 2013. ARV referrals were provided to those who were HIV-infected. In 2011, follow-up testing and counseling was instituted for discordant couples.

Results: 11,277 discordant couples were identified in government clinics in Lusaka, Copperbelt, and Southern Provinces. Although more than half of HIV+ partners in discordant couples reported having been previously tested as individuals, only 1829 (16.2%) had initiated ARVs prior to CVCT. Follow-up testing rates were low, ranging from 10%-25% across cities. Of 1161 HIV-infected partners who were not on ARVs at the initial CVCT visit, 980 (84.4%) had not initiated ARVs at subsequent follow-up visits. Higher rates of ARV use were observed in discordant couples where the woman was HIV-infected.

Conclusion: Couples’ HIV testing and counseling with follow-up testing for HIV discordant couples should be provided and promoted in government clinics. Obstacles to ARV uptake among Zambian discordant couples should be investigated and may include fear of stigma, unwillingness to take medication if asymptomatic, and limited ARV stocks with priority given to those with more advanced disease. Discordant couples benefit from reduced HIV transmission after CVCT whether or not they initiate ARV. Future HIV vaccine efficacy trials should include discordant couples.
P04.25

Integration of Prevention, Screening, and Treatment for Common Diseases for Vaccine Trial Target Populations: The Good Health Package in Zambia

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Background: In addition to the high HIV prevalence in Sub-Saharan Africa, the region is burdened with a majority of the world’s neglected tropical diseases (NTDs). Millions of people live without access to clean water, and non-communicable diseases (NCD) are an emerging problem. The Good Health Package (GHP) offers prevention, screening, and treatment for common diseases to couples seeking HIV testing.

Methods: Zambia Emory HIV Research Project (ZEHRP) has established Couples’ Voluntary HIV Counseling and Testing (CVCT) services in 50 Zambian government clinics. Sero-concordant negative and sero-discordant couples are invited for HIV retesting at regular intervals and referred to ZEHRP for vaccine trials and studies. In 2012, ZEHRP began offering blood pressure screening, chlorine for drinking water, deworming medication for household members, rapid syphilis testing, antibacterial hand soap, and urine dipstick screening for diabetes and schistosomiasis. Couples choose one service at each follow-up visit.

Results: In 16 months, more than 7,500 services have been received by couples returning for follow-up. The top three choices were soap (76%), chlorine (12%), and deworming (7%); the least popular was urinalysis (<1%). Thirteen percent of individuals who had blood pressure tested were referred to clinics for hypertension treatment and 20% tested for syphilis were referred to clinics for hypertension treatment and received free treatment.

Conclusion: The GHP provided an opportunity to teach proper hand-washing, correct chlorine use, and periodic household deworming. Hypertension and diabetes are on the rise; education is needed as they are not recognized as important problems. Couples appreciated syphilis screening as it is otherwise available only to pregnant women. Although 4/26 HIV vaccine trial participants referred from ZEHRP CVCT were diagnosed with active schistosomiasis during this time frame, neither couples nor clinic health care providers are aware of the high prevalence in urban adults. Prevention, screening, and treatment for common diseases should be offered to potential HIV vaccine trial participants.

P04.26

PENNAX-B DNA Vaccine via Electroporation Drives Potent Cellular Immune Responses and Synthesis of Granzyme B, Perforin: Data from 3 Clinical Trials

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Background: Together with the HVTN, we recently reported the safety and comparative immunogenicity data from two clinical trials (HVTN-070/080) of the PENNVAX-B DNA vaccine (PVB; SynCon® HIV Gag, Pol, and Env) with plasmid IL12 and impact of intramuscular electroporation (EP) using the CELLECTRA® device for delivery. In HIV negative cohorts, PVB+IL12 via EP provided superior immunogenicity to the non-EP trial and had a significant dose-sparing effect. 71.4% of the vaccinees with EP produced a CD4+ or CD8+ T cell response after only 2 vaccinations rising to 88.9% after 3. High levels of CD8+ T cells in the majority of subjects were a notable impact of EP delivery likely in combination with IL12. Importantly, 85.7% of the responders with CD8+ and 71.4% with CD4+ T cells had memory responses persisting over 6 months.

Methods: Induction of potent and sustained levels of anti-HIV CD8+ T cells could lead to successful immunotherapy for infection. We therefore evaluated PVB + EP (weeks 0, 4, 8, 16) in 12 HIV infected volunteers on HAART in a Phase I study. Enrollment criteria included HIV RNA<75 copies/mL, CD4 > 400/µL with nadir >200/µL. This study did not include IL12.

Results: All 12 subjects showed strong vaccine-specific T cell responses (IFN-γ ELISPOT) to at least one antigen. Furthermore 9 of 12 subjects responded to 2 antigens; 4 subjects to all 3 antigens. Flow cytometry showed that vaccination led to increased HIV specific CD8+ T cell activation after peptide stimulation (CD137 expression) and increased frequency of CTL-related markers Granzyme B and Perforin.

Conclusion: Vaccinations in all 3 trials were safe and well tolerated. These trials illustrate that improved DNA vaccination with CELLECTRA EP can drive the expansion of HIV-specific CD8+ T cells with a CTL phenotype and suggest this as a viable approach for HIV immunotherapy in combination with HAART or PrEP regimens.
P04.27

Eligibility Assessments of Healthy Adults for Eleven HIV Vaccine Clinical Trials Conducted Through a Screening Protocol: 2002–2013

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Background: The VRC/NIAID screening protocol, NIH #02-I-0127 (NCT00031304), was developed to offer a well-structured process through which recruitment of potential participants could occur, with opportunity for volunteers to learn about investigational HIV vaccines and to be evaluated for eligibility.

Methods: The protocol was IRB-approved at the NIH Clinical Center (Bethesda, MD). Participation began after informed consent to be screened for an HIV vaccine study was obtained. Evaluations included medical history, physical exam, laboratory tests and collection of research samples. Subjects could screen for more than one HIV vaccine protocol. Screening outcomes and reasons for ineligibility were documented. After receipt of placebo in an HIV vaccine study or if potentially eligible for an HIV vaccine booster study, subjects could re-enroll in the screening protocol.

Results: Eligibility screening for 11 HIV vaccine protocols was completed 2002-2013. Cumulatively, 1324 adults enrolled one or more times for 1359 enrollments total. The study population included 425 (32%) women and 899 (68%) men. Distribution by race was 69% white, 23% black/African American, 4% Asian, and 4% other races; overall, 6% were Hispanic/Latino. Of 1494 screening assessments, 29% resulted in enrollment into an HIV vaccine protocol. Other outcomes were ineligibility (44%), withdrawal (19%), lost to follow-up (7%) and closed-out (1%). Reasons for HIV vaccine study ineligibility included abnormal laboratory test (53%), medical history (22%), physical exam (10%), protocol-specific HIV risk criteria (8%) and other (7%). After 2007, pre-existing immunity to a candidate vaccine vector, adenovirus titers (the candidate vaccine vector), and 2 did not return for the trial (20%) and 2 enrolled. Two others were HIV-infected but unaware of their status. LCPR may be enhanced by increasing awareness of HIV vaccine trials, by directing participants to initial studies where comprehensive education about HIV and by recruiting both HIV-infected and uninfected individuals into efficacy trials with study phase-appropriate eligibility criteria, but unaware of their status. LCPR may be enhanced by increasing awareness of HIV vaccine trials, by directing participants to initial studies where comprehensive education about HIV and by recruiting both HIV-infected and uninfected individuals into efficacy trials with study phase-appropriate eligibility criteria, and also by recruiting both HIV-infected and uninfected individuals into initial studies where comprehensive education about HIV research may encourage potentially eligible individuals to consider longitudinal follow-up.

Conclusion:

Conclusion: The screening protocol was successful in facilitating enrollment of 11 VRC HIV vaccine protocols over 11 years at the NIH Clinical Center and allowed vaccine protocols to begin accrual quickly after IRB approval. Careful attention to screening outcomes improves the study education process and ensures a well-informed pool of volunteers for HIV vaccine protocols.

P04.28

Long-Chain Peer Referral to Recruit Black MSM and Black Transgender Women for an HIV Vaccine Efficacy Trial

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Background: Face-to-face outreach and online recruitment strategies for biomedical HIV prevention trials have identified limited numbers of black men who have sex with men (MSM) and black male-to-female transgender women (transwomen) enrollees, two populations disproportionately affected by HIV. Long-chain peer referral (LCPR) has been used effectively to recruit hard-to-reach populations into cross-sectional and longitudinal studies. We therefore evaluated the feasibility of LCPR to recruit black MSM and transwomen into an ongoing HIV vaccine efficacy trial.

Methods: We conducted key-informant interviews, focus groups, and community consultations to develop an LCPR recruitment protocol and educational materials, and to identify initial participants to encourage black MSM and transwomen in their social networks to undergo vaccine study screening. From August 2012-March 2013, initial participants from San Francisco and Alameda counties were asked to refer peers who were ages 18-50 years and HIV uninfected or of unknown status.

Results: An initial 11 MSM and 6 transwomen participants were identified. Fifteen 1st or 2nd wave recruits underwent prescreening. Seven recruits advanced to medical screening. Of these, 3 were eligible for the trial (20%) and 2 enrolled. Two others were HIV-infected but previously unaware of their status; 1 was ineligible due to high adenovirus titers (the candidate vaccine vector), and 2 did not return to complete screening.

Conclusion: While we had limited success enrolling black MSM and transwomen in this application of LCPR, the recruitment strategy remains promising given its ability to tap into at-risk populations as seen by presentation of participants who were HIV-infected but unaware of their status. LCPR may be enhanced by increasing community awareness of HIV vaccine trials, by directing participants into efficacy trials with study phase-appropriate eligibility criteria, and by recruiting both HIV-infected and uninfected individuals to initial studies where comprehensive education about HIV research may encourage potentially eligible individuals to consider longitudinal follow-up.
P04.29

Retention, ARV Use, and Dual Contraception in Discordant Couples in Government Clinics in Kigali, Rwanda

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Background: HIV discordant couples benefit from joint HIV testing, with seroconversion rates of 3-4%/year in Rwanda compared with 11%-12%/year among uncounseled discordant couples in the region. Treatment-as-prevention (TAP) with ARV has been recommended for discordant couples. HIV- women participating in vaccine trials are asked to avoid pregnancy, ideally with long acting contraceptives (LARC) such as the copper IUD and the contraceptive implant.

Methods: Projet San Francisco provides training, monitoring and evaluation, and reporting of discordant couple management and LARC insertion in government clinics in Kigali, Rwanda. We describe follow-up rates, ARV uptake, and LARC use among HIV discordant couples.

Results: Of 2322 discordant couples identified during routine CVCT in antenatal clinics, 53% (1220) were on ARV. Sixty three percent (1452) of couples enrolled in the follow-up program. Of these, 54% (777) were on ARV at first couples testing and 51% (347/675) initiated ARV after CVCT. It is not clear why more than a third of discordant couples did not enroll in follow-up, nor why 328 who did enroll in followup did not initiate ARV. Following an intensive LARC training program, the proportion of family planning clients choosing LARC increased from <2% to >55%. However, only 9% of discordant couples in the followup program report LARC use (7% implant, 2% IUD): 9% use depo-provera, 2% use oral contraceptives, and 80% use condoms alone.

Conclusion: Rwanda is the only country in Africa to implement CVCT on a national scale, with >90% of pregnant women now tested with their partners. Even in the capital city however, many discordant couples do not avail themselves of followup services or ARV. Integrating full-service family planning with dual-method counseling into discordant couple follow-up would help couples avoid unplanned pregnancies and provide an added benefit to those participating in future HIV vaccine efficacy trials.

P04.30

Performance of the Determine HIV 1/2 Ag/Ab Combo Rapid Test on Serial Samples from an Acute Infection Study (RV217) in East Africa and Thailand

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Background: Several small studies have demonstrated low sensitivity of the Determine HIV 1/2 Ag/Ab Combo Rapid Test (Combo RT) during acute HIV infection (AHI). We evaluated Combo RT performance on well-defined panels of serial samples collected from AHI cases identified by the Early Capture HIV Cohort (ECHO) Study, RV217.

Methods: Individuals at high risk of HIV infection from Tanzania, Uganda, Kenya and Thailand were screened twice-weekly by Aptima HIV-1 Qualitative RNA assay (Gen-Probe, Inc. San Diego, CA). Following a reactive Aptima result, serial samples were collected twice weekly from consenting participants. Serial specimen panels were screened with Combo RT (Alere, Inc), HIV-1 p24 Antigen (Ag) Test, GS HIV-1/2 Plus O EIA (3th Gen), and HIV1/2 Multispot (Bio-Rad Laboratories, Redmond, Washington).

Results: Antigen and antibody (Ab) performance of the Combo RT were evaluated separately. Of 29 AHI cases, only four (13.7%) were identified by p24 target alone (Ag range: 210-5,500 pg/ml) despite device claims of a limit of detection of 12.5-25 pg/ml. Anti-HIV Ab detection by Combo RT was highly sensitive. 24/29 (82.7%) cases were identified either before, or at the same time point as a 3rd generation EIA. Combo RT AB was reactive on median day 14 (range 3-25) while 3rd generation EIA reactivity occurred at day 16 (range 10-33) (p=0.76). In 23/29 (79.3%) cases, the Combo RT antibody test was reactive before Multi-spot, which was reactive on median day 20 (range 14-40)(p<0.0001).

Conclusion: Combo RT performance for AHI case detection was poor as previously described and is likely due to omission of a p24 Ab/Ab dissociation step. Anti-HIV Ab detection was comparable with 3rd generation EIA and showed improved sensitivity over the Multi-spot rapid test. Ag detection sensitivity and, thus, enhanced AHI case detection may be vastly improved by addition of a p24 dissociation step.
Discordant Couples Cohort Is Still Relevant for HIV Vaccine Studies Despite Low Transmission Rates: Canadian–Nigerian Experience

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Background: Discordant couples have been known to be medium risk group for HIV transmission. This group is suitable for HIV vaccine trials due to the natural exposure and high retention rates recorded in the past. A cohort of discordant couples is being developed in Nigeria to record HIV incidence, STI infections, retention rates, risk factors and behavioral data. The data collected from this cohort will establish baseline information on the general Nigerian population.

Methods: A prospective cohort study to enroll and follow up 500 HIV serodiscordant couples for 2 years was established in Jos, Nigeria. Relevant ethical approvals were obtained. Following informed consent, standardized questionnaires were administered, clinical examinations done, and samples collected. Data on risk behavior from participants; and CD4 counts and viral from their partners were collected.

Results: A total of 540 HIV negative volunteers were enrolled, 282 (52.2%) females and 258 (47.8%) males. 215 enrollees (39.8%) reported use of condoms at all times while 159 (29.4%) never use condoms. The average CD4 count was 428 cells/µl. Most importantly, 258 (69.4%) of the HIV positive partners had detectable viral load levels with 67 (26.0%) having >10,000 copies/ml. Three of the 540 volunteers seroconverted giving this cohort an incidence of 0.56%.

Conclusion: Our study indicates that despite the scale of antiretrovirals, seronegative partners continue to be at risk of being infected by their HIV+ partners in resource limited settings. Our study recorded detectable virus in the seropositive partners and inconsistent condom use despite risk reduction counseling. In addition, this fairly healthy serodiscordant couple cohort is an important natural adult model to study immune responses in HIV exposed adults. This baseline immune response might impact the interpretation of the effectiveness of HIV vaccine trials.

A Recombinant Vesicular Stomatitis Virus (rVSV) HIV-1 Gag Vaccine Is Safe and Immunogenic in Healthy, HIV-1 Uninfected Phase I Trial Participants

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Background: Highly attenuated recombinant vesicular stomatitis virus (rVSV) is a replicating viral vector-based vaccine which takes advantage of limited seroprevalence to VSV. Here we present safety and immunogenicity data from a phase 1a first-in-human trial.

Methods: HVTN 090 enrolled sixty healthy, HIV-1-uninfected adults in a randomized, double-blinded, placebo-controlled dose escalation study. Groups of 12 participants received rVSV HIV-1 Gag vaccine at 5 dose levels (4.6 x 10 3 to 3.4 x 10 7 PFU) (N=10/group) or placebo (N=2/group), delivered intramuscularly as bilateral injections at 0 and 2 months. Reactogenicity over 7 days, adverse events (AEs), and VSV cultures from blood, urine, saliva and swabs of oral lesions were assessed. HIV-1-specific CD4+ /CD8+ T cell responses to Gag peptides were measured at 2 weeks post-boost by intracellular cytokine staining (ICS) and by IFN-γ ELISpot. Neutralizing antibody responses to the rVSV vector were also assessed.

Results: The median age was 24; 45% were female and 37% were non-white. Local and systemic reactogenicity symptoms were mild to moderate and increased with dose. No severe reactogenicity, encephalitis, or product-related SAEs were reported. All rVSV cultures were negative. All vaccine recipients became seropositive for VSV after 2 vaccinations indicating successful vaccine ‘take’. Gag-specific T cell responses were detected in 63% of participants by IFN-γ ELISpot at the highest dose post-boost. ICS revealed that responses were predominantly mediated by CD4+ T cells expressing CD40L, showing a clear dose-response from 11% of participants responding at the second dose level to 50% at the highest dose.

Conclusion: A replication-competent rVSV Gag vaccine has an acceptable safety profile in healthy adults. Gag-specific T cell responses were detected in ~60% of study participants after 2 vaccinations at the highest dose. HVTN 087 is currently exploring whether priming with a multigene HIV-1 DNA vaccine given with plasmid IL-12 adjuvant by electroporation will increase the immunogenicity of the rVSV vaccine.
**P04.33**

**Performance of HIV Rapid and HIV ELISA Test Kits on Samples from a Preventative HIV Vaccine Trial to Evaluate Vaccine Induced Sero-Reactivity (VISR)**

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**Background:** HIV uninfected participants in HIV vaccine trials may develop HIV-specific antibodies as a result of receipt of HIV vaccine, which can lead to vaccine induced sero-reactivity (VISR), i.e. reactive results by standard HIV diagnostic test kits. Correct HIV diagnosis therefore requires confirmatory testing. It is essential to determine the best suited HIV testing algorithm for a particular HIV vaccine to facilitate appropriate HIV test counselling, avoid mis-diagnosis and mitigate social harm. The performance of 4 HIV test kits for detection of VISR was determined using stored sera from post vaccination samples.

**Methods:** Study participants were HIV-uninfected and had received either Ad26-ENVA or Ad35-ENV product or both or placebo in a prime-boost regimen. Samples stored from 32 vaccine and 8 placebo recipients at 2 months and 9 months post last vaccination visit were tested on 4 different HIV test kits at a research centre in Nairobi.

**Results:** 96.9 (31/32) of vaccine recipient samples showed reactive results on both the 4th generation BioMerieux Vironostika Ag/Ab Microelisa kit and Mini-VIDAS HIV Ultra Duo Ag/Ab kit for both post vaccination visits. 37.5% (12/32) 2 months and 9.4% (3/32) 9 months post last vaccination samples were reactive when using the 3rd generation Alere HIV 1/2 Rapid test. 50.0% (16/32) 2 months and 3.1% (1/32) 9 months post last vaccination visit were reactive when using the 3rd generation Trinity Unigold 1/2. No placebo recipients tested positive on any assay for VISR.

**Conclusion:** Selection of optimal kits for HIV testing algorithm(s) following receipt of HIV vaccine may be challenging. 3rd generation HIV Rapid test kits were much less sensitive in detecting VISR compared to 4th Generation ELISA kits. Post vaccination samples from HIV vaccine trials are useful when designing HIV testing algorithms for subsequent trials and following up of persistence of VISR.

**P04.34 LB**

**Immunogenicity of MVA-B in HIV-1-Infected Volunteers**

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**Background:** Previous studies suggested that poxvirus-based vaccine regimens may be instrumental in the therapeutic HIV field. Here, we have characterized the T cell mediated immunity elicited in 22 chronic HIV-1-infected patients undergoing highly active antiretroviral therapy (HAART) pre- and post-vaccination with a clade B-based HIV-1 vaccine candidate expressing Env, Gag, Pol and Nef antigens (MVA-B).

**Methods:** A total of 30 chronic HIV-1-infected patients on HAART with CD4 cell counts above 450 cells/mm³ and undetectable viremia were enrolled in a phase I, double-blind, placebo-controlled trial. The volunteers were randomly allocated to receive three injections of MVA-B (10⁸ PFU/ dose) (n=20) or placebo (n=10) by intramuscular route at weeks 0, 4 and 16 followed by interruption of the HAART therapy at week 24. The magnitude, quality and phenotype of the cellular immune responses were assayed in 22 out of 30 volunteers by polyfunctional flow cytometry (IL-2/IFN-γ/TNF-α/CD107a) on cryopreserved peripheral blood mononuclear cells (PBMC) at weeks 0, 6, 18 and 24.

**Results:** High, broad and polyfunctional HIV-specific CD8 T cell responses with effector memory (TEM) phenotype were detected in all of the 22 seropositive individuals pre- and post-vaccination, with preference in the order of GPN=Gag>Env. A significant increase in the frequencies of HIV-specific CD4+ T cell responses with TEM phenotype were detected after 2 (p-value=0.0014) or 3 (p-value=0.008) doses of MVA-B in vaccine group compared with placebo. At all the time points assayed the CD4+ T cell responses were polyfunctional, with preference in the order of Gag>GPN>Env.

**Conclusion:** MVA-B vaccination represents a feasible strategy to improve T cell responses in individuals with existing HIV-specific immunity.
Background: Strategies for HIV eradication will hinge on the ability to purge latently infected cells. The levels of HIV DNA (pDNA) in chronic patients on ART are stable for years but serve as a reservoir for re-emergence of HIV in the absence of ART.

Previously we reported results of a clinical study testing AGS-004 in chronic HIV patients. AGS-004 is an autologous immunotherapy consisting of dendritic cells expressing autonomous HIV RNAs. Twenty-five subjects received four immunizations in combination with ART followed by an analytic treatment interruption (ATI). Twenty-one subjects reached the primary endpoint with 16 of 21 demonstrating a mean reduction in viral load set point of 1.21 logs. In this study we evaluated the impact of AGS-004 on the pDNA levels.

Methods: The pDNA was measured by repetitive sampling PCR. Samples at a baseline (week 0) and after four immunizations with AGS-004 (week 14) for this analysis were available from 20 of 21 subjects meeting the primary endpoint. Samples from 10 subjects were available after 10 weeks of ATI (week 24).

Results: In the analysis of week 14 versus baseline, 5 of 20 subjects had an increase, 8 had stable levels and 7 had decreased in pDNA levels. Subjects with lower baseline levels of pDNA were more likely to experience a decrease in pDNA levels at week 14. In the analysis of samples after 10 weeks of ATI, 8 of 10 had increased pDNA levels coinciding with elevated plasma VL. However, in 2 of 10 subjects measured, pDNA levels continued to decline during ATI coinciding with elevated plasma VL. However, in 2 of 10 subjects measured, pDNA levels continued to decline during ATI.

Conclusion: We believe this is the first report of the HIV pDNA levels modulated in response to DC-based therapy. Thus AGS-004 is a good candidate for combination with ART and latency re-activating agents in pursuit of HIV eradication.
P04.37 LB
Safety, Immunogenicity and Dynamics of Viral Load Rebound After cART Interruption in Chronic HIV Infected Patients Receiving MVA-B Vaccination

Background: Poxvirus-based vaccines have shown great potential as HIV vaccines. We present the safety and immunogenicity results of a phase I, double blinded, placebo-controlled therapeutic vaccine trial of an MVA vector expressing HIV-1 antigens from clade B (MVA-B) in successfully cART-treated HIV-infected patients who underwent cART interruption.

Methods: Patients were randomly allocated to receive 3 intramuscular injections of MVA-B at 0, 4 and 16 weeks (n=20) or placebo (n=10). cART was discontinued in all patients 8 weeks after the last dose of MVA-B and viral rebound dynamics were assessed during the first 12 weeks of cART interruption. Immunogenicity to the vaccine insert and the rest of the HIV proteome was assessed using IFNγ ELISPOT.

Results: Vaccinations were well tolerated with no grade 3 or 4 side effects reported and viral load (VL) was maintained below detectable levels in all patients while receiving cART and MVA-B challenges. No major changes in total magnitude or breadth of HIV-specific responses were detected between vaccinees and placebos. Only a minor significant increase in the responses targeting vaccine inserts of Gag and Env-gp120 was seen after 2 vaccinations and was maintained over time (median Gag responses of 290, 403 and 435 SFC/M PBMC at baseline, w6 and w24 respectively, p=0.02 and p=0.04). However, all patients rebounded after cART interruption. At week 12 after cART interruption, median reduction in VL (as compared to setpoint VL before any cART) was -0.24 vs -0.53 log copies/ml in MVA-B vs placebo, respectively (p=0.74). CD4 T cell counts declined similarly between groups. The dynamics of VL rebound did not correlate with the responses detected before cART interruption.

Conclusion: MVA-B vaccination was a safe strategy to increase Gag and Env-gp120 specific T cell responses in individuals with existing HIV-specific immunity but did not avoid nor impact VL rebound after cART interruption.

P04.38 LB
HVTN 505: Efficacy of a Multi-Gene DNA Prime/Recombinant Adeno 5 (rAd5) Vector Boost Vaccine in Men & Transgender Women (TGW) Who Have Sex with Men

Background: The DNA/rAd5 vaccine regimen developed by VRC/NIAID elicits HIV-specific, multifunctional CD4+/CD8+ T-cell responses and antibodies (Ab) to HIV Env. Preclinical and clinical data demonstrated safety and immunogenicity and supported efficacy potential.

Methods: Primary objectives of this multicenter (U.S.), double-blind, placebo-controlled, randomized trial were vaccine efficacy (VE) for HIV acquisition (diagnosed between Wk 28 through month 24), viral load (VL) setpoint (mean HIV-1 RNA level obtained Wks 10-20 post diagnosis), and safety. Eligibility included being Ad5 Ab seronegative, circumcised, MSM or TGW, 18-50 years, and meeting behavioral risk criteria. The 6-plasmid DNA vaccine [Gag, Pol, Nef (clade B) and gp145 Envs (clades A, B, C)] was administered at Wks 0, 4 and 8. The rAd5 boost [4-valent vector with full E1 and E4 and partial E3 deletions, encoding Gag/Pol fusion protein (clade B) and gp140 Env (clades A, B, C)] was administered at Wk 24.

Results: 2504 participants enrolled 6/11/09 through 3/27/13 with 1253 randomized to vaccine (V) and 1251 to placebo (P). Characteristics included median age 29; 70% White, 16% Black; 8% Hispanic; risk factors balanced across arms. On 4/22/13 the DSMB recommended halt of vaccinations based on pre-specified efficacy futility criteria. The 6-plasmid DNA vaccine [Gag, Pol, Nef (clade B) and gp145 Envs (clades A, B, C)] was administered at Wks 0, 4, 8 and 24. The rAd5 boost was re-challenged with vaccine arms at Wk 28. Results at end of blinded follow-up showed 27 (V) and 21 (P) Wk 28+ HIV infections [VE -25.0%; (95%CI -121.2,29.3); p = 0.44]. Mean VL setpoints were 4.46 (V) and 4.47 (P) log RNA copies/mL. MITT analysis, including all infections [41 (V);31 (P), p = 0.28] was concordant with the primary analysis. The vaccine regimen was well tolerated and immunogenic for both T and B cell responses.

Conclusion: The VRC DNA/rAd5 regimen neither reduced HIV acquisition nor post-acquisition VL setpoint in MSM and TGW. The greater number of infections in the vaccine compared to the placebo arm is not statistically different but unblinded follow-up continues for further assessment.
P04.39 LB

Vaccination with Heterologous HIV Envelope Sequences and Heterologous Adenovirus Vectors Increases T Cell Responses to Conserved Regions: HVTN 083

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Background: Several approaches have been proposed to improve the ability of HIV vaccine-induced responses to recognize diverse circulating HIV strains, among them increasing the breadth of immune responses or directing them towards conserved regions. HVTN083 tested how prime-boost strategies employing heterologous vectors, heterologous inserts or combinations thereof affect the breadth and specificity of cellular immune responses.

Methods: 180 subjects were randomized among five prime-boost combinations of vectors (Ad5 or Ad35) and HIV-1 Env inserts (clade A or B). T-cell responses to individual 15mers were mapped by IFN-γ ELISpot using insert-matched peptides. Shared epitopes were defined as sequences ≥8 amino acids (AA) with ≥8 identical AA for EnvA and EnvB. Alternatively, optimal 8-10 AA epitopes were predicted using a computational HLA binding predictor (NetMHCpan) and participants’ HLA class I types with similar results.

Results: All regimens were well tolerated with no vaccine-related serious adverse events. Response rates to EnvA (included in all groups) were similar across groups (58.1%-75.9%). Heterologous and homologous insert regimens elicited responses to similar total numbers of epitopes (ratio of means=1.0, 95% CI=[0.6, 1.6], p=0.91), however, heterologous insert regimens targeted more epitopes shared between EnvA and EnvB than homologous insert regimens (ratio of means=2.7, 95% CI=[1.2, 5.7], p=0.01). Shared responses included responses to regions conserved between EnvA and EnvB, or cross-reactive T cells targeting variant EnvA and EnvB epitopes. Heterologous vector regimen responses had significantly higher numbers of total, EnvA, EnvB and shared epitopes than homologous vector regimen responses (p=0.02, 0.044, 0.045, 0.065 respectively).

Conclusion: Combinations of Ad5-EnvA, Ad5-EnvB, and Ad35-EnvA appeared safe and immunogenic. Vaccination with heterologous vector-insert pairs has minimal impact on overall response rates; however, heterologous vector regimens increased the total number of targeted epitopes. Notably, heterologous insert prime-boosting increased T-cell responses to shared epitopes. Increased responses to conserved epitopes may translate into better coverage of circulating viruses.

P04.40 LB

Preliminary Results of a Brazilian Clinical Trial for DC-Based Immunotherapy Against HIV-1

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Background: Immunotherapy based on monocyte-derived dendritic cells (moDC) is a promising strategy for treating HIV-infected individuals. Clinical trials using this product have shown to be safe and well tolerated, but individual response is variable. Currently, we are conducting a phase I/II clinical trial aiming to test the effect of moDC pulsed with chemically inactivated autologous HIV in a Brazilian cohort. We present preliminary results concerning safety and evolution of plasma viral load during the first 90 days after vaccination.

Methods: Nineteen chronically HIV-infected patients, naïve for antiretroviral treatment, were randomized in three groups (G1-G3) to receive three doses (15 days intervals) of 3x10⁷ un-pulsed DCs (G1, n=4), 3x10⁸ HIV-pulsed DCs (G2, n=9) or 3x10⁷ HIV-pulsed DCs (G3, n=6). Safety and immunovirological parameters will be monitored for one year.

Results: We hereby report data from the first 90 days of follow up. Forty-five days after the first dose of vaccination, two out of three patients in G1 and four out of six in G3 have shown a decrease in plasma viral load (PVL) (mean of 0.056 log and 0.323 log in G1 and G3 respectively). Ninety days after vaccine initiation, 3/3 subjects in group G1, 2/7 in G2, and 3/6 in G3 have presented reduced PVL (mean of 0.056 log and 0.323 log in G1 and G3 respectively). As for the CD4 and CD8 T cell counts, so far, we have not observed significant changes after vaccination.

Conclusion: Until now, our results have demonstrated that the vaccine is safe, for no major adverse–related events have been reported. These preliminary results suggest a slight advantage for subjects under the 3x10⁷ HIV-pulsed DCs regimen with respect on PVL. A more conclusive response to this immunotherapy will be better defined once the ongoing follow-up is concluded.
**P04.41 LB**

**Immunogenicity and Efficacy of a Therapeutic HIV-1 Vaccine (HIVAX™) in HIV Patients on HAART**

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**Background:** HIV-1 specific cellular immunity contributes to the control of viral replication. In this first-in-human therapeutic vaccination study, a replication-defective HIV-1 vaccine was tested in HIV-1 infected subjects under antiretroviral treatment intermittent (ATI) warranting further evaluation of protective efficacy by this therapeutic vaccine.

**Methods:** A010 is a randomized, placebo-controlled dose-escalation trial to evaluate the safety and immunogenicity of two doses of a replication-defective HIV-1 vaccine (HIVAX™) in subjects receiving stable highly active antiretroviral therapy (HAART) who have an HIV-1 RNA <50 copies/ml and CD4 cell count >500 cells//mm3. Fifteen subjects were randomized 2:1 to vaccine or placebo in low dose group. Following the randomized 24-week vaccination phase, subjects who received active vaccine and who met eligibility underwent a 12-week analytical antiretroviral treatment interruption (ATI). Subjects with viral load less than 5,000 copies/ml and stable CD4 cell count at week 36 have option to continue ATI. Cellular immunity was measured by ELISPOT assay. Plasma cytokines were monitored during the vaccination phase. CD4 cell count and viral load were monitored throughout the trial.

**Results:** HIVAX™ was well tolerated. Mild injection site reaction with transient induration occurred in 9 subjects. High levels of cell-mediated immune response, measured by ELISPOT assay, were observed in all subjects receiving HIVAX. Subjects receiving placebo have low background of ELISPOT responses. Surprisingly, pro-inflammatory cytokines (IL-6, IL-1β, TNFa, IL-1α, IL-8, IL-15) were all significantly reduced in vaccine recipients compared to pre-vaccination level. The median viral load set point (2,841 copies/ml) was significantly lower in vaccine recipients compared to pre-vaccination level. Two subjects who continue ATI for over 10 months still maintain low viral load and stable CD4 cell count.

**Conclusion:** HIVAX™ vaccine is safe and highly immunogenic in these HIV-1 infected subjects. HIVAX™ was effective in controlling viral load during ATI warranting further evaluation of protective efficacy by this therapeutic vaccine.

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**P04.42 LB**

**Transcriptional Profiling of RV144 Participants Reveals a Gene Expression Signature that Correlates with Immunogenicity**

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**Background:** The Thai HIV Vaccine Clinical Trial (RV144) showed partial protection from infection. Our objective was to define transcriptional correlates of immunogenicity in the trial via antigen-specific stimulation of participant peripheral blood mononuclear cells (PBMCs).

**Methods:** We used Illumina HumanHT-12 BeadChips to produce two microarray datasets, initially on samples from 40 RV144 vaccinees and 10 placebo subjects. PBMCs collected at pre-vaccine, 26wk and 52wk post-enrollment were stimulated with 15h using 92TH023ENV peptide pools or DMSO control. We also arrayed 426 case-control study samples from 213 participants after 15h stimulation with ENV or DMSO at 26wk post-enrollment. Because our group was blinded to case-control status, our bioinformatic analysis was performed on both datasets with the purpose of identifying transcriptional profiles that correlate with intracellular cytokine staining (ICS) data.

**Results:** We compared responses to ENV in vaccinees after subtracting the DMSO baseline between pre-vaccine and 26wk revealing a 119 gene signature (P<0.01, <20% False Discovery Rate) that distinguished a cluster of vaccinees from placebo subjects. This signature was associated with response status upon correlation with ICS data (Fisher’s exact test, P<10^-3). To validate this signature, we analyzed the case-control cohort dataset by regressing ICS data with differential gene expression (P<0.05, Fold Change>1.3) for stimulated vaccine-recipient PBMCs (DMSO background subtracted). Hierarchical clustering of the top genes by r² value that most significantly correlated with ICS revealed clusters of putative responding and non-responding subjects. Genes highly associated with the ICS data included interferon response genes (STAT1, CXCL9/10, SERPING1, TAP1/2, WARS) and TNFA (inflammasome) in the case-control dataset.

**Conclusion:** Our study represents the first functional genomics analysis of RV144 trial subject samples and has identified potential mechanisms underlying the development of protective immune responses in RV144 participants. Funding by NIAID/DAIDS (HVVTN) and the Henry M. Jackson Foundation.
A Late Third HIV-MVA Vaccination Boosted Strong and Potent Immune Responses in Tanzanian Volunteers Previously Immunized with HIV-DNA and HIV-MVA

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Background: A successful vaccine against HIV requires effective and long-lasting cellular and humoral immune responses. We explored the duration of immune responses and the effect of a third HIV-modified vaccinia Ankara (MVA) boost in HIV-DNA and HIV-MVA Tanzanian vaccinees.

Methods: Twenty healthy volunteers previously primed with HIV-DNA plasmids encoding HIV-1 subtypes A, B, and C at months 0, 1 and 3 and boosted with HIV-MVA expressing CF10-AE at months 9 and 21 were given a third HIV-MVA boost three years after the second HIV-MVA immunization. Despite pre-existing immunity to vaccinia, a late third HIV-MVA vaccination boosted strong and potent anti-HIV cellular and humoral immune responses in previously HIV-DNA and HIV-MVA immunized volunteers.

Conclusion: Anti-HIV immune responses were still detectable three years after the second HIV-MVA immunization. Despite pre-existing immunity to vaccinia, a late third HIV-MVA vaccination boosted strong and potent anti-HIV cellular and humoral immune responses in previously HIV-DNA and HIV-MVA immunized volunteers.

P04.44 LB

A Phase I/II Trial of Preventive HIV Vaccination with DNA and Poxviral-Vector in Healthy Subjects EV03/ANRS VAC20: Cytokine Production by T Cells


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Background: The EV03/ANRS Vac20 phase I/II trial investigated the optimal prime/boost strategy using DNA/NYVAC combination, both expressing HIV-1 clade C Env and Gag/Pol/Nef. 147 healthy volunteers were randomized in 8 European centres to 3 DNA/1 NYVAC (weeks 0/4/8/24; group 1) or 2 DNA/2 NYVAC (weeks 0/4/20/24; group 2). At week 26 and/or 28, T cell responses (IFN Elispot assay) were present in 91% and 80% of volunteers in group 1 and 2, respectively (P=0.053). The magnitude of responses was higher in group 1 than in group 2 (P<0.001).

Methods: Functionality of T cell responses was assessed using ICS assay performed at week 26/28 in responders to Env plus at least to one of the Gag/Pol/Nef pools. Cells were stimulated 6h with the same pools used for IFNy Elispot in the presence of anti-CD28 antibody and BFA, and stained with mAb CD3/CD4/CD8/IL2/IFNy/TNFa. Responses were defined as % cytokine+ in CD4+ or CD8+ T cells > 0.03% and 3 fold background.

Results: Among 66 Elispot responders (n=43 group 1, n=23 group 2), the frequency of CD4+ T cell responders to at least one HIV pool was 68%. The magnitude of CD4+ T cell responses (median [IQR]) was 0.62% [0.55-0.86] without difference between groups. The majority of CD4+ T cell responses were directed against Env (72% and 56% in groups 1 and 2, respectively) while only 9% were directed to Gag/Pol/Nef. 38% of vaccinees were CD8+ T cell responders and the magnitude of CD8+ T cell responses to Gag/Pol/Nef was 68%. The magnitude of CD4+ T cell responses (median [IQR]) was 0.50% [0.40-0.72] without difference between groups. The majority of CD4+ T cell responses were directed against Env (72% and 56% in groups 1 and 2, respectively) while only 9% were directed to Gag/Pol/Nef. 38% of vaccinees were CD8+ T cell responders and the magnitude (median [IQR]) was 0.50% [0.40-0.72] without difference between groups. In each group, 28-30% of vaccinees responded to Env and 13-16% to Gag/Pol/Nef. Among HIV-specific CD4+ and CD8+ T cells, 33% and 17% produced at least 2 cytokines, respectively.

Conclusion: DNA/NYVAC combination elicits both polyfunctional CD4+ and CD8+ T cell responses with a majority of CD4+ T cells directed against Env protein.
Background: Closed-ended methods, such as true/false tests, for assessment of understanding (AoU) may not measure true understanding of important trial concepts. For HIV vaccine trials, complex concepts require more comprehensive assessment methods.

Methods: Eighty men and women were enrolled at two clinical research centers in South Africa and Uganda, which were recruiting for phase I HIV vaccine trials. A within-subjects, repeated measures design was used. Consenting volunteers were given three AoUs with different formats: a true/false test, a narrative, and a mixed-method approach using true/false questions and scenarios. Performance was compared using correlations and repeated-measure t-tests, and inter-rater reliability analysis. Interviews were recorded and transcribed. Volunteer feedback on the AoUs was collected with a questionnaire. Focus groups with study staff investigated experiences implementing the different formats. AoU transcripts were scored by multiple raters using standardized criteria.

Results: The true/false and the narrative scores differed significantly, as did the true/false and scenario scores. Narrative and scenario scores were well correlated. The true/false test appeared to overestimate understanding, compared to the qualitative measures. Inter-rater reliability scores showed a significant variance. Results from the volunteer questionnaires and staff focus groups showed that the scenario format was preferred because of volunteer comfort level and increased dialogue about the trial between volunteers and staff.

Conclusion: The results suggest that qualitative methods may be better suited to assessing volunteer understanding in clinical trial settings, particularly for concepts that are considered essential for genuine informed consent. Given that the inter-rater reliability showed significant differences in how scorers were evaluating the AoU responses, it seems prudent to incorporate more training for individuals who administer and score AoUs in order to increase consistency. An AoU toolkit is being developed that will incorporate findings from this study and additional experience using the tool in recent HIV vaccine clinical trials.

Targeting HIV Env gp140 to LOX-1 in Combination with Replication Competent NYVAC-C Elicits Humoral and Cellular Responses in Rhesus Macaques

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Background: Protective immunity against HIV will likely require vaccination with a combination of a viral or DNA vector with HIV Env protein plus adjuvant. To this end, we have developed as a candidate protein component cLOX-1.HIV Env gp140, a dendritic cell (DC)-targeting vaccine bearing gp140 from clade C ZM96 fused to a humanized cLOX-1 monoclonal antibody.

Methods: Non-human primates (NHP), either naïve (4 per group) or primed twice (wk0, 4) with NYVAC-KC viruses encoding CN54 Gag, Nef, Pol and ZM96 Env gp140 sequences, were injected three times (wk12, 16, 20) intradermally with 250 micrograms of cLOX-1.HIV Env gp140 plus 1 milligram poly ICLC (Hiltonol) or 20 micrograms of GLA as adjuvant. ELISA was used to assay Env-specific antibody responses, while IFNγ ELISPOT was used to detect Env-specific T cell responses.

Results: In the naïve NHP groups, anti-Env IgG titers were elicited to maximal levels at 2 weeks after the third vaccination and were significantly more robust with poly ICLC (~400 spots per 10^6 cells) at 2 weeks after the first vaccination and were only observed with poly ICLC adjuvant. In the NYVAC-C-primed groups, anti-Env IgG titers were maximal (~400 spots per 10^6 cells) at 2 weeks after the second vaccination and were only observed with poly ICLC adjuvant. In the NYVAC-C-primed groups, anti-Env IgG titers were maximal (~400 spots per 10^6 cells) at 2 weeks after the first cLOX-1.HIV Env gp140 vaccination and were similar with both adjuvants. However, Env-specific T cell responses were more robust (200-300 spots per 10^6 cells) in the poly ICLC group. Overall, the DC-targeting vaccines elicited both CD4+ and CD8+ T cell responses against multiple Env epitopes, and especially for the CD4+ T cell responses, the quality was high.

Conclusion: Thus, cLOX-1.HIV Env gp140 plus poly ICLC is a promising protein and adjuvant combination for eliciting potentially protective Env-specific antibody and T cell responses in both priming and boost settings.
P05.01 D

Rapid Detection of Early HIV-1 Infections by Direct Sequencing of a Hypervariable Segment of the Viral Env Gene

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Background: In the 75% of HIV transmission events, only one viral variant establishes the infection. To know this specific variant allows performing studies to increase our understanding of transmission. Clinical data analysis can identify patients with evidence of recent infection but are unable to define whether a high degree of viral diversification have already occurred. Here, we propose that direct sequencing of a short hypervariable segment of env allows rapid identification of newly infected patients with very early infection times.

Methods: We studied 44 newly diagnosed individuals with clinical evidence of infection time (30-270 days). Blood samples were collected and the viral RNA was extracted from plasma with QIAamp Viral RNA Kit. An hypervariable segment of the env gene (nt. 6858 to 6883 of HXB2) was amplified by nested RT-PCR and sequenced with Big Dye terminator Kit and ABI Prism 3100 sequencer. Chromatograms were analyzed with Sequencher 4.10.

Results: From the analysis of the sequences of the segment we found that the number of ambiguities present in the sequences increased with the number of days estimated post-infection. The average number of ambiguities was 0.125 in patients with less than 60 days of infection, 3.71 for 90, 13.6 for 120, 17.33 for 150 and largely superior than 17.3 for 270 days post-infection. In addition, in a set of 5 patients we observed that the intra-host diversity of the gag gene (assessed by cloning and sequencing of the quasispecies) was also correlated with the number of ambiguities in the env segment.

Conclusion: We show that it is possible to rapidly identify patients where the founder virus might still be present by sequencing of an hypervariable region of the env gene, which provides an accurate correlate with the infection time estimated from clinical and serological data.

P05.02 D

Mechanism of HIV Entry into the Columnar Epithelium of the Female Genital Tract

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Background: During male-to-female transmission HIV must bypass the mucosal epithelium of the female reproductive tract and gain access to underlying target cells. Previously, we illustrated that HIV is able to penetrate intact columnar and squamous genital epithelia in both ex vivo and in vivo systems. Furthermore, we also previously illustrated that virus enters the squamous epithelium in a diffusion-based mechanism. By utilizing a similar series of approaches, we illustrate endocytosis as a possible mechanism of HIV entry into the simple columnar epithelium of the endocervix.

Methods: Human cervical explants were exposed to V-ATPase inhibitor Bafilomycin A1 (BafA1) and cell-free PA-GFP HIV of various tropisms. Control samples were incubated with virus in the absence of BafA1 treatment. Following, samples were removed, snap frozen, sectioned, stained and imaged accordingly through deconvolution fluorescent microscopy. Comparison of the image z-stacks pre and post-photoactivation revealed viral signal, accounting for background.

Results: In our human ex vivo control samples, PA-GFP HIV was found to enter the simple columnar epithelium of the endocervix to depths up to 50 microns. We also confirmed that virions penetrated to depths were target cells reside. In contrast, BafA1 treated samples, independent of viral tropism, had a smaller number of penetrating virions. Likewise, deep penetrators (>20 microns) were rarely observed with the majority of virions seen within the simple columnar barrier itself.

Conclusion: Overall, our control samples confirmed PA-GFP HIV was found to enter the simple columnar epithelium of the endocervix to depths up to 50 microns. We also confirmed that virions penetrated to depths were target cells reside. In contrast, BafA1 treated samples, independent of viral tropism, had a smaller number of penetrating virions. Likewise, deep penetrators (>20 microns) were rarely observed with the majority of virions seen within the simple columnar barrier itself.
P05.03 D

Impact of Host Cell-Specific Glycosylation Differences on SIV Infectivity and Mucosal Transmission

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Background: The dense glycosylation of the human immunodeficiency virus (HIV) envelope protein (Env) shields potential epitopes from neutralizing antibodies. Furthermore, the interaction of Env glycans with immune cell lectins can enhance viral transmission to target cells. The major viral target cells, macrophages and T cells, glycosylate Env differentially, but the consequences of these glycosylation differences for HIV biology are unknown. We seek to identify host cell specific Env glycosylation differences on a molecular level and to elucidate their impact on infectivity and transmission in vivo using the simian immunodeficiency virus (SIV)/rhesus macaque model.

Methods: SIVmac239/316ENV produced in primary rhesus macrophages (M-SIV), primary rhesus CD4⁺ T cells (T-SIV), HEK293 (293-SIV) and 293S GnTI⁻/- cells (GNTI-SIV) was characterized for glycan composition by EndoH and PNGaseF digest and virion incorporation of gp120. Viral infectivity and interactions with dendritic cell lectins were analyzed in standard assays. Mucosal transmission was determined by rectal challenge of rhesus macaques (n=3/virus).

Results: Western blot analysis revealed that M-SIV and T-SIV incorporated similar amounts of Env. Nevertheless, T-SIV Env contained more oligomannose glycans and the virus was less infectious in vitro than M-SIV, indicating that oligomannose glycans might negatively impact infectivity. Similarly, GNTI-SIV, which bears exclusively oligomannose glycans, was markedly reduced in infectivity relative to 293-SIV. However, decreased Env incorporation of GNTI-SIV and not differential glycosylation might explain this phenotype. M-SIV was better transmitted by dendritic cell lectins than T-SIV suggesting that specific glycan motifs might be more important for lectin interactions than high amounts of oligomannose. We are currently investigating whether differential lectin interactions result in differential mucosal transmission of SIV in rhesus macaques. The results of these studies will be presented.

Conclusion: We show that host cell specific glycosylation differences impact SIV infectivity and transmission by lectins. Mass spectrometry must reveal the position and nature of the responsible glycans.

P05.04 D

The V1V2 Domain Preserves Envelope Functionality During Free Virus Transmission but Is Largely Dispensable for Cell-Cell Transmission

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Background: The variable loop 1 and 2 (V1V2) domain of the HIV-1 envelope glycoprotein gp120 has a dualistic role in the envelope’s functionality. Its high variability allows tailored shielding against evolving neutralizing antibodies (nAbs) rendering it the key regulator of neutralization escape. Yet, V1V2 has been indicated to play also an important role in conserving entry functionality of the envelope trimer, largely attributed towards its influence on trimer stability. Here, we aimed to precisely dissect the steps in the HIV-1 entry process steered by the V1V2 domain during both free-virus and cell-cell transmission.

Methods: We generated 12 subtype B and C HIV-1 envelope mutants with V1V2-deletion or specific point mutations, including mutants of the glycosylation site at position 160, and compared wt and mutant envs for entry fitness and nAb shielding capacity during free-virus and cell-cell transmission.

Results: We found that key point mutations or deletion of V1V2 strongly reduced free-virus but not cell-cell transmission. By long-term passaging of a replication-competent V1V2-deleted strain we derived a series of envelope mutants that allowed a detailed characterization of the adaptation strategy HIV chooses to overcome loss of V1V2. We found that V1V2 steers trimer stability, virus entry kinetics and the number of trimers required for entry into target cells. Of note, shielding from nAbs is compromised during both free-virus entry and cell-cell transmission if V1V2 integrity is lost.

Conclusion: Our results underline the role of V1V2 as a major regulator of the HIV-1 entry process during free-virus transmission. The fact that cell-cell transmission is less affected by changes in V1V2 highlights a further possible survival route of the virus. V1V2 mutations required for neutralization escape that render the virus less fit for free-virus transmission may replicate strictly in a cell-cell transmission manner until compensation mutations emerge which ensure both neutralization escape and entry fitness.
### P05.05

**Genetic Characteristics of HIV-1 Env in the Genital Tract of African Heterosexual Transmission Pairs**

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**Background:** A better understanding of characteristics that define mucosally-transmitted HIV-1 may inform vaccine design. Commonly, a single CCR5-tropic virus establishes infection. In cross-sectional studies, histidine in Env leader peptide position 12 (His-12) is more frequent in blood-derived subtype B viruses from early compared to chronic infection, but few data exist for non-clade B or genital tract-derived viruses.

**Methods:** Full-length HIV-1 env was sequenced from multiple viral templates in seminal plasma and endocervical swabs from 9 African male-to-female transmission pairs in the Partners in Prevention HSV/ HIV Transmission Study. Viral genetic diversity, chemokine co-receptor usage, and Env position 12 residues were evaluated.

**Results:** A mean of 14 sequences (SD = 6) was obtained from each participant. Couples were infected with HIV-1 subtypes A or A1/C (N=4), C (N=3), and D (N=2). Seroconverters had less variable sequences compared to transmitters (pairwise genetic distance 0.5% versus 1.4%, Student’s t-test p = 0.007). Within transmission pairs, mean pairwise distance between the most closely related sequences was 1.3% (SD = 0.7%). Viruses were predicted to be CCR5-tropic, except in one transmitter with 7/16 CXCR4-tropic viruses. At Env position 12, 17 participants had homogenous viral templates, encoding glutamine (N = 8), asparagine (N = 3), histidine (N = 4), and arginine (N = 2). Partners in these transmitting couples shared Env position 12, with the exception of one couple with asparagine in 15/15 seroconverter and 17/19 transmitter sequences, and aspartate in 2/19 transmitter sequences.

**Conclusion:** Genital tract viruses within African male-to-female HIV-1 transmission pairs show evidence of a genetic bottleneck and predominant CCR5 co-receptor usage. In this small study, multiple variants were rarely observed at Env position 12 in transmitters and His-12 was not found with increased frequency in seroconverters. Further studies are underway to determine the significance of Env position 12 in transmission of non-clade B viruses.

### P05.06

**Incidence of HIV-1 Superinfection Is Similar to Primary HIV-1 Incidence in a Cohort of Female Sex Workers in Uganda**

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**Background:** Understanding the host, viral and immunological correlates of HIV-1 superinfection are crucial for vaccine design and development. Several studies have shown varying incidence rates of superinfection among different risk behavior populations. We report on the incidence of HIV-1 superinfection in a cohort of female sex workers in Kampala, Uganda.

**Methods:** In 2008, the Good Health for Women Project (GHWP) recruited a cohort of 1027 women involved in commercial sex work in Kampala, Uganda. The baseline HIV-1 prevalence was 37% and HIV incidence rate in the first 3 years was 3.7/100-person years. A validated next-generation sequencing technique was used to determine the rate of HIV superinfection by examining two regions of the viral genome (p24 and gp41) in two longitudinal samples from women who entered the cohort as HIV prevalent cases (n=125).

**Results:** 70% (88/125) of the women screened had amplifiable sequences from at least one genomic region for both time-points analyzed (204.2 total person years). The samples amplified well with good sequence coverage for time-point 1 (mean of total sequence reads for p24=8901, gp41=9606) and time-point 2 (p24=12625, gp41=8301). Seven cases of superinfection and eight cases of women initially diagnosed with dual HIV-1 infection were identified. The rate of superinfection in this population was calculated to be 3.4/100 person years. Together with the dual infections, 17% of the women in the cohort experienced infection with multiple strains of HIV during the study. The rate of superinfection was more than double the rate observed in a lower-risk rural general population in Uganda (IRR=2.4 95% CI=0.7-7.9; p=0.06).

**Conclusion:** The rate of superinfection was highly similar to the primary HIV incidence rate in this high-risk population, which agrees with previous data from a lower-risk general rural population. The high rate of superinfection is most likely a reflection of continued risky behavior in this population.
P05.07

Phenotypic and Functional Characterization of CD4+ T Cells in the Foreskin

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Background: Male circumcision is known to reduce HIV incidence by ~60% in heterosexual men from Sub-Saharan Africa. Therefore, a better understanding of the immune correlates of HIV acquisition in the foreskin is needed to design additional male-focused HIV prevention strategies. We characterized putative HIV target cells in the foreskin using multi-parameter flow cytometry.

Methods: We collected foreskin tissue and blood from 34 HIV-uninfected Ugandan men. Foreskin T cells were isolated using previously described mechanical and enzymatic digestion. Phenotyping was performed using the following antibodies: CD3, CD4, CCR5, CD69, HLA-DR, CD38, CCR6, IL17a, IL22, IFNγ, α4, β7, and CLA.

Results: The phenotype of CD4+ T cell subsets differed between blood and foreskin. As previously described, foreskin CD4+ T cells expressed much more CCR5, CD69 and HLA-DR than blood cells (p<0.0001). Th1, Th17, and Th22 cells from the blood all expressed higher levels of CCR5 than did bulk CD4 T cells, with highest expression on Th17 cells (CCR6+/IL17a+). In the foreskin, CCR5 expression was greatest on Th1 cells (p=0.003), elevated to a lesser extent on Th17 cells (p=0.001), and decreased on Th22 cells (p=0.008) compared to bulk CD4 T cells. Foreskin CD4 T cells were more polyfunctional than blood, with a higher proportion of cells producing ≥2 cytokines. Foreskin CD4+ T cells expressed low levels of α4β7 compared to blood (1.68% vs. 6.93%, p<0.0001), but very high levels of the skin homing marker CLA (59.0% vs. 18.45%, p<0.0001).

Conclusion: The phenotype of functional CD4+ T cell subsets differs substantially between the blood and foreskin, with implications for HIV susceptibility. Homing marker expression in the foreskin is more characteristic of skin than mucosal-homing T cells. Further characterization of foreskin T cells may assist the development of better HIV prevention tools.

P05.08

Transmission and Ultradeep Sequence Evolution of CXCR4 Using HIV-1 in an Individual Homozygous for the CCR5 Delta-32 Mutation

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Background: Following an explosive mid-1990s HIV-1 outbreak, the incidence of X4 infections among Vancouver injection drug users was 13%, one-half of which mapped to a single phylogenetic cluster. Within this cluster, we identified a putative case of X4-HIV transmission from a CCR5wt/wt donor to a CCR5Δ32/Δ32 recipient, and characterized this rare event using deep-sequencing.

Methods: Phylogenetic relatedness of donor and recipient HIV was confirmed by bulk sequencing of plasma RNA gag, pol, nef and env-V3. Host CCR5 genotyping was performed using sequence-based methods. The donor’s estimated infection date was Mar/00; plasma/PBMC were available 10, 16 and 50 months thereafter. The estimated transmission event occurred Aug/01; recipient plasma/PBMC were available 5, 6 and 12 months thereafter. Env-V3 from plasma-RNA and PBMC-DNA were triplicate amplified, pooled and deep-sequenced.

Results: V3 deep-sequencing suggested transmission of a minimum of two highly related variants in both donor and recipient. Exact matches for the recipient’s two predominant variants were not observed in donor plasma at any timepoint; instead, one matched a minority (0.04%) PBMC variant 8 months earlier. Whereas the CCR5wt/wt donor exhibited rapid progression (Log10pVL>5 copies/ml and CD4=20 cells/mm3 within 1.5 years of infection), the CCR5Δ32/Δ32 recipient’s CD4 counts remained >400 cells/mm3 for two years with Log10pVL<4.2. Whereas the donor’s coreceptor genotype evolved towards increased CCR5 usage in the first year (X4 prevalence from 100% to 52% by the time of transmission), the recipient’s HIV evolution appeared constrained and remained consistently X4. Within one year, the recipient’s dominant virus population was replaced with a single V3 R25K variant, with novel X4 minority variants exhibiting positively-charged residues at codon 24 and others, also emerging.

Conclusion: Results highlight HIV’s extensive capacity for host adaptation. Discordant clinical progression in the donor vs. recipient despite infection with a highly similar virus underscore the influence of host genetics on HIV pathogenesis.
Evidence of Selection During HIV-1 Transmission in Heterosexual Couples in Zambia

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Background: HIV infection occurs by the transmission of one or a few of the variants present in the donor. This process is poorly understood and it is not known if this transmission bottleneck is mediated by an active selection process or is the consequence of a random event.

Methods: In a group of 169 epidemiologically-linked transmission pairs from a Zambian cohort, we studied the frequency and dynamics of HIV polymorphisms in Gag, Pol and Nef. The frequency of each residue (based on 350 chronically-infected patients) was correlated with the rate of transmission in the transmission pairs.

Results: Gag, Pol and Nef proteins of transmitting partners had a large number of non-consensus amino acid polymorphisms (medians of 36, 41 and 24, respectively) and the majority of them (80%) were transmitted to the epidemiologically-linked partner. Overall, the odds of transmission was significantly correlated with the polymorphism frequency in the cohort, with residues present in 95% of donors transmitting with 99% frequency vs 87% transmission frequency for rare polymorphisms. A similar bias was observed at positions where there was a mixture of polymorphism and consensus residues, with polymorphisms being transmitted at a significantly lower frequency than predicted (43% vs 50%; p<10⁻¹⁰). Polymorphisms were 1.6x more likely to be transmitted from male-to-female than from female-to-male (p=4x10⁻¹¹) and, in the female-to-male transmission pairs, we identified 9 positions among Gag, Pol and Nef where the rate of transmission was significantly different than expected from the frequency of the residues (p<0.005; q<0.2).

Conclusion: While a majority of Gag, Pol and Nef polymorphisms are transmitted, there is a dose dependent relationship between cohort prevalence of a residue and odds of transmission. Moreover, female-to-male transmission results in higher negative selection on polymorphisms. These observations argue in favor of a selective transmission process that favors certain residues.

Human Leukocyte Antigen Class I (HLA-I) Adapted Epitopes Can Induce Functional Primary CD8+ T Cell Responses During Acute HIV-1 Infection

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Background: HIV CD8+ T cell escape mutations can be predicted based on the human leukocyte antigen class I (HLA-I) allele of the infected individual, whereby the epitope adapts to HLA-restricted pressure. Although these adapted epitopes may still be able to induce CD8+ T cell responses, the extent to which this occurs has not been accurately assessed.

Methods: We derived the transmitted founder virus (TFV) from 13 clade B HIV acutely infected patients. This strategy allowed us to differentiate primary vs. cross-reactive responses, which is not possible when studying chronic patients. Using IFN-γ ELISPOT assay, we compared the ability of TFV-encoded adapted or non-adapted epitopes to induce primary CD8+ T cell responses.

Results: Non-adapted epitopes were more likely to elicit a primary response (p=0.046). Similarly, individuals with more non-adapted epitopes encoded by their TFV had a greater breadth of primary responses (p=0.028). Furthermore, in a cross-sectional study of a larger cohort of Zambian patients with acute clade C HIV infection, those with TFV encoding fewer adapted epitopes had lower set point viral loads (p=0.007). Thus, protective CD8+ T cell responses may be maintained in an individual who’s TFV contains more non-adapted epitopes. Although responses to adapted epitopes were seen less frequently, they shared similar magnitude, functional avidity, and polyfunctionality compared to those elicited to the non-adapted counterparts. Interestingly, the few adapted epitopes that elicited a response were predicted to exhibit higher HLA binding affinity compared to the non-responsive ones (p<0.0001). Testing these non-responsive adapted epitopes in a larger cohort of chronic patients confirmed the lack of specific HLA-restricted responses.

Conclusion: Therefore, while some adapted epitopes induced primary CD8+ T cell responses that were indistinguishable from their non-adapted counterparts, the majority did not elicit any response. These results have important implications for T cell-based HIV vaccine strategies.
**P05.11**

**Impact of Training Peer Educators Ex-Fighters BURUNDI as a Strategy to Reduce Risk Behaviors Favoring the Transmission of HIV/AIDS**

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**Background:** AIDS affects not only the physical health of individuals but also their family and community identity. Indeed, the stigma and discrimination surrounding the disease are equally destructive as the disease. This stigma is so strong in groups that have been affected by armed groups such as ex-combatants conflict. To improve the status of veterans in their home communities in reducing risk behaviors favoring the transmission of HIV/AIDS, FACOSASE wished form 360 PEEXC to fight against this scourge and the objective Show is PEEXC on how to improve the strategy of reducing risk behaviors favoring the transmission of HIV/AIDS.

**Methods:** To implement this strategy as training PEEXC reduction of risk behaviors facilitating the transmission of HIV/AIDS, we first conducted a raid on land in order to identify PEEXC who will participate in the training.

**Results:** At the end of training, there is the development of knowledge of older PEEXC 20 to 35 years on a better integration to reduce risk behaviors favoring the transmission of HIV/AIDS educators - A general awareness of the issue of this strategy through media coverage.

**Conclusion:** This training helped PEEXC on developing knowledge PEEXC as a strategy to reduce risk behaviors facilitating the transmission of HIV/AIDS.

It was anticipated identification of best practices that could be applied elsewhere in order to reduce risk behaviors favoring the transmission of HIV/AIDS among groups that have been affected by armed conflict in general and in particular the ex-combatants BURUNDI and drive in the psycho-social care and the medical PEEXC having made a voluntary tracks and living with HIV/AIDS to improve their quality of life and more of the 360 who have been trained and have the screening 60% were seropositive.

**P05.12**

**Cryptic Determinant of α4β7 Binding in the V2 Loop of HIV-1**

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**Background:** The peptide segment spanning positions 166-181 of the V2 loop of HIV-1 has been found to harbor two functionally important sites. The first, spanning positions 179-181, binds the human α4β7 integrin receptor that plays a role in dissemination of infecting HIV-1 virions to gut-associated lymphoid tissue (GALT). The second, at positions 166-178, is the target of anti-V2 antibodies elicited by the vaccines used in the RV144 clinical trial. Here, we present evidence that this second site affects the functionality of the first site.

**Methods:** We generated short V2 peptides mimicking the A244/TH023 and MN strains of HIV-1, the immunogens tested in the RV144 trial, and measured the interaction of these peptides with the α4β7 integrin receptor on the surface of CD4+ and CD8+ T cells in an in vitro cellular assay. We also generated a set of diverse V2-derived peptides and analyzed the pattern of their binding to the α4β7 integrin receptor.

**Results:** Peptides bearing the same sequence at positions 173-185 as RV144 immunogens did not bind α4β7 on both CD4+ and CD8+ T cells. However, the addition of amino acids QRV at positions 170-172 restored binding. Specific amino acids at 170-172 were also required for binding of diverse V2-derived peptides.

**Conclusion:** We identified a second, cryptic determinant of α4β7 binding located at positions 170-172 of the V2 loop. Interestingly, the anti-V2 antibody response elicited by the RV144 vaccine, along with immune pressure inferred from a sieve analysis, is directed specifically to these same amino acids within the V2 loop. The anti-V2 antibodies that apparently reduced the risk of infection in the RV144 trial may have functioned by neutralizing α4β7-mediated dissemination to GALT via this cryptic determinant.
Siglec-1 Expressed on Activated Monocytes Captures and Transfers HIV-1 Through Recognition of Sialyllactose Exposed on Viral Membrane Gangliosides

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**Background:** HIV-1 cell-to-cell transmission is a potent infectious pathway that could boost viral dissemination in tissues. We have recently identified that the Sialic acid-binding Ig-like lectin 1 (Siglec-1, CD169) expressed on dendritic cells captures HIV-1 through recognition of sialyllactose exposed on viral membrane gangliosides. Siglec-1 is up-regulated upon dendritic cell exposure to immune activation signals, such as Interferon-alpha (IFNα) or lipopolysaccharide (LPS). However, if other myeloid cells, such as activated monocytes, mediate HIV-1 transmission via Siglec-1/sialyllactose recognition, is still not known.

**Methods:** Monocytes from seronegative donors were treated or not with LPS or IFNα and analyzed for Siglec-1 expression by flow cytometry. Fluorescent HIV-1 viral like particles lacking the envelope glycoproteins were used to monitor Siglec-1 viral capture via sialyllactose recognition of viral membrane gangliosides. HIV-1 trafficking in activated monocytes was followed by confocal microscopy. Siglec-1 role during HIV-1 transmission was further assessed in functional blocking assays. Finally, the expression of Siglec-1 on monocytes of HIV-1 infected patients was detected before and after antiretroviral treatment. Statistical analysis was performed using paired.

**Results:** Both LPS and IFNα-activated monocytes increased Siglec-1 expression compared to non-activated cells. Siglec-1 on IFNα-activated monocytes increased viral capture and transmission to target cells (P<0.0001 and P=0.021, respectively). Moreover, pre-treatment of IFNα-activated monocytes with a monoclonal antibody against Siglec-1 diminished HIV-1 capture and transmission (P<0.0001 and P=0.0013, respectively). Confocal microscopy revealed trafficking of Siglec-1 and HIV-1 to the same cytoplasmic sac-like compartment. Finally, antiretroviral treatment in HIV-1 patients decreased Siglec-1 expression (P=0.0024).

**Conclusion:** Both LPS and IFNα-mediated immune activation in response to HIV-1 infection induce Siglec-1 expression on monocytes. Siglec-1 expressed on activated monocytes recognizes sialyllactose exposed on HIV-1 membrane gangliosides, facilitating infection of target cells. Thus, Siglec-1 expressed on myeloid cells, such as monocytes and dendritic cells could boost HIV-1 dissemination in inflamed immune tissues.

Altered Immune Activation in HIV-Negative High-Risk Men Who Have Sex with Men (MSM) Compared to Low-Risk HIV-Negative Men

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**Background:** Sexually transmitted infections and inflammation in the female genital tract can raise the risk of HIV acquisition in women. Men who have sex with men (MSM) are often at increased risk of HIV infection due to behavioral and biological factors. In order to assess whether high-risk behavioral preferences associated with increased markers of immune activation, we recruited a cohort of high-risk HIV-negative MSM (unprotected sex, intravenous drug use) and compared markers of immune activation to low-risk HIV-negative males.

**Methods:** HIV-negative MSM at high risk for HIV-1 acquisition (HR-MSM) and HIV-negative low-risk males (controls) were enrolled at the Fenway Health Center and the Ragon Institute, respectively. Peripheral blood mononuclear cells isolated from blood samples were analyzed for markers of T lymphocyte activation by flow cytometry. Plasma samples were analyzed for soluble markers of inflammation by bead-based multiplex assay.

**Results:** Subject demographics were as follows: HR-MSM (n=20; mean age=35.7[20-57]; 50% Caucasian, 30% Black/African American, 20% other/unknown), controls (n=11; mean age=40.9[23-63]; 91% Caucasian, 9% Black/African American). The percent of CD4+ and CD8+ T cell populations followed a Gaussian distribution in controls but not in HR-MSM, with the latter also displaying a significantly lower CD4/CD8 ratio owing to a significantly higher proportion of CD8+ lymphocytes (p<0.05). Furthermore, HR-MSM presented with decreased percentage of CD38+CD4+ and CD25+CD8+ lymphocytes. Soluble marker analysis revealed significantly higher levels of IP-10 (p<0.05), as well as a trend toward increased IL-12p40 and IL-12p70 in HR-MSM. Several of the measured inflammatory makers showed a Gaussian distribution in controls, but not in HR-MSM.

**Conclusion:** Our preliminary observations suggest increased immunological heterogeneity and altered levels of immune activation in HIV-negative HR-MSM compared to low-risk controls. These differences could potentially indicate a subset of MSM who are at an increased risk of HIV acquisition following exposure due to an altered inflammatory state.
Background: The HIV-1 epidemic in former Soviet Union (FSU) countries is dominated by a monophyletic subtype A variant (AFSU). In spite of its epidemic importance, no functional envelope clones of this variant have been reported in the literature. Here we aim to obtain a panel of functional AFSU envelope clones from plasma of HIV-1-infected individuals and to characterize their neutralization properties and coreceptor usage.

Methods: Plasma samples collected in Russia from 28 HIV-1-infected individuals were used. Full-length HIV-1 envelopes were RT-PCR-amplified using a single genome amplification assay and cloned into an expression vector. Envelope clones were cotransfected with an Env-deficient HIV-1 plasmid, generating pseudovirions, which were tested for envelope functionality in TZM-bl cells. Phylogenetic sequence analyses were done via maximum likelihood.

Results: Highly functional envelope clones of the AFSU variant were obtained from 18 individuals. All 18 pseudoviruses were sensitive to VRC01 (mean IC50, 0.54 µg/ml); most were sensitive to 2F5 (n=17; mean IC50, 11.49 µg/ml) and 4E10 (n=16; mean IC50, 11.24 µg/ml); and a few were sensitive to b12 (n=5) and 2G12 (n=2). Of 15 pseudoviruses tested for coreceptor usage, 14 were of R5 and 1 was of R5X4 phenotypes.

Conclusion: We report the first panel of highly functional HIV-1 envelope clones of the AFSU variant with characterization of neutralization properties and phenotypic coreceptor usage. Neutralization sensitivities to broadly neutralizing mAbs were similar to those reported for African subtype A-enveloped pseudoviruses. Most clones were of R5 phenotype. These clones may be useful for neutralization assays in vaccine-related studies and for analyses of coreceptor usage and of efficacy of cell entry inhibitor drugs.

**Keywords:** HIV-1, envelope clones, AFSU variant, neutralization, coreceptor usage.
**P05.17**

Dissecting HIV-1 Quasispecies Dynamics Within Intestinal Mucosal Tissues from Primary Infected MSM Subjects

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**Background:** The global spread of HIV-1 has been fueled by mucosal infection through sexual transmission. Gut associated lymphoid tissue (GALT) contains the majority of the body’s CD8+ T cells and is the primary site of viral replication in early HIV-1 infection. Despite recognition of its importance in HIV-1 pathogenesis, characterization of the HIV-1 quasispecies in the GALT during early HIV-1 infection is limited. This understanding may be critical in revealing determinants for viral entry and for a general understanding of virus trafficking and virus turnover.

**Methods:** Endoscopic intestinal biopsies from the colon, ileum and duodenum were obtained from two HIV infected subjects during acute infection (Fiebig III and IV) and 6-months later. Whole-genome amplification coupled with 454 deep sequencing allowed the characterization of intra-host diversity across the entire HIV genome from longitudinal biopsy specimens, plasma and PBMCs.

**Results:** Colon demonstrated the highest viral diversity, with its spectra of variants temporally following those in plasma, including a number of likely CTL escape mutations. Duodenum appeared to harbor unique variants demonstrating a strong preference for the minority variant in plasma. Conversely, ileum was more homogenous, lagging behind the pace of diversification of other tissue compartments and plasma. We also observed a number of tissue-specific viral variants over time.

**Conclusion:** This study highlights the complex dynamic interplay between plasma and mucosal resident virus early after infection. Deep sequencing revealed sequestration of minority variants residing within each tissue compartment, which appeared transient in nature, and may be attributed to local bursts of viral replication. The longitudinal analysis of CTL escape mutants suggests that the intestinal tissues may represent sequestered immunologic compartments that are distinct from one another and the systemic circulation. The compartmentalization of immune pressure may then lead to development of unique tissue-specific viral populations.

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**P05.18**

The Impact of HLA-Class-I-Mediated Control of HIV-1 in a Japanese Cohort

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**Background:** HIV-1 immune evasion from cytotoxic T lymphocytes (CTLs) causes the emergence of escape mutations, resulting in persisting escape variants that shape HLA-associated viral diversity at the population level. Recent studies with Caucasian and African cohorts showed that HLA-associated escape mutations in Gag impose fitness cost and that Gag-mediated viral replication capacity (Gag RC) is compromised in patients with protective HLA alleles. However, the protective alleles well-studied in Caucasian/Africa cohorts, including B*57:01, are not prevalent in Japan, and some studies suggested an important role of Pol as an immune target by CTLs in Asian cohorts. To address these issues, we investigated HLA-associated changes Gag RCs in treatment-naive HIV+ Japanese patients.

**Methods:** Gag RCs were examined by generating chimeric HIV-1 NL4-3 encoding plasma-derived gag-protease from 350 untreated Japanese patients chronically infected with HIV-1 subtype B. RCs were examined in vitro by infecting a GFP+ T cell line.

**Results:** We examined HLA-associated changes in Gag RCs in vitro. Unlike Caucasian/African cohort studies, Gag RCs showed only a weak association with CD4 count and none with plasma viral load (pVL) in our Japanese cohort even though CD4 count and pVL were strongly associated. In addition, Gag RCs for individual HLA alleles did not reflect lower or higher pVL associated with particular HLA alleles and no advantage of rare HLA alleles or disadvantage of homozygosity at HLA class I loci.

**Conclusion:** Even though African and Caucasian-cohort studies previously indicated a significant association between Gag RCs and patients’ clinical outcome, Gag RCs were not predictive of disease prognosis in our Japanese cohort. Our observations suggested a possibility that immune responses against Gag may not play a dominant role in HIV+ Japanese patients, unlike African/Caucasian cohorts, possibly due to different HLA prevalence and unique HLA footprints on the viral evolution.
No Evidence for Selection of HIV-1 with Enhanced Gag-Pro or Nef Function Among Breakthrough Infections in the CAPRISA 004 Tenofovir Microbicide Trial

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Background: The use of antiretroviral drugs for HIV-1 prophylaxis could provide partial protection that inadvertently selects for more fit viral variants among incident infections. To examine this, we assessed in vitro Gag-Protease and Nef protein function in early viral sequences obtained from the CAPRISA 004 1% tenofovir gel microbicide trial.

Methods: Baseline plasma samples (median [IQR] 37.5 [23.9-62.6] days post-infection) were studied for 31 treated and 51 placebo participants (84% of total study infections). Gag-Protease recombinant viruses were constructed (30 treated and 45 placebo participants). Viral replication capacity (RC) was assessed using a GFP reporter T cell assay and results normalized to WT NL4-3. One representative Nef plasma sequence per patient was cloned into pSELECT-GFP vector (31 treated and 48 placebo). Nef clones were transfected into CEM T cells expressing CD4 and HLA-A*02. CD4 and HLA down-regulation functions were strongly correlated (Spearman’s r=0.2; p=0.05).

Results: No major phylogenetic clustering and no differences in Gag-Protease (p=0.2) or Nef CD4 down-regulation function (p=0.2) were observed in sequences from patients who received tenofovir vs. placebo gel prophylaxis. There was a trend towards tenofovir recipients having lower HLA down-regulation function (p=0.06). Nef CD4 and HLA down-regulation functions were strongly correlated (Spearman’s r=0.007); however, neither function correlated with Gag-Protease replication capacity. We observed a modest association between Gag-Protease replication capacity and plasma viral load set-point (Spearman’s r=0.2; p=0.05).

Conclusion: In vitro Gag-Protease and Nef function was similar in breakthrough viruses obtained from individuals in the tenofovir and placebo arms of the CAPRISA 004 study. Results suggest that the 1% tenofovir microbicide gel did not select for founder viruses with significantly altered functional characteristics in these genes.

HIV-1 Gag Evolution During Dual Selective Pressure by Drugs and HLA- Class I Immune Responses in Long-Term Treated Patients

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Background: Although HLA class I immune pressure is the stronger contributor to HIV-1 evolution, the generalized introduction of antiretroviral treatment (ART) to control virus replication, will favour the convergence of drug and immune selective pressures against HIV-1. In this scenario, dual drug/immune pressure could modify the patterns of natural HIV-1 evolution at important immunogenic and enzymatic sites. The accurate understanding of the evolutionary pathways of HIV-1 under ART would be crucial to optimize immunotherapeutic strategies directed to treated individuals.

Methods: Viral RNA was extracted from plasma samples in four patients before and a media of four years after ART introduction. A total of 50 viral clones covering HIV-1 Gag and Protease (Gag-PR) were obtained per patient by Single-Genome Amplification (SGA) and sequence. Selection of HIV-1 drug resistance mutations in the PR and CD8+ T cell epitope variation in Gag was monitored overtime. All subjects were HLA-class I typed and CD8+ T cell responses measured by Elispot against a panel of Gag and Protease overlapping peptides.

Results: Longitudinal HIV-1 Gag-PR sequencing reveals a concomitant accumulation of mutations in Gag and Protease during long-term antiretroviral treatment. However, SGA data demonstrate differences in HIV-1 evolution between patients. For patient 1, HIV-1 Gag evolution was fast and marked by the absence of measurable CD8+ T cells responses during ART. Meanwhile, patients 2, 3 and 4 maintained measurable Gag CD8+ T cells responses during ART and have a low accumulation of Gag mutations with overall lower viral loads. Appearance of drug resistance mutations in the protease was observed in all patients.

Conclusion: Our data demonstrate concomitant Gag-PR evolution and suggest that CD8+ T –cell responses against Gag may contribute to slow viral evolution during ART. These findings provide a better understanding of the evolutionary events during long-term ART and are crucial for the accurate design of immune therapeutic strategies in treated patients.
**P05.21**

**Models of HIV-1 gp120 Complexed with CXCR4 and CCR5**

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**Background:** During cellular entry, the HIV-1 gp120/gp41 viral envelope glycoprotein mediates the sequential attachment of HIV-1 to the CD4 host immune cell receptors, which are the primary receptors, and to the CCR5 or CXCR4 chemokine co-receptors. While X-ray crystallography studies provided details of the interaction between the gp120 core and a two-domain fragment of human CD4, a three-dimensional model in which gp120 simultaneously binds CD4 and either CCR5 or CXCR4 does not exist at this time.

**Methods:** We used in silico molecular docking to find the optimal 3D structural fit between the crown of gp120’s V3 loop and pockets on the molecular surface of the core of gp120 in its liganded conformation. Based on the location of optimal fit, molecular modeling was used to connect the loops between the V3 loop crown and the V3 stem emerging from the core. This new conformation of gp120 was then docked to molecular models and crystal structures of CXCR4 and CCR5.

**Results:** The V3 loop crown exhibited a strong structural preference for a particular docking site on the molecular surface of the gp120 core domain, near a region known as the “bridging sheet”. Using this location to guide the modeling of the complete CD4-ligated conformation of gp120, we found that the new conformation is structurally compatible with the N-terminal sulfated helix of CCR5 and with the ligand-binding surface of the crystallographic surface of CXCR4. Models of the whole complex of gp120 with CXCR4 and CCR5 were refined from this starting point.

**Conclusion:** The interface of gp120 and CCR5/CXCR4 is primarily between the V3 loop crown and the N-terminal helix of the chemokine receptors, which exhibits sulfated tyrosines that make critical V3 loop and gp120 core contacts. According to our model, the C-terminal beta-strand of the V3 crown docks into the central surface extracellular pocket of CCR5/CXCR4.

**P05.22**

**Siglec-1 on Macrophages Is a Major Infectivity Receptor for HIV-1: Differential Effects of GM-CSF and M-CSF on HIV-1 Entry and Replication**

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**Background:** The environmental milieu around macrophages has a profound influence on their phenotype, and permissiveness to HIV-1 infection and replication. The effect of granulocyte macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) on the phenotypic profiles and permissiveness to HIV-1 infection of primary human monocyte-derived macrophages (MDM) were evaluated.

**Methods:** MDM were generated from PBMCs of HIV-1 seronegative donors following in vitro culture in media supplemented with GM-CSF or M-CSF. Expression of cell surface markers and intracellular p24 were determined by flow cytometry. Chemokines were assayed by ELISA. HIV-1 gp120 sequences derived from infected MDM were characterized using single genome amplification. Virus entry and intracellular replication were assessed by PCR using strong stop and LTR/Gag primer/probe sets.

**Results:** MDM cultured in GM-CSF induced high levels of β-chemokines (80-120 pg/ml) compared to M-CSF (p <0.001). Incubation of M-CSF cells with supernatants from GM-CSF cultures reduced the infectivity by 85% (p <0.002). Blocking CD4 or CCR5 receptors with monoclonal antibodies did not prevent HIV-1 infection. However, blocking Siglec-1 (CD169) receptor with anti-CD169 abrogated HIV-1 infection. Siglec-1 expression was decreased by 50% in GM-CSF cultured MDM. MDM from three donors differentiated in GM-CSF were 8-10-fold less permissive to primary viruses compared to MDM grown in M-CSF (p <0.001). Differences in HIV-1 infectivity were not due to genetic differences in viral sequences (p = 0.1845). Infected GM-CSF cultures showed decreases in HIV-1 entry (3-128-fold) and replication (8-64-fold) at 24 and 96-hr time points relative to corresponding M-CSF infected cultures (p <0.01).

**Conclusion:** The differences in infectivity observed in these two models were not due to selective virus variation or fitness, but due to decreased entry and replication of HIV-1 in GM-CSF cultured MDM. The decreased expression of Siglec-1 and the presence of soluble factors in the GM-CSF cultured MDM could have contributed to decreased entry.
Background: Nef clones derived from HIV-1 elite controllers display reduced activity compared to those from chronic progressors, but the relevance of host and viral factors to this phenotype remain unknown. To investigate this, we assessed the CD4 and HLA class I down-regulation activities of acute/early Nef clones derived from individuals who suppressed plasma viremia to <2000 RNA copies/mL in the first year (Acute Controllers [AC]) and those who did not (Acute Progressors [AP]).

Methods: Nef sequences were cloned from N=10 AC (median [IQR] 72 [57-99] days post-infection [DPI]) and N=56 AP (54 [35-73] DPI). Clones were transfected into CEM T cells expressing HLA-A*02, and surface CD4 and A*02 levels were quantified at 24h using flow cytometry. The ability of each Nef clone to down-regulate CD4 and HLA was normalized to Nef-SF2 control.

Results: Nef sequences from AC and AP displayed no substantial phylogenetic clustering or significant imbalance in amino acid frequency. Nevertheless, Nef clones from AC had reduced ability to down-regulate CD4 (median [IQR] 0.94 [0.87-0.99] vs. AP (0.99 [0.98-1.02]), p=0.004. Similarly, HLA down-regulation activity was reduced in 2 AC and 1 AP over the first year of infection, while HLA down-regulation activity diminished in all 6 participants over the same period.

Conclusion: Acute/early Nef clones derived from AC displayed lower CD4 and HLA down-regulation activities. Impaired CD4 down-regulation in AC expressing protective HLA supports the contribution of both host and viral factors to this phenotype.

Background: The appearance of HLA-associated HIV-1 polymorphisms (HLA-APs) represents an HLA footprint effect. Since the distribution of HLA alleles is quite different between regions or races, it is assumed that the comparison of HLA-APs between Asian and Caucasian individuals infected with the clade B virus can clarify HIV-1 evolution between both populations. However, there are no large-scale HLA-AP studies in Asian individuals.

Methods: We analyzed the sequence of Gag, Pol, and Nef genes in 430 treatment-naive Japanese chronically infected with HIV-1 clade B. Identification of associations between HIV-1 amino acid polymorphisms and 4-digit HLA class I alleles was performed by using a phylogenetically corrected logistic regression model and multiple tests were corrected by using q-value. Then, we compared our results with those for the Caucasian cohort (IHAC).

Results: We completely determined 397, 363, and 306 sequences of Gag, Pol, and Nef, respectively, and then analyzed polymorphism associated with 37 4-digit HLA alleles. At the threshold of q<0.2, we identified 284 HLA-APs (94 in Gag, 86 in Pol, and 104 in Nef). These HLA-APs were more frequently detected in Nef codons (21.8%; 45 of 206) than in Gag (10.2%; 51 of 500) or Pol (5.1%; 51 of 947). Furthermore, we found that the number of HLA-associated mutations in Pol was inversely correlated to viral load. Analysis of HLA-APs among HIV-1 subtypes demonstrated different HLA-APs between HIV-1 subtypes among which substitutions were found outside the peptide-binding groove. Comparative analysis between Japanese and Caucasian cohorts showed that 85 (53.5%) of 159 HIV-1-APs associated with 18 HLA alleles shared in both populations were not observed in the IHAC. Approximately 66% of HLA-APs were identified only in Japanese individuals.

Conclusion: This study demonstrated the different HIV-1 evolution between Japanese and Caucasian individuals and highlighted the complexity of HLA-AP formation and the existence of novel mechanisms for HLA-APs.
P05.25

Envelope Gene Analysis Shows Migration of HIV-1 A to India from East Africa Early During Epidemic

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Background: The global diversity of HIV-1 poses an alarming challenge to HIV vaccine development. Subtype A of HIV-1 is the second globally most prevalent variant (12%) and its recombinants CRF02_AG and CRF01_AE contribute 8% and 5% respectively. The subtype is mainly found in Africa, Europe and Asia. Among the five sub-clades (A1-A5) of HIV-1, A1 is the most predominant. Although reported for last two decades, HIV-1 subtype A represents a small proportion of circulating HIV viruses in India. We report the results of analysis of env sequences from Indian HIV1 A to study genetic diversity and original source of infection in India.

Methods: Phylogeographic analysis was carried out using the Bayesian MCMC approach based on 233 globally representative env sequences including 6 isolates (1998–2011) from Western India. MCC tree was generated under the relaxed uncorrelated lognormal clock model with Bayesian skyline tree prior.

Results: The evolutionary rate of HIV1-A was estimated as 4.3x10⁻³ substitutions/site/year with time to the most recent common ancestor at ~1950. All Indian sequences formed a monophyletic cluster within sub-clade A1. The genetic diversity within HIV-1A1 was found to be 10.3% and within Indian cluster, 6.5%. Though, Central Africa was determined to be the ancestral origin of A (A1-A5), the Indian A1 cluster had Eastern Africa as the ancestral source and dated to 1979 (1976–1982).

Conclusion: The age of HIV-1A is comparable to an earlier estimate (~1945) based on multi-subtype analysis. The introduction of subtype A into India may be subsequent to HIV-1C reported to be ~1975. The ancestral origin of Indian subtype A viruses was determined to be East Africa. This analysis was based on small number of available data and needs to be confirmed with larger dataset.

P05.26

CD25+FoxP3+ Memory CD4 T Cells Are Frequent Targets of HIV Infection In Vivo

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Background: Interleukin 2 (IL2) facilitates homeostatic proliferation and maintenance of IL2 receptor alpha chain+ (CD25) FoxP3+ CD4+ regulatory T cells (Tregs). In vitro, IL2-dependent cell cycle induction and CD25 expression is closely linked to HIV replication in CD4 T cells. We hence hypothesized that Tregs may constitute a highly suitable target for HIV infection in vivo.

Methods: Using polychromatic flow cytometry, Treg frequencies, absolute numbers, CCR5 expression (n=381) and markers of cell proliferation (Ki67, n=38) were studied ex vivo in relation to HIV infection status. HIV-DNA content was then quantified in different sorted CD45RO+ memory CD4 T cell populations, including CD25+FoxP3+ Tregs from HIV+ PBMC samples (n=22).

Results: Tregs cell numbers in HIV+ subjects were depleted (10 cells/µl) compared to HIV- subjects (18 cells/µl, p<0.0001), with a linear correlation between Treg and total CD4 T cell counts (p=0.0009). Independent of HIV infection, a substantial fraction of Tregs (median>50%) expressed the HIV co-receptor CCR5, potentially supporting viral entry into Tregs. Importantly, chronic HIV infection was associated a substantial increase of proliferating (Ki67+) Tregs cells (median: 27.6% (HIV+) and 18% (HIV-); p=0.004) and memory CD4 T cells (4.1% (HIV+) versus 1.3% (HIV-), p<0.0001). Finally, sorted CD45RO+ Treg cells had a 15- fold HIV-DNA content compared to memory CD4 T cells (p=0.0032).

Conclusion: Our data demonstrate that during chronic HIV infection, a large proportion of Treg cells express the CCR5 and are actively proliferating, which might facilitate HIV infection of memory CD25+, FoxP3+ CD4 T cells in vivo. These data support the hypothesis that specific cellular characteristics of the Treg cell life cycle make then a highly suitable target for HIV replication in vivo.
**P05.27**

**Enrollment Characteristics in a Potential HIV Vaccine Efficacy Population Among Fishing Communities Around Lake Victoria, Uganda**

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**Background:** HIV vaccine efficacy trials will require high levels of participant enrollment and retention in order to maximize power to detect differences in endpoints. We conducted a randomized study to assess participant retention in a potential efficacy population of fishing communities of Lake Victoria, Uganda.

**Methods:** HIV negative individuals aged 13-49 years, resident in 4 fishing communities around Lake Victoria, Entebbe, Uganda, were enrolled and randomized to either mobile phone reminder or physical contact tracing arm. Participants were followed up at 1, 2, 3, 6, 12, and 18 months post-baseline and data on socio-demographic characteristics, and HIV/STI risk behavior were collected using interviewer administered questionnaires. Venous blood was drawn for HIV testing.

**Results:** A total of 879 participants were screened (41.7% females), of which 662 (75.5%) were enrolled, giving a screening/enrollment ratio of 1.3:1. The overall mean (SD) and median (IQR) age were 28.2(6.8) and 27(23-32) years respectively. 94.3% had attained formal education, majority (57.8%) having attained primary education. 45.3% had spent less than 5 years in fishing communities. Only 35.9% were spending most of their time in fishing activities including fishing, fish selling and processing. 47% reported monogamous marriages. Only 2.8% reported consistent condom use in the past 3 months in extra marital affairs. 71% reported having less than 5 new partners in the past 3 months, among these, only 4.8% consistently used condoms (p<0.0001). 18.4% (162/879) reported STI symptoms of which 61.1% (99/162) reported having sex while having STI symptoms.

**Conclusion:** Enrollment characteristics for this cohort show that fishing communities along Lake Victoria, Uganda are a diverse population with different social and economic status and at a higher risk for HIV infection and other STIs. Research and medical interventions in this population should cater for this diversity.

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**P05.28**

**First Cell(s) Infected by Vaginal Transmission of Immunodeficiency Virus in Rhesus Macaques**

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**Background:** Male-to-female HIV infection occurs after exposure of the female reproductive tract (FRT) to viral inoculum in semen. The initial target cells infected and their specific localization remain to be defined.

**Methods:** To identify the initial sites of infection we have developed a single round infection system where a SIV based vector expressing luciferase and fluorescent protein reporters are pseudotyped with different envelopes. High titre vector is administered intravaginally and/or intrarectally and animals are sacrificed after 48 hours. The luciferase expressing cells are identified in a photon detector, then dissected into foci of infection and frozen for imaging of thin sections. Individual transduced (infected) cells within the tissue can be unequivocally identified by the spectrum specific to the fluorescent protein and staining for luciferase.

**Results:** Examination of the target cells reveals that they are primarily CD4+, CD3+ T cells. However, CD3-, CD4+ cells are also detected. The ratio of CD3+/CD3- cells varies between animals. Characterization of the infected CD3- cells is ongoing, but some of them have been identified to be tissue resident macrophages. Foci of transduced cells identified in 7 animals are distributed throughout the FRT. Most infected cells are found in the vaginal vault. Unexpectedly, we find that ovaries are commonly infected under the conditions of exposure in this model. The infection of cells within the endocervix is also detected, but it is not the predominant site of infection as proposed by some models.

**Conclusion:** This system reveals that after vaginal exposure of virus, all aspects of the upper and lower FRT are susceptible to infection. This study identifies the interaction of the mucosal exposure of SIV after rhesus macaque vaginal challenge. A better understanding of the initial steps of HIV transmission will inform and facilitate efforts to develop a vaccine to block HIV sexual transmission.
P05.29
HIV-1 Replicative Capacity Independently Predicts CD4+ T Cell Decline and May Play a Role in Early Immune Activation and Memory Cell Depletion

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Background: Determining host and viral factors of HIV-1 pathogenesis is paramount for rational vaccine design. In individuals recently infected with HIV-1 subtype C, viral replicative capacity (vRC), determined by the transmitted Gag sequence, was correlated with both early set point viral load (VL) and CD4+ T cell (CD4) decline. However, it was unclear if vRC impacts HIV-1 pathogenesis beyond its effect on early set point VL.

Methods: To answer this question, we doubled the size of the cohort under study. vRC was assessed for 127 acutely infected Zambians with longitudinal CD4 counts up to 6 years post-infection. Kaplan-Meier survival curve analysis and Cox proportional hazard models were utilized to identify independent correlates of CD4 decline.

Results: Individuals with low vRC viruses exhibit significantly slower disease progression up to 5yr post-infection for CD4 counts <350 (p = 0.02), < 300 (p = 0.002), and <200 (p = 0.033). After statistical adjustment for set point VL, vRC remains an independent predictor of CD4 decline.

Conclusion: It is possible that HIV-1 isolates with low vRC scores deplete memory T cell subsets to a less severe extent and/or do not induce high levels of immune activation, which may delay disease progression. Ongoing studies evaluating the levels of inflammatory cytokines in plasma, cellular activation, and memory T cell depletes should uncover the mechanistic basis for vRC’s contribution to early stage pathogenesis and further our understanding of the complex interactions between HIV-1 and the human immune system.

P05.30
Evidence of a Less Pathogenic HIV Infection Associated with High Adaptation to HLA-I Mediated Immune Response in a South American Native Community

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Background: HIV escapes cellular immune response by selecting mutations that are associated to the HLA alleles carried by an individual. Our objective was to study the impact that selection of escape mutations has on disease progression in a native HIV-positive population from Oran, Argentina that exhibits a restricted HLA profile. Our initial hypothesis was that HIV would rapidly select escape mutations to the limited number of HLA alleles and impair the immune response against the infection.

Methods: We performed high-resolution HLA-I typing and near-full length HIV genome sequencing on 65 chronically infected individuals. Escape mutations linked to the most frequent HLA alleles (considered signatures of viral adaptation) were identified and correlated with viral load and CD4 count. CD8 subsets and immune activation were assessed by flow-cytometry.

Results: The phylogenetic analysis of the HIV genomes showed 12 monophyletic clades (3-9 sequences/clade; bootstrap support=100), suggesting highly circulating strains, as well as virus sequences located in isolated branches of the tree. We identified 24 HLA-linked escape mutations (p<0.05; q<0.1) that showed to be more prevalent in the viruses present in clades than in the isolated ones (p=0.0114). In addition, patients infected with the former viruses exhibited a higher proportion of CD8+ naïve cells (CD45RA+CCR7+) and a less exhausted (PD-1+) phenotype. Moreover, in a subset of 41 antiretroviral-naive patients, we found that the number of escape mutations was positively correlated with the CD4 count (p=0.044) and negatively correlated with the viral load (p=0.023).

Conclusion: Adaptation of HIV to the HLA-I mediated immune response in the native community of Oran is leading to a less pathogenic infection. Our current hypotheses are focused on a potential negative impact of escape mutations on viral fitness and/or redirection of the immune response to novel/subdominant epitopes where escape is more difficult.
P05.31 LB D

Relative Contribution of Gag, Nef, and Env to Minority Variant Transmission Revealed by Deep Sequencing of Transmission Pairs

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Background: A single transmitted/founder (T/F) virus initiates infection in the recipient in over 70% of heterosexual transmissions; a few transmitted variants are detected in the remaining cases. Several studies suggest that there is selection for certain envelope features upon transmission, e.g., viruses with shorter and less glycosylated envelopes are preferentially transmitted, and the vast majority of T/F variants use CCR5 for entry into the host cell. It is important to identify what features may be selected upon transmission, in order to target vaccines to those features which may be more likely to establish infection in new hosts.

Methods: We performed full-genome HIV 454 deep sequencing of plasma virus from five epidemiologically linked transmission pairs from a Zambian cohort. In all cases a single T/F virus in the recipient could be inferred. Using global haplotype reconstruction for gp120, gp41, Nef, p24 and p17 we tested whether the T/F virus was likely to be selected by chance from the donor population. We combined our observations across all five pairs to identify regions of the genome potentially under selection at transmission.

Results: In gp120 the T/F variant in the recipient is distinct from the majority donor population across all transmission pairs, and not selected at random from the donor population (Test of Random Transmission, p < 0.05). There were signatures of non-random transmission in 4/5 pairs in the gp41 fusion domain and in 3/5 pairs in p24, however the probability of minority variant transmission was not significant in these proteins.

Conclusion: Our study shows (1) deep sequencing, together with global haplotype reconstruction can be used to analyze the genetic signatures of selection upon transmission (2) the T/F variant is not randomly selected from the donor population (3) both gp120 and gp41 are likely to influence the ability of a virus to initiate infection.

P05.32 LB

Are Fishing Communities in Uganda Potential HIV Vaccine Efficacy Trial Populations? Results on HIV Incidence, Retention and Willingness to Participate

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Background: HIV prevention efficacy trials (including vaccine trials) require populations with high incidence, retention rates and willingness to participate levels

Methods: A community-based cohort study in 8 fishing communities and a randomized controlled trial (RCT) in 4 fishing communities were conducted, to determine population representative HIV rates, willingness to participate in hypothetical HIV vaccine trials (WTP) and retention. A cohort random sample of 2191 consenting participants aged 18-49 years were enrolled at baseline and followed up ~12 months later. Data were collected on HIV risk behaviors and WTP, and venous blood was collected for HIV testing using rapid HIV tests with EIA confirmation. In the RCT, retention rates were determined in a simulated vaccine efficacy follow up schedule with visits at 0, 1, 2, 3, 6, 12, and 18 months. Adjusted incidence rate ratios (Adj. IRR) of HIV acquisition were estimated by multivariable Poisson regression

Results: HIV incidence was 3.4/100py overall but among those who drank alcohol it was 7.67/100py (95% CI; 4.62 - 12.7) in the 25-29 year olds and 5.67/100py (95% CI; 3.14 - 10.2) among 18-24 year olds. Compared to non-drinkers, the adj.IRR was 3.18 (95%CI; 1.18 - 8.57) and 4.93 (95%CI; 1.91 - 12.8) among non-regular and regular drinkers respectively. Cohort follow up was 77% and was significantly higher among HIV negative at baseline (80%), age 30+ years (82.2%), and those with 5+ years of stay in the community (88.5%). WTP was 89.3%, higher in men than women (91.2% vs 87.3%, p=0.004) and among island communities than lakeshore ones (90.4% vs 85.8%, p=0.004). Retention rates in RCT were 78.9%, 87.5%, 85.1% and 85.1% at 1, 2, 3, and 6 months respectively.

Conclusion: With high HIV incidence, retention and WTP, fishing communities along L.Victoria, Uganda, appear to be a potential population for HIV prevention efficacy trials including vaccine trials.
**P05.33 LB**

**Development of Advanced Oligonucleotide-Based Microbicides: Driving HIV into Suicide**

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**Background:** HIV is globally transmitted primarily by sexual intercourse. Therefore, a major unmet medical need is seen in the self-protection of women, particularly in societies where condoms are not accepted. Thus, user-controlled microbicides may represent promising intervention strategies that are currently being developed.

**Methods:** We are performing methods like RT/RNase H cleavage assay in vitro to show directly the specific oligodeoxynucleotide-dependent cleavage of the viral RNA sequence via viral RNase H in the highly conserved polypurin tract. Furthermore we are using luciferase-based infection assays to demonstrate reduced ability of the virus to infect cells after treatment with the oligodeoxynucleotides.

**Results:** We previously designed a short hairpin-looped oligodeoxynucleotide (ODN) that activates HIV-1 RNase H by mimicking ongoing reverse transcription, leading to efficient degradation of the viral RNA genome. ODNs have been shown to target cell-associated as well as free viral particles. Moreover, pronounced antiviral activity has been demonstrated in vitro and in several in vivo (animal) models. Therefore, current development focuses on strategies to increase overall ODN stability and to investigate different strategies of ODN delivery by chemical modifications of the ODNs. We could show that different chemical modifications of the ODNs did not alter the effect on RNase H dependent RNA cleavage and uptake of the ODNs into cells.

**Conclusion:** Taken together, these data suggest that ODNs may be valuable components of advanced antiviral microbicides that literally drive HIV into suicide.

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**P05.34 LB**

**New Insight in HIV-1 Evolution During Acute Infection Gained Through Dense Sampling and Targeted Deep Sequencing (TDS)**


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**Background:** Initial adaptive immune responses to HIV-1 can be detected during the first weeks post-infection and viral escape variants can emerge soon after. Due to technical and logistical obstacles these processes are hard to define. Here we combine frequent sampling with targeted deep sequencing (TDS) to study viral evolution during very early acute HIV-1 infection (AHI).

**Methods:** A consented volunteer from RV217/ECHO with documented nucleic acid testing (NAT)-conversion and seroconversion was studied (day 0: first NAT-positive time-point). Nine consecutive plasma samples (days 2-31; mean sampling interval = 3.6 days) were studied by combination of full- and partial-genome SGS and TDS (Ion Torrent). Ex vivo CTL responses to autologous peptides were analyzed at days 7, 17, and 53. TDS sequences were analyzed using Nautilus (Kijak et al., ARHR2013).

**Results:** The individual was originally infected with two highly-related T/F variants (genetic distance: 1.5%; minor variant frequency: 3%). Peak viremia occurred at day 10. The peak CTL response to immunodominant LV9 epitope (gp41; HXB2: 814-822) was detected on day 17. The first evidence of escape at this epitope was also detected at day 17 (frequency: 0.8%). By day 21, there were 16 different escape variants (frequencies: 0.6%-13.9%) all of them defined by a single non-synonymous substitutions in any of 8 positions of the nonamer. Between day 17 and day 31 plasma viral load remained constant (range: 5.71-6.04 log$_{10}$ copies/ml); the original variant decreased exponentially from 99.2% to 10.5%, while the two major escape variants grew exponentially to 12.8% and 33.7%, respectively. The original sequence of both T/F variants was identical at the epitope and flanking regions, and escape variants emerged simultaneously on both variants. Six months post-infection a severe genetic bottleneck was observed: the dominant variant (frequency: 79.5%) was one that at day 31 had been found at 7.4%, followed by the first detected escape variant (frequency: 18.2%), while the original variant was present at 0.3%.

**Conclusion:** During very early AHI viral escape variants can emerge very rapidly and by exploration of a vast genetic landscape, which calls for the combined use of dense sampling and TDS. This data can be used to more accurately model dynamics of immune escape and inform vaccine design.
**P05.35 LB**

**Impact of HLA Selection Pressure on HIV-1 Evolution and Fitness During the North American Epidemic**


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**Background:** Despite HIV's extraordinary mutational capacity, immune escape pathways are broadly predictable based on host HLA. However, the extent to which HIV has adapted to HLA-driven selection over the pandemic's course, and its pathogenic implications, remain unclear. Persistence of escape mutations following transmission could facilitate their gradual accumulation in circulating sequences, compromising host immunologic capacity as the epidemic progresses. A paucity of historic host/viral genotypes, combined with incomplete knowledge of epidemic founder viral sequences, render these challenging questions to answer.

**Methods:** We investigate immune-driven HIV-1 evolution in North America, and its functional implications, by genotypically and functionally comparing linked host (HLA) and HIV (Gag/Nef) sequences from >350 novel historic specimens spanning 1979-1989, to a modern dataset spanning 2000-2011. Specimens derive from four key epidemic cities (New York, Boston, San Francisco and Vancouver). To minimize bias, HIV-1 sites under HLA-mediated selection were defined using independent sources.

**Results:** Reconstructed North American ancestral (founder) sequences are consistent with a late 60s/early 70s epidemic origin and are nearly identical to the continental consensus, arguing against widespread fixation of escape mutations in circulating sequences. Nevertheless, an observed ~3-4 fold increase in HIV diversity over the past >30+ years appears to be driven in large part by HLA-mediated selection. Furthermore, median "background frequencies" of HLA-associated polymorphisms increased by 35-50% during this time. Though our estimates of the time to accumulation to dominance are on the order of centuries for most escape pathways are broadly predictable based on host HLA. However, the extent to which HIV has adapted to HLA-driven selection over the epidemic progresses. A paucity of historic host/viral genotypes, combined with incomplete knowledge of epidemic founder viral sequences, render these challenging questions to answer.

**Conclusion:** Persistence of escape mutations following transmission could facilitate their gradual accumulation in circulating sequences, compromising host immunologic capacity as the epidemic progresses. A paucity of historic host/viral genotypes, combined with incomplete knowledge of epidemic founder viral sequences, render these challenging questions to answer.

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**P05.36 LB**

**Low Antibody-Dependent Cellular Cytotoxicity in HIV-1 Intrasubtype C Superinfected Zambian Seroconverters**


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**Background:** Although the role of antibody-dependent cellular cytotoxicity (ADCC) in HIV-1 superinfection and vaccine-elicited protection is unclear, this effector activity can result in early control of virally-infected target cells. Previous studies of superinfected Zambian seroconverters showed poor anti-HIV-1 humoral responses, including low autologous neutralizing antibodies, gp120- and V1V2-binding antibodies prior to superinfection. Here, we compared ADCC responses between superinfected and non-superinfected groups, prior to superinfection. We also investigated CMV-specific antibody responses to evaluate potential global antibody defects.

**Methods:** We tested pre-superinfection antibodies for the ability to elicit ADCC killing in the presence of Subtype C env-coated target cells and unstained effector cells. We used flow-cytometry to measure killed targets and compared these responses between groups. We measured CMV-specific IgG responses approximately 3-months after seroconversion via a CMV-specific ELISA.

**Results:** ADCC responses prior to superinfection were significantly lower in the superinfected group as compared to the non-superinfected group. Two of three superinfected individuals had no measurable ADCC activity, compared to 1/10 non-superinfected controls. ADCC responses also directly correlated with gp120-specific IgG binding titer, viral load, and autologous neutralization titer, prior to superinfection. All study participants showed CMV seropositivity with no significant difference in CMV-specific binding IgG antibody reactivity between superinfected and non-superinfected groups.

**Conclusion:** Superinfected individuals exhibited lower levels of HIV-specific ADCC, but similar CMV-specific binding IgG antibody responses, as compared to non-superinfected controls. This, with our previous data, suggests that HIV-specific antibody responses may be compromised prior to superinfection, leading to potentially higher susceptibility to reinfection, yet superinfected individuals do not seem globally defective in antibody responses to viral pathogens. Moreover, it implies that within an area with a predominant subtype infection (subtype C), primary HIV-1 infection may induce some level of protection from superinfection.

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P05.37 LB

Enhanced Fusion and Virion Incorporation for HIV-1 Subtype C Envelope Glycoproteins with Compact V1/V2 Domains

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Background: HIV-1 encodes an envelope glycoprotein (Env) that triggers the fusion of viral and cellular membranes while minimizing exposure of its key protein domains to the adaptive immune system. Envs with long variable domains are generally less sensitive to antibody neutralization but also appear less fit to establish a de novo infection. By exchanging the V1/V2 domains between Envs of the same infected person or between two persons linked by a transmission event, we investigated the links between V1/V2 length, Env incorporation and fusion to primary CD4 T-cells or monocyte-derived dendritic cells.

Methods: From a set of cloned Envs from four male-to-female transmission pairs (Lusaka cohort), we engineered 19 mutants in which sequences from V1 or V2 domains were exchanged between different Envs sequences from the same person or within the same transmission pair. The impact of V1/V2 domain length on Env incorporation was measured by Western blot while viral fusion was measured using the FRET-based fusion assay, which specifically quantifies fusion of HIV-1 virions containing β-Lactamase-Vpr to primary cells.

Results: Envs with compact V1/V2 domains mediated higher levels of fusion to both cell types while Envs with longer V1/V2 domains fused with lower efficiency. Unexpectedly, the length of V1/V2 greatly impacted Env incorporation into HIV-1 virions. Long V1/V2 domains in Env were associated with lower levels of virion Env. The number of potential N-glycosylation sites in V1 or V2 also inversely correlated to Env incorporation and fusion.

Conclusion: Our study points to Env incorporation into virions and fusion as limiting steps for transmission of HIV-1 to a new host, and suggests that the length and/or the N-glycosylation profile of V1/V2 domain influences these steps. Exploiting the fitness cost incurred by long V1/V2 could lead to the development of more effective vaccine interventions to curb the HIV-1 epidemic.
Background: Altered susceptibility to HIV1 infection has been observed in multiple cohort studies. One of the best characterized HIV1 exposed yet uninfected groups is a commercial sex worker cohort from Nairobi, Kenya. (Fowke, et al, 1996; Plummer, et al, 1999; Ball, et al, 2007) A gene expression analysis conducted showed differential regulation of the glycolysis gluconeogenesis pathway in HIV-1 exposed yet seronegative CSWs. (Songok, et al, 2012)

Glucose is utilized by lymphocytes as their primary fuel source for cell survival, size, activation and cytokine production. The first critical regulatory step in glucose metabolism is glucose entry into cells through facilitated diffusion by proteins of the glucose transporter (GLUT) family. (Fox et al, 2005; Jacobs, et al, 2008) Over-expression of GLUT1 leads to increased glucose uptake and glycolysis which is required to mount a functional immune response necessary for rapid cell growth and proliferation in T lymphocytes. (Maciver et al, 2008)

Methods: The study population was drawn from the Pumwani Sex Worker Cohort, Nairobi including: HIV highly exposed yet seronegative (HESNs) CSWs (>7 years); newly enrolled HIV-uninfected (<7 years); HIV-infected and lowly-exposed HIV negative antenatal clinic attendees (low risk group).Total RNA was extracted from PBMCs using Trizol; cDNA was synthesized and relative mRNA expression determined using SYBR Green by quantitative real time PCR.

Results: Each assay was normalized using 18s rRNA gene. We observed a significant difference between highly exposed yet uninfected (HESNs) and newly enrolled HIV uninfected CSWs. (p=0.0056) There was no significant difference between HESNs with the HIV negative antenatal clinic attendees (p=0.8628) and HIV infected CSWs (p=0.5399).

Conclusion: We observed significantly lower mRNA expression of Glut1 in HESNs when compared to their uninfected yet susceptible counterparts. Following studies of Glut 1 protein expression and uptake studies are underway to understand the role Glut1 in glucose metabolism in HIV resistance.

Background: HIV-1 exposed seronegative individuals (HESN) have been documented in diverse cohorts. Recent genome-wide studies capturing common genetic variation in European populations failed to identify HESN-associated single nucleotide polymorphisms (SNPs). We compared whole genome sequences (WGS) of African HESN and HIV-1 seroconverters (SC) in order to identify genetic variation underlying an HESN phenotype.

Methods: HIV-1 exposure scores (ES) were generated through regression modeling of SC and HESN in the context of prospective follow-up of 3893 African HIV-1 serodiscordant couples. We selected 50 HESN with consistently high ES matched to 50 SC by gender and self-reported ethnicity. Complete Genomics, Inc. generated 100 WGS with a median of 49 reads per base obtained over these genomes. We evaluated 38 candidate SNPs previously reported as associated with HESN versus SC. We also evaluated 1) single SNP associations with HIV-1 acquisition using logistic regression, and 2) gene-level associations using two distinct algorithms (SKAT and Morris-Zeggini). All analyses controlled for population stratification using the first 3 principal components. P-values are uncorrected for multiple comparisons.

Results: Nearly 22 million high quality autosomal SNPs (454,176 exonic SNPs) were identified across 100 African WGS. Only one candidate (CCL2, rs1024610) was significantly associated with HESN compared to SC (p=0.01, identified in 1 SC and 11 HESN); although, the power to evaluate some candidate variants was limited. The by-variant analysis identified 13 variants having 10 -5P>1.8x10 -7; the by-gene analysis identified 36 genes with 10 -3P>1.34x10 -7 (SKAT) or 28 genes with 10 -3P>4.2x10 -5 (Morris-Zeggini).

Conclusion: Our WGS analysis of African HESN and SC has identified variants not previously associated with HIV-1 acquisition phenotypes that are of high priority for further study. Among 38 candidate SNPs tested, only one was significantly associated with protection from HIV-1. We are currently validating these associations through genotyping in an independent cohort of HESN and SC.
P06.03

Higher DEFA1A3 Gene Copy Number and α-Defensins 1-3 Secretion by Dendritic Cells Are Associated with HIV Protection

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Background: Some exceptional individuals are exposed to HIV virus but remain uninfected (HIV-exposed uninfected, EU). Higher α-defensins1-3 (HNP-1-3) secretion is known to be one of the innate host-resistance factors to HIV-1-infection. HNP-1-3 are peptides expressed mainly in neutrophils, but also in DCs, which display anti-HIV-1 activities. DEFA1A3, the gene coding for HNP1-3 is present in a copy number variation (CNV). We hypothesize that an increased CNV would be a key factor inducing a protective HIV-1-immune response.

Methods: Healthy as well as EU monocyte-derived DCs supernatants were collected and HNP1-3 protein and 25 cytokine were quantified. HIV-DCs were collected and HNP1-3 protein and 25 cytokine were quantified. HIV-DCs-infection assays were performed in healthy individuals with high CNV (≥9) versus low CNV (≤4) being DCs infected at low doses and matured levels quantified. HIV-1-pulsed-DCs were co-cultured with autologous-monocyte-depleted PBMCs and HIV-specific responses were analyzed.

Results: EU individuals display a higher DEFA1A3 copy number variation (CNV), resulting in a higher HNP1-3 secretion in their immature dendritic cells (iDCs). Higher DEFA1A3 CNV and levels of HNP1-3 in iDCs correlate with the amount of virus EU-individuals are exposed to, suggesting a protective role (p<0.01). CNV critically and significantly modulate iDCs functions, as observed with healthy individuals with high CNV (≥9) who display a more mature DC status, including increased cytokine and chemokine (IL-8, IL-6, IL-1β, MCP-1 and MIP-1β) secretion. These DCs have a decreased capacity to be HIV-1-infected and are able to induce a higher HIV-specific CD8+ T cell response than their lower CNV counterparts (p<0.05).

Conclusion: Elevated DEFA1A3 CNV genotype confers HIV-1 protection while DCs from these individuals secrete higher amounts of HNP1-3 which by its antiviral activities and immune-mediators induction inhibit HIV replication on DCs. Together, elevated HNP1-3 CNV and HNP1-3 secretion potentiate and improve DC-HIV presentation lowering the risk of infection and providing an enhanced HIV-1-specific response.

P06.04

HLA-Associated Variation in HIV Viral Load in a South American Native Community with Limited HLA Diversity

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Background: Pathogen-driven balancing selection has been proposed as an explanation for the high diversity of the HLA system. Different HLA alleles have been associated with variations in HIV viral load. Our aim was to determine the HLA-A and -B diversity in a South American native HIV-positive community and their association with clinical markers of disease progression.

Methods: We developed and validated a sequence-based technique for high resolution typing of HLA-A and -B genes. This technique was implemented to determine the diversity of these genes in a group of 65 HIV-positive native individuals from Oran, Argentina. The comparison between the patient’s HLA alleles and the HIV viral load was made by Mann-Whitney test and the False-Discovery Rate approach was applied to correct for multiple comparisons.

Results: The HLA profile of the population under study was consistent with that observed for other native communities from South America. HLA-A diversity was restricted at both group (Heterozygosity Index (H)=0.791) and subtype (H=0.841) level. Alleles A*02:01:01:01, A*24:02:01 and A*31:01:02 accounted for 83.4% of the genetic frequency. HLA-B diversity was found to be restricted at the group level (H=0.870) but highly diverse at the subtype-level (H=0.969). The analysis of the viral load in each HLA allele group showed that HLA-B*39-positive patients exhibit significantly higher viral loads (p=0.038, q=0.17) while HLA-B*35Px-positive patients exhibit significantly lower viral loads (p=0.014, q=0.13) than the negative counterparts.

Conclusion: The HLA profile of the HIV-positive native community of Oran showed to be restricted except at the subtype-level in HLA-B, consistent with the high prevalence of endemic infectious diseases in the area. In particular, B*39 is a native allele that mediates a novel association with a better control of the HIV infection.
**P06.05**

**IL-4, IL-10 and TNF-α Promoter Gene Polymorphism in North-Eastern Ukrainian HIV-1 Infected Individuals**

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**Background:** The objective of the research was to study distribution character of the allel variants of IL-4 promoter gene area in position -590, IL-10 in position -592, TNF-α in position -308 in HIV-1 infected Ukrainians of North-Eastern region.

**Methods:** Data for the study were DNA samples, received from peripheral blood leukocytes of 200 inhabitants of North-Eastern region of Ukraine: 78 HIV-infected, 22-HIV-negative individuals from the group of high risk of contamination, 100 healthy blood donors. Gene polymorphism detection was made with PCR-RFLP method.

**Results:** IL-4 (rs 2243250), IL-10 (rs 1800872) and TNF-α (rs 1800629) gene polymorphism has been studied for the first time in the population of HIV-infected Ukrainians. By analysis of frequency of IL-4 gene allel variants it has been discovered that homozygotes by the main allel were the dominant variant. It has been found out that among people with HIV there were 9.0 % of T/T minor gene carriers and were 4.5 more often met in comparison with control group (p<0.05) that can prove the tendency to association of the mentioned genotype with infection. Distribution of allel variants of IL-10 gene promoter region in position -592 is characterized by homozygote dominance by the main gene. It has been established that among the individuals with HIV A/A minor allel carriers were 3.4 more often met in comparison with control group (p<0.05). The occurrence of the homozgyous combination of the allel variant G/G of the promoter of TNF-α has been shown to prevail almost twofold over the occurrence of the variant G/A among all groups. High frequency of heterozygote by the main allel has been recorded among the individuals with HIV.

**Conclusion:** Our data suggest that IL-4, IL-10, TNF-α variations may contribute to the acquisition of HIV infection and encourages carrying out of further populations studies in this sphere of HIV-infection immunogenetics.

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**P06.06**

**Predictors of HVTN 503 MRK AD5 HIV-1 Gag/Pol/Nef Vaccine-Induced Immune Response**


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**Background:** Phambili, the HVTN 503Phase IIb efficacy study of MRK-Ad5 HIV-1 clade B vaccine conducted in South Africa, neither prevented HIV-1 infection nor lowered viral load setpoint. However, immune responses recognizing Clades B and C HIV-1 subtypes were elicited. We investigated predictors of vaccine-induced HIV-1 specific T cell immune responses.

**Methods:** An analysis of vaccine-induced immunogenicity by interferon-γ ELISPOT assays was conducted on the first 186 enrolle all vaccine and placebo recipients four weeks post second vaccination. Descriptive and frequency analysis stratified by study arm and gender were performed on baseline demographics (gender, age, BMI, study site, Ad5 titre, HSV2, circumcision status) and risk behaviours. Multivariate logistic regression determined predictors of immune response to any Clade B or C antigens in the vaccine arm using backward selection. Each analysis was two-sided with 5% level of significance.

**Results:** Of the 186 participants, 53.7% (n=100) were female, median age was 23 years [IQR:21-27], median BMI was 22.5 [IQR:20.4-27.0], 53.7% (n=100) were from Soweto, 85.5% (n=159) were Ad5-seropositive, 18.8% (n=35) were heavy drinkers; 31.7% (n=59) reported drinking/drug use with sex, 61.3% (n=114) had unprotected vaginal sex and 79% (n=147) reported a main partner. All participants in the vaccine arm (n=93, 50%) developed T cell responses to either Clade B (n=87, 47%) and/or Clade C antigens (n=74, 40%), p=0.17. In multivariate analysis, normal/underweight BMI [OR: 5.926, CI: 1.069-32.84, p = 0.0417], female gender [OR: 0.172, CI: 0.033-0.883, p=0.0350], and one-log increase of Ad5 titre [OR: 0.374, CI: 0.164-0.850, p=0.0189] significantly predicted immune response to any Clade C antigens. Heavy drinking [OR: 0.224, CI: 0.056-0.891, p=0.0336] inhibited immune response.

**Conclusion:** Gender, BMI, Ad5 titre and heavy drinking affected vaccine-induced HIV-1 specific immune responses to Clade C antigens. The role of BMI in blunting these immune responses requires elucidation. Whether these factors affect HIV vaccine efficacy remain to be determined.
**P06.07**

The Molecular Interaction of HIV’s Nef Protein with β-catenin in the Wnt Signaling Pathway

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**Background:** The Wnt signaling pathway is implicated in major physiologic cellular functions such as proliferation, migration, cell fate specification, maintenance of pluripotency and induction of tumorigenicity. Proliferation and migration are important responses of T cells, which are major cellular targets of HIV infection.

**Methods:** Using an informatics screen, we identified a previously unsuspected interaction between HIV’s Nef protein and β-catenin, a key component of the Wnt pathway. The three-dimensional structural compatibility of Nef peptides with the β-catenin ligand binding site was then tested more stringently by computational molecular docking. A pull-down assay using purified recombinant proteins and co-immunoprecipitation experiments in HEK293 cells were also used to confirm the Nef/β-catenin interaction. Finally, we used a β-catenin responsive luciferase reporter assay to determine if Nef inhibited the Wnt signaling pathway in cells.

**Results:** A segment in Nef contains identical amino acids at key positions and structurally mimics the β-catenin binding sites on endogenous β-catenin ligands. The interaction between Nef and β-catenin was confirmed in vitro and in cells. Moreover, the introduction of Nef into cells specifically inhibited a Wnt pathway reporter in cell-based assays.

**Conclusion:** Structural and biochemical observations confirm that HIV’s Nef protein interacts with human β-catenin. This interaction is functionally significant in cells. The association of Nef with β-catenin potentially implicates the Wnt signaling pathway in T cell transmigration and immune activation phenomena characteristic of HIV infection and AIDS.

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**P06.08**

HLA Class I and KIR Polymorphisms Associated with Protection from HIV Infection and Disease Progression in a MSM Cohort in Lima, Perú

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**Background:** Increasing data suggest that highly-exposed subjects can remain HIV uninfected despite prolonged viral exposure. It is also well known that relative viral control can be achieved by some infected individuals, especially those expressing particular HLA class I alleles and KIR genes.

**Methods:** To assess potential associations between particular genetic markers and risk of HIV infection or HIV viral load and CD4 counts, we followed a cohort of 468 individuals (222 HIV-, 246 HIV+) with high-risk sexual behaviour in Lima (Perú), who were typed for HLA class I alleles. Of these, KIR genotypes were available for 73 HIV- and 170 HIV+ subjects, with 35 of the latter also being assessed for the HLA-C upstream -35 variant.

**Results:** Three HLA class I allele (A*2301, B*3543, B*5703) and 1 KIR gene (2DS1) were significantly enriched among the HIV infected group, while one HLA allele (B*4002) was associated with relative protection from HIV infection. Eight HLA alleles (A’*0201, B’*0801, B’*1801, B’*3501, B’*3505, B’*3509, C’*0401, C’*0702) and one KIR gene (2DS4) were associated with higher viral loads and/or reduced CD4 counts in the HIV infected group while 11 HLA alleles (A’*0222, A’*1101, A’*3303, B’*1501, B’*1516, B’*3903, B’*3913, B’*3914, B’*4004, B’*5703, C’*0801) and 2 KIR genes (2DP1 and 2DL1) were associated with lower viral loads and/or higher CD4 counts (all based on uncorrected p-values < 0.05).

**Conclusion:** Despite Peruvian cohorts being well represented in past and current HIV vaccine trials; this is the first study to define high-resolution HLA and KIR typing for this population. The description of the genetic background of this population will be of importance for immunopathological studies and to interpret disease outcome and HIV vaccine trial data in this country and the rest of South America.
P06.09

Mechanisms of Abrupt HIV Disease Progression in a Cohort of Previous Elite and Viremic HIV Controllers


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Background: Extensive cross-sectional immune data from individuals able to control HIV replication to undetectable or low levels and with normal CD4+ T cell counts in the absence of cART (elite and viremic controllers) is already available. However, a small proportion of controllers end up progressing and relatively little is known about the factors responsible for the abrupt loss of HIV control (LoC) in these individuals.

Methods: We identified 14 HIV patients who experienced an abrupt transition from a non-progressive to progressive HIV infection defined as >1 log VL increase and loss of >=30% CD4 cell counts or drop below 350 cells/ml within 1 year. All individuals were screened longitudinally for T cell responses to the entire HIV proteome by ELISPOT and flow cytometry. Emergence of CXCR4-using virus and presence of HIV superinfection were evaluated. Presence of HLA footprints in the autologous viral population pre- and post-LoC was assessed. All immune data was compared to long-term Elites (n=32) and Viremic Controllers (n=19) without signs of HIV disease progression.

Results: Tested individuals had stable viremia <3log copies/ml before LoC without protective HLA alleles being overrepresented. CXCR4-tropic plasma virus was detected in 4 individuals’ post-LoC of which 2 had R5 virus pre-LoC. There was no evidence for superinfection except one individual who is being investigated further. In 7/9 individuals tested to date, HLA footprints were maintained after LoC or even emerged newly post-LoC. Only one patient showed reversal of pre-LoC HLA footprints at the post-LoC timepoint. Immune analysis of detailed CTL escape mutations as well as changes in polyfunctionality is being currently assessed.

Conclusion: With a number of analyses still ongoing, emergence of CXCR4-using HIV, but not HIV superinfection nor reversion of viral adaptation to immune pressure, might be involved in loss of HIV control in subjects with previous spontaneous control of HIV-1 infection.

P06.10

KIR2DS4 Confers Susceptibility in Mother-to-Child HIV-1 Transmission

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Background: HIV-1 mother-to-child transmission (MTCT) remains the primary source of HIV-1 acquisition amongst children and can take place in utero (IU), intrapartum (IP) or postnatally through breastfeeding. Killer cell immunoglobulin-like receptors (KIRs) present on natural killer (NK) cells have previously been associated with HIV-1 transmission. KIR genes can be classified into A or B haplotypes, which respectively contribute to a more inhibitory or activating NK cell potential. KIR2DS4 is the only activating KIR gene within the A haplotype, and alleles of KIR2DS4 can be divided into functional receptors (KIR2DS4-f) or non-functional variants (KIR2DS4-v) devoid of a transmembrane region. This study questions the role of KIR2DS4 in the context of MTCT.

Methods: We KIR and HLA-C genotyped 217 Black South African mother-infant pairs: 145 were HIV-1 non-transmitting mothers (NT) and their exposed uninfected infants (EU); 72 were HIV-1 transmitting mothers (TR) and their infected infants [IU, IP or IU-2 (an IU enriched infected group)].

Results: KIR2DS4-v gene frequency was significantly higher in IU-2 infants compared to EU infants (P=0.010, OR=3.41). This association was more significant when comparing infants homozygous for the A haplotype (P=0.004, OR=18.4). Mother-infant concordance (M+I+) for KIR2DS4-f, KIR2DS4-fv or KIR2DS4-v were not associated with an increased risk of HIV-1 transmission. However in M-I+ discordance, KIR2DS4-f was associated with a higher risk of IP transmission (P=0.005, OR=3.84); whilst in M+I- discordance, KIR2DS4-v was associated with an increased risk of IU transmission (P=0.002; OR=6.40). Comparisons involving the described HLA-C ligands for KIR2DS4-f did not reveal any significant associations.

Conclusion: Our findings add to studies of discordant couples identifying KIR2DS4-f as associated with risk of transmission through mucosal routes, and further identify the lack of a surface KIR2DS4 as a risk factor in IU HIV-1 acquisition. This study highlights an important role for KIR2DS4 in HIV-1 susceptibility/protection.
**P06.11**

May Host Genome Affect the Response to Dendritic Cell Based Immune Treatment Against HIV-1?

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**Background:** Background. Promising studies have been made in recent years around the world, addressing the epidemic of Acquired Immune Deficiency Syndrome (AIDS) and including a new line of treatment against HIV-1 by using autologous dendritic cells (DC) vaccination. In December 2004, the Brazilian phase I clinical trial of DC-based immune therapy against HIV-1 was published, reporting the data of 18 untreated HIV+ patients vaccinated with autologous DC pulsed with autologous inactivated HIV showed that half of the patients presented a good control of viral load. DC-based vaccine was demonstrated to increase HIV specific cellular immune response; however, in some HIV-infected patients, the response to the vaccine resulted to be not fully effective. In order to understand if the outcome of the vaccination may be influenced by the host’s genome, we studied the genetic background of 18 HIV-infected patients previously vaccinated with DC.

**Methods:** Methods. Genomic DNA of 18 HIV-infected patients included in the Brazilian phase I clinical trial were analysed through Illumina Exome chip and Taqman SNP genotyping assays. SNPs frequencies distribution was compared between good- and bad- responder to the immune treatment.

**Results:** Results. We identified genes (PARD3B, PCDHB6 and B9, PKD1L2, SPOCK3, OR8U8 and U1, FOLR4, WDR66) and Single Nucleotide Polymorphisms (SNPs) potentially associated with the response to the immune-treatment.

**Conclusion:** Conclusion. Our findings suggest that, independently and/or in addition to other variables, the host’s genome could significantly contribute to the modulation of the response to the DC vaccine. It will be interesting to investigate the distribution of these SNPs in other immune therapies trials.

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**P06.12**

Host Genetics and Susceptibility to HIV-1 Infection: Novel MHC Associations Among Serodiscordant Couples

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**Background:** Human genetic variations known to influence HIV-1 acquisition have been restricted primarily to the CCR5 gene that encodes a major HIV-1 coreceptor. Two recent genome-wide association studies of African cohorts have failed to identify additional genetic loci. Our analyses of HIV-1 serodiscordant couples suggest that further investigation of variants within the human major histocompatibility complex (MHC) may help identify novel factors.

**Methods:** Single nucleotide polymorphisms (SNPs) within the extended human MHC region were genotyped using the ImmunoChip. Two alternative analytical methods, single marker models and penalized (HyperLasso), were applied to detect SNPs differentially distributed between either recent seroconverters (SCs) or seroprevalent partners (SPs) and HIV-1-exposed seronegative subjects (HESNs) with adequate follow-up and testing. Both types of models assumed additive effects adjusted for sex, age, visit interval, and previously reported risk factors in index and recipient partners. For Bonferroni correction, the number of independent comparisons was determined by simpleM.

**Results:** Analyses focused on 6,865 MHC SNPs with minor allele frequency >2.5% among 212 SCs, 437 SPs, and 227 HESNs. When SCs were compared to HESNs, single marker models failed to identify any variant associated with acquisition at a significance threshold of p=2.8x10^-5. Penalized regression models revealed three independent association signals: rs2451731 (intergenic between ABT1 and ZNF322A) (OR=0.55, p=0.001), rs7744381 (ncRNA within HLA-DPB2) (OR=0.56, p=0.002), and rs9469003 (intergenic, between MICA and HLA-X) (OR=2.04, p=0.0008). Further comparison between SPs and HESNs identified one haplotype block between IER3 and DDR1 (tagged by rs25352335) as another region of interest (OR = 1.72, p = 0.0006).

**Conclusion:** High-dimensional statistical approaches may identify novel relationships between HIV-1 acquisition and variants in the MHC where their potential for functional contribution is considerable.
P06.13

APOBEC3G and -3F Induced Cytidine Deamination and Association with Viral Control in a Population with High Frequency of the APOBEC3G H186R Variant

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Background: In the absence of HIV-1 Vif, APOBEC3G mutates the viral DNA during viral reverse transcription. Previous data suggests that the polymorphic variant, APOBEC3G H186R, may predispose to accelerated HIV-1 disease progression, however the mechanism remains unknown. It is possible that genetic variation within APOBEC3G may hinder HIV-1 DNA editing in vivo.

Methods: Participants in this study were twenty two females recruited with known acute or recent HIV-1 infection in Durban, South Africa. Participants were genotyped for the APOBEC3G H186R polymorphism by Taqman genotyping. The HIV-1 env gene was amplified from proviral DNA samples obtained from participants at 36 months post-infection and directly sequenced. The Hypermut 2.0 Tool (Los Alamos HIV Sequence Database) was used to detect APOBEC3G- and -3F induced hypermutations relative to a consensus subtype C reference sequence.

Results: There was no difference in viral DNA hypermutation according to APOBEC3G genotype. Interestingly, HIV-1 env sequences contained a higher number of APOBEC3F compared to APOBEC3G-induced mutations (p=0.003), however, there was significant correlation between the numbers of APOBEC3G and -3F-induced mutations that were present (r=0.5, p=0.03). Additionally, the number of APOBEC3F- but not APOBEC3G-induced mutations correlated negatively with viral load (r=-0.6, p=0.006) and positively with CD4 T cell counts (r=0.6, p=0.004). One participant, homozygous for the 186H variant and a viremic controller (HIV-1 RNA copies below 2,000 copies/ml) at evaluation had extensive APOBEC3G-induced hypermutations, resulting in multiple stop codons across the gene.

Conclusion: Our findings suggest that in our study population with a high frequency of the detrimental APOBEC3G H186R polymorphism that occurs predominantly within African populations there may be more significant contribution of APOBEC3F to viral hypermutation and viral control. Further studies are needed on the contribution and mechanisms of viral control by diverse cytidine deaminases in heavily burdened countries.

P06.14

Copy Number Variation of the Immunoglobulin Heavy Chain Variable Gene 1-69 in HIV-1 Infected Individuals

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Background: Immunoglobulin heavy chain variable region gene IGHV1-69 is commonly used by anti-HIV-1 antibodies including those to the CD4i epitope and the neutralizing antibodies 4E10, 8ANC195 and CH12, from a participant in the CAPRISA Acute Infection Cohort, CAP206. IGHV1-69 can vary from 2 to 4 copies per genome because of duplications on 14q, which occur in an estimated 50% of people. IGHV1-69 usage has been shown to be linked to the number of gene copies.

Methods: We developed a droplet digital PCR assay in which copy number was determined by counting the absolute number of target and reference DNA molecules. Reaction mix was partitioned into droplets and amplified to endpoint with primers and fluorescently labeled probes. The IGHV1-69 reverse primer was positioned in the 3' flanking region to ensure that only germline DNA was amplified. The DNA from 12 CAPRISA participants, whose IGHV1-69 allele number is known from cloning and ultra-deep sequencing experiments, was used.

Results: Copy number among the 12 participants varied from 2 to 4 per diploid genome. When compared to the allele numbers, copy number equaled these values in 10 cases but exceeded these values in 2 participants. Five individuals had a copy number of 3, four had a copy number of 2 and three had a copy number of 4. All three individuals with 4 copies produced broadly cross-neutralizing antibodies against HIV. CAP206 had 3 copies and 3 alleles, all of which were confirmed by antibody isolation, including IGHV1-69*02 used by CH12.

Conclusion: The IGHV1-69 insertion polymorphism of 14q is common in the South African population with 67% (8/12) CAPRISA women having more than 2 copies. Isolation of neutralizing antibodies from these individuals, including those with 4 copies of IGHV1-69, will determine whether increased copy number is associated with a greater likelihood of IGHV1-69 neutralizing antibodies.
P06.15

Polymorphisms in the IRF1 Gene Associated with Reduced HIV Susceptibility and Their Impact on Mrna Splicing and IRF1 Protein Expression

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Background: Interferon regulatory factor 1 (IRF1) is induced by HIV early in the infection process and serves two functions: transactivation of the HIV-1 genome and thus replication, and eliciting antiviral innate immune responses. Previous work has shown association of three polymorphisms in IRF1, located at 619, the microsatellite and 6516 of the gene, with decreased susceptibility to HIV-1 infection and a reduced likelihood of seroconversion. Peripheral blood mononuclear cells from individuals with protective IRF1 genotypes exhibited significantly lower basal IRF1 expression and a reduced likelihood of seroconversion. DNA sequence variations can cause phenotypic changes by multiple mechanisms, including mRNA splicing and turnover. This study will further characterize the effect of identified polymorphisms on IRF1 expression and its effect on HIV-1.

Methods: Alternative splicing and the functional impact of IRF1 polymorphisms on expression of IRF-1 regulated genes was analysed using the Affymetrix Human Exon 1.0 ST microarray. Exon splicing assay was performed in order to establish a direct link between identified polymorphisms and alternative splicing of the IRF1 mRNA.

Results: Data from this work shows an association of protective IRF1 polymorphisms with increased expression of exon 7/8 and decreased IRF-1 protein stability. IRF1 polymorphism (microsatellite GT repeat) was shown to directly regulate mRNA splicing. Overall our results show a direct link between identified IRF1 polymorphisms and alternative splicing of the IRF1 gene, leading to altered IRF1 protein levels. Additionally, we observed an increase in antiviral immune responses in cells from individuals with protective IRF1 genotypes.

Conclusion: Individuals with protective IRF1 genotypes are able to regulate the nature and strength of the immune response to HIV through altered IRF1 mRNA splicing. Resulting decrease in IRF1 protein stability and associated increase in antiviral immune responses could represent an important mechanism in preventing the establishment of HIV infection.

P06.16

Functional Comparison of the Promoters of the Two Genes (CCL3; CCL3L) that Encode for CCL3, the Natural Ligand of CCR5

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Background: The CCL3 chemokine is encoded by two functional genes, CCL3 and CCL3L. Numerous studies have shown a role for CCL3 in HIV-1 disease, however the individual contributions of the two genes to CCL3 production is largely unknown since the two isoforms cannot be easily differentiated. The 5'UTR regions of CCL3 and CCL3L share a 77% nucleotide similarity even though the two genes share a 96% amino acid similarity; this is largely due to CCL3L 5'UTR harbouring a ~310bp Alu element absent in the CCL3 5'UTR.

Methods: We compared the function of the 5' UTR regions of CCL3 and CCL3L using an in vitro luciferase assay. Six representative CCL3 promoters and two variations of the CCL3L promoter were amplified, sequenced and cloned upstream of the firefly luciferase gene in the pGL4.10 vector. HEK 293-T and four blood cells (Jurkat, K562, U937 and THP-1) were transfected with the constructs and relative luciferase activity was taken as a measure of promoter strength/function.

Results: Promoter strengths differed substantially depending on cell type suggesting differences in transcription factor usage between the cell types. The two CCL3L promoters did not differ significantly from each other or from the wildtype (WT) CCL3 promoter in the blood cell lines. In HEK 293-T cells however, the CCL3L promoter was significantly (P<0.001) 1.7 to 1.9 fold stronger than CCL3 WT promoter. This result is suggestive of a transcriptional interaction of the SV40 large T antigen in 293-T cells and the CCL3L promoter, and we hypothesise that the Alu element is a likely site for this interaction.

Conclusion: This result may explain why certain viral infections (e.g. HTLV-2) selectively up-regulate CCL3L and why certain viral-HIV-1 co-infections have been reported to exert a protective effect in HIV-1 disease. This study forms the basis for investigating the role of the CCL3L Alu element on promoter function.
**P06.17**

**Novel HLA Associations with HIV Control in Two Different Immunogenetic Contexts: A Multivariable Analysis**


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**Background:** Associations between HLA and HIV control have been well characterized. Unique HLA frequency distributions in uncharacterized populations represent an opportunity to find novel factors for HIV control. We aimed to study HLA effects on HIV control in a previously uncharacterized cohort comparing with a large Caucasian cohort.

**Methods:** HLA-A, -B and -C sequence-based typing was performed on 1187 and 1500 chronically HIV-1 B clade-infected, ART-naïve individuals from Mexico/Guatemala and from the mainly Caucasian British Columbia HOMER cohort. Associations between HIV plasma viral load (pVL) and expression of HLA alleles were evaluated using false discovery rate analyses. Univariable and stepwise multivariable linear regression were used to identify factors associated with pVL in the Latin American cohort.

**Results:** There were significant differences between HLA frequencies of two cohorts reported here and the HOMER cohort. The universal associations between B*5701 and B*2705 and HIV control were evident in both cohorts. As previously described for B-infected Caucasian cohorts, B*0702 and B*5501 were associated with higher pVL, while B*1401, B*1302 and B*5101 showed a protective effect in HOMER cohort. In the Mexican/Guatemalan cohort, B3501/02 showed a risk effect and B*4102/1401/1402 showed a protective effect. New associations between HLA and HIV control were also observed in the Mexican/Guatemalan cohort, including B*39:02 and C*08:01/02 associated with lower pVL and B*35:12 associated with higher pVL (all p<0.05, q<0.15). We have found a multivariable model that explains the variation in pVL in Mexico/Guatemala with the variables: CD4 count, being male, number of protective HLA-A and HLA-B alleles associated with pVL, age and B*5701/3502/4001 alleles by themselves. Finally, HLA frequency-dependent effects in Mexico/Guatemala were observed.

**Conclusion:** Our data confirms previous associations between HLA alleles and HIV viral control, but also suggests the existence of new protective and risk associations and mechanisms in the context of a previously uncharacterized population.

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**P06.18**

**Host Factors, Viral Replicative Capacity, and Viral Adaptation Work in Concert to Define HIV-1 Subtype C Disease Progression**

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**Background:** Efforts to elucidate protective host factors often rely on cross-sectional viral loads (VL) or CD4 T cell counts to determine factors influencing pathogenesis. Here, we study a cohort of 127 acutely infected Zambians with longitudinal plasma VL and CD4 counts up to 5 years post infection as a more sensitive method to identify novel host and viral characteristics directly associated with disease progression.

**Methods:** Plasma VL and CD4 counts were assessed at 3-month intervals after seroconversion for 127 acutely infected Zambians for up to 5 years. HLA genotyping was performed using genomic DNA and PCR-based techniques. Viral replicative capacity (vRC) was assessed by cloning the gag gene from acute time points into MJ4, a subtype C proviral vector, and infecting a CEM T cell line with Gag-MJ4 chimeric viruses.

**Results:** Kaplan-Meier analyses and Cox proportional hazard models with an end-point defined by CD4 counts <300 were used to investigate protective and deleterious HLA class I and II alleles. HLA-B*1401, B*81, B*57/C*18, DQB1*02, and DRB1*15 were found to provide significant protection from CD4 decline, while HLA-A*03, A*24, and C*17 were deleterious. Moreover, the effects of these alleles were found to be additive, such that individuals with 2 or more protective alleles experienced significantly slower CD4 decline. Meanwhile, sharing HLA-B alleles with a transmitting partner resulted in a significant risk for accelerated CD4 decline, reflecting the impact of pre-transmission viral adaptation on immune control. In multivariable models, HLA-I/II, set point VL, gender, HLA-B sharing and vRC were found to be independent predictors of time to CD4 <300.

**Conclusion:** This study of a well-characterized cohort of acutely infected individuals provides unique insight into the independent contributions of host immunogenetics, viral characteristics, and viral adaptation to HIV-1 pathogenesis and will help to elucidate mechanisms of control, which may in turn define correlates of protection.
Background: RV144 is the first HIV-1 vaccine clinical trial to show an effect on HIV-1 acquisition. A follow up study discovered two immune correlates as predictors of risk of infection. Binding of plasma IgA antibodies to HIV-1 envelope (Env) correlated directly with acquisition whereas binding of IgG antibodies to the variable regions 1 and 2 (V1V2) of Env correlated inversely with acquisition. We hypothesized that HLA class II molecules expressed on antigen presenting cells modulate CD4 T cell stimulation of antibody production by B cells involved in vaccine-induced responses.

Methods: HLA class II genes were genotyped in the case-control group of 205 uninfected and 41 infected RV144 vaccinated volunteers. The interaction of HLA-DPB1, DQB1 and DRB1 alleles with IgA and IgG immune responses was tested for an effect on acquisition by logistic regression.

Results: DQB1*06 had a significant interaction with IgA antibody responses (interaction p=0.002, q=0.089) on acquisition. Increased IgA antibody levels associated with increased risk for infection in the presence of DQB1*06, while no association was observed in the absence of DQB1*06 (OR=9.35, p=0.002 and OR=1.08, p=0.704, respectively). Corroborating this finding, in a categorical analysis DQB1*06 had a significant interaction with IgA antibody levels associated with increased risk for infection in the subgroup of vaccinees with low IgA. These data suggest that vaccine-induced Fc receptor (FcR)-mediated Abs function could play an important role in lowering infection risk. Thus, we hypothesize that the FcR genotypes may also influence VE because they affect Abs binding affinity. Furthermore, a follow up study discovered two immune correlates as predictors of risk of infection. Binding of plasma IgA antibodies to HIV-1 envelope (Env) correlated directly with acquisition whereas binding of IgG antibodies to the variable regions 1 and 2 (V1V2) of Env correlated inversely with acquisition.

Conclusion: Evidence herein suggests that HLA class II genes are modulating the effect of antibody responses to the vaccine and impact HIV-1 acquisition. A next step may be to define the CD4 T cell clones that could mediate these HLA/antibody interactions. Understanding the mechanism of HLA class II effects on antibody responses to this vaccine will enable improved HIV vaccine.
Host and Viral Factors Associated with HIV-1 RNA Set-Point Among HIV-1 Seroconverters from sub-Saharan Africa

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Background: HIV-1 set-point predicts disease progression and is partially determined by the source partner's HIV-1 level and adaptive immune responses. However, these factors fail to capture most set-point variation. We quantified the collective impact of virus characteristics, human leukocyte antigen (HLA) alleles, and innate responses through toll-like receptor (TLR) alleles on HIV-1 set-point.

Methods: We analyzed data from HIV-1 seroconverters and their HIV-source partners in a large African HIV-1 serodiscordant couples cohort. Linear regression was used to determine associations with seroconverter set-point and $R^2$ to estimate variation explained by HLA and TLR alleles, transmitting partner virus levels and other factors.

Results: Among 128 HIV-1 seroconverters, the strongest predictors of set-point were HLA alleles and HIV-1 level of the transmitting partner, but HLA concordance between partners and TLR variation were also associated with set-point. Specifically, each log10 increase in source partner plasma HIV-1 RNA was associated with 0.4 log10 copies/mL increased set-point (95% CI:0.2-0.5; p<0.001). HLA-B*5301 was associated with 0.6 log10 copies/mL higher (95% CI:0.3-0.9; p<0.001), and B*1401 and B*2703 with 1.1 (95% CI:0.4-1.9; p=0.002) and 0.7 (95% CI:0.1,1.6; p=0.07) log10 copies/mL lower set-point, respectively. HLA-A allele sharing between partners was associated with 0.5 log10 copies/mL increase (95% CI:0.4,1.0; p<0.001). Finally, TLR2-rs3804100 was associated with 0.4 (95% CI:0.3-1.0; p=0.3) log10 copies/mL higher and TLR7-rs179012 with 0.4 (95% CI:0.2,0.7; p=0.001) log10 copies/mL lower set-point. HLA alleles and transmitting HIV-1 level accounted for 13% and 10% of set-point variation, respectively, and HLA-A concordance and TLR polymorphisms contributed 6% and 5%. Overall, these factors and genital factors of the transmitter explained 46% of variation in set-point.

Conclusion: We found that both innate immune responses, as captured by TLR variants, and acquired immune response, as determined by HLA alleles and HLA concordance with the transmitting partner, together with plasma HIV-1 levels of the transmitting partner, explain almost half of the variation in viral load set-point.
Background: Multiple Adenovirus (Ad) vectors are currently being evaluated for clinical development and have been shown to differ in their innate stimulation profiles. The mechanism by which Ad26 and Ad35 differentially stimulate greater innate responses than Ad5 remains poorly characterized. We therefore assessed the innate pathways stimulated by these Ad vectors and the influence of Ad vector trafficking.

Methods: Human PBMC were pre-incubated with acidification or cathepsin inhibitors and then infected with 10^3 vp/cell Ad5, Ad35, or Ad26. Supernatant cytokines were measured by luminex. At 48 hours post-infection, Ad5, Ad26, Ad35, or fiber/knob chimeric vectors. Cells were analyzed by immunofluorescence histochemistry for EEA1, LAMP1, Mannose-6 Phosphate Receptor (M6P) and nuclei (DAPI).

Results: Pre-incubation of PBMC with acidification and cathepsin inhibitors (Bafilomycin A1, Chloroquine, Ammonium chloride, C2C2Me, CAA0225, z-Fa), which block essential events in late endosomal innate immune sensing, inhibited induction of IFNγ (41- and 334-fold higher, respectively; p=0.0286, both). ALVAC induced higher fold-change responses in human PBMC 24h post-infection compared to MVA and NYVAC both for proinflammatory, antiviral cytokines e.g. IFNα (109- and 166-fold higher, respectively; p=0.0002, p<0.0001, respectively), IL-6 (83- and 6.3-fold higher, respectively; p=0.0286, both), and TNFα (48- and 29-fold higher, respectively; p=0.0002, p<0.0001, respectively). In Rhesus PBMC in vitro ALVAC, relative to MVA and NYVAC 24h post-infection, induced higher levels of IL-1β (66- and 125-fold higher, respectively; p=0.0286 both), IL-6 (7.9- and 21-fold higher, respectively; p=0.0286, both), and TNFα (6.8- and 12-fold higher, respectively; p=0.0286 both), as well as the antiviral cytokines MIP-1α (7.2- and 30-fold higher, respectively; p=0.0286 both) and IFNy (109- and 166-fold higher, respectively; p=0.0286 both). ALVAC induced higher fold-change responses in human PBMC 24h post-infection compared to MVA and NYVAC both for proinflammatory, e.g. IL-1β (68- and 310-fold higher, respectively; p=0.0286 both) and anti-viral cytokines e.g. IFNy (41- and 334-fold higher, respectively; p=0.0286 both).

Conclusion: Our results suggest that the differential innate stimulation by Ad vectors occurs by triggering innate signaling pathways accessed preferentially in late endosomotis by Ad35 and Ad26. Trafficking to late endosomes is influenced by both vector fiber and capsid components. These data demonstrate a mechanism by which various Ad serotypes differentially trigger innate immunity.
### P07.03

**Antigen Presenting Cells Characterization at Local Site of Injection After MVA and Poly (I: C) Intramuscular Vaccination in Non-human Primates**

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**Background:** Better understanding of early events at site of intramuscular vaccine injection is needed for a rational design of future vaccines.

**Methods:** The attenuated vaccinia virus Ankara (MVA) is an ideal replication-deficient viral vector for HIV-1 vaccines due to the excellent safety profile in humans and Polyinosinic-polycytidylic acid (Poly(I:C)) is an adjuvant known for its high tolerance and immunogenicity in animal trials. Cynomolgus macaques were immunized by the intramuscular route with either PBS, MVA (5x10⁷ plaque-forming-unit) or Poly(I:C) (200µg). Muscle biopsies were sampled at 24h after injection for Poly(I:C) and at 48h after injection for MVA to assess antigen presenting cells (APC) characterization by flow cytometry and APC localization by immunohistology.

**Results:** Perimysium inflammation due to massive APC arrival (mainly macrophages and polynuclear cells) was observed by HES coloration and immunohistochemistry, at the injection site, after MVA or Poly(I:C) intramuscular injection. In muscle biopsies of immunized animals, leukocyte (CD45+) frequency was increased (49.6%±5.1 and 54.4%±21.8 of total alive cells for Poly(I:C) and MVA, respectively; mean±SEM) compare to non-immunized animals (16.8%±9.0). Interestingly, macrophages (CD45+HLADR+CD163+) were the most abundantly recruited cells (46.7%±10.3 and 39.6%±1.1 of total CD45+ cells for Poly(I:C) and MVA, respectively) whereas dendritic cells (CD45+HLADR+CD11c+) were recruited in lower proportion (6.7%±5.7 or 0.5%±0.4 of CD45+ cells for Poly(I:C) and MVA, respectively). Moreover, MVA injection leaded to more prominent polynuclear cells recruitment (25.5%±8.2 of total CD45+ cells) compare to Poly(I:C) (19.9%±0.8).

**Conclusion:** Our results highlight the importance of macrophages following intramuscular vaccination and their possible role in the antigen-specific immune response induction, suggesting the potential benefit of a vaccine targeting macrophages as candidate against HIV.

### P07.04

**Vaccine Adjuvants Induce Distinct Innate Immune Activation Profiles In Vivo**

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**Background:** Understanding the innate mechanisms dictating vaccine responses and why some adjuvants induce more potent responses than others would help optimize the design of new vaccine formulations. Here, utilizing a non-human primate (NHP) model we investigated the activation and recruitment of immune cells to the injection site as well as vaccine uptake efficiency after administration of three distinctly different adjuvants in vivo.

**Methods:** Rhesus macaques received intramuscular injections of fluorescently-labeled HIV-1 envelope glycoprotein (Env) alone or together with one of the adjuvants; alum (benchmark), alum containing a TLR7 ligand or MF59 (emulsion). Muscle tissue, lymph nodes (LNs) and blood were collected for flow cytometry and confocal analysis.

**Results:** Robust infiltration of multiple immune cells into the muscle at the site of injection was seen with all adjuvants at 24 hrs. Neutrophils were the most frequent infiltrating cell type with all the adjuvants. Monocytes, myeloid and plasmacytoid dendritic cells (DCs) were also recruited. Env uptake was readily detectable in these cell populations in the muscle and in the draining, but not in non-draining, LNs. While alum-TLR7-ligand exclusively induced strong DC maturation and type I interferon responses, MF59 promoted neutrophil migration to LNs. Env+ neutrophils isolated from the LNs were found to present Env to memory T cells although at much lower capacity than did DCs.

**Conclusion:** In line with findings that both MF59 and alum-TLR7-ligand are more potent adjuvants compared to alum in immunogenicity studies, our data indicate that these adjuvants induce stronger innate immune activity than alum. Therefore, a more comprehensive picture of how adjuvants regulate innate immune processes can assist in a more rational selection of adjuvants that are best suited for a given vaccine. As NHP cell subsets are similar to humans, this is a powerful model for translating data into optimizing future vaccine formulations and delivery strategies.
Background: DNA vaccines are a new promising approach in the research of preventive and therapeutic vaccination against chronic infections such as HIV. However, despite their effectiveness reported in small animal studies, DNA vaccines showed lower immunogenicity in humans. In a previous study, we demonstrated that auxoGTU-multiHIV DNA vaccine immune response is strongly improved by electroporation (EP) treatment of the injection site in non-human primates (NHP). A better understanding of cellular behavior at the injection site during the first hours after vaccination is needed to increase and direct the immune response to DNA vaccines. In this study we used in vivo fluorescence imaging to visualize skin antigen presenting cells (APC) immune response to DNA vaccines. In this study we used in vivo fluorescence imaging to visualize skin antigen presenting cells (APC) and monitor their spatio-temporal dynamics in NHP after vaccination.

Methods: AuxoGTU-multiHIV plasmid was injected intradermally and associated with noninvasive EP at vaccinated sites in anesthetized NHP (n=3). Fluorescent labeled anti-HLADR antibody was injected at vaccinated sites before in vivo fluorescence imaging. Epidermal APCs were tracked with noninvasive in vivo fibered confocal fluorescence microscopy at 24h, 48h, 72h, and 96h post vaccination. Furthermore, epidermal and dermal APCs were monitored continuously for 24h by confocal fast laser scanning microscopy on skin explants.

Results: When electroporation was associated to intradermal injection of DNA plasmid, the density of epidermal APCs decreased significantly between 48h and 96h after vaccination. Experiments on skin explants confirmed the epidermal APC depression associated to a rise of dermal APC number. Moreover, we showed an increase of APC velocity and displacement in this condition.

Conclusion: Using in vivo fluorescence imaging approaches, we monitor epidermal APC behavior changes, induced by DNA vaccination associated with EP. In vivo imaging experiments on distinct innate immune cell populations are ongoing, in order to understand the cellular orchestration that shapes the adaptive immunity and thus optimize the DNA vaccines efficacy.

Background: DC-SIGNR is a C-type lectin receptor that interacts with a plethora of pathogens including HIV-1. DC-SIGNR is proposed to play a vital role in binding to HIV-1 gp120 thereby facilitating transmission of HIV to CD4+ T cell targets. To determine dendritic cell count and its subsets and study the expression of DC-SIGNR on PBMCs and its polymorphic variants.

Methods: Blood from 230 seronegative healthy individuals, 200 injecting drug users, and 230 patients infected with HIV-1 was collected. The repeat region polymorphism in DC-SIGNR was performed by PCR. DC-SIGNR expression on PBMCs was determined by real time PCR and flow cytometer. DC-SIGNR expression on PBMCs was determined by real time PCR and flow cytometer in antiretroviral naïve HIV-1 infected patients and healthy individuals and in culture of monocyte derived dendritic cells from healthy individuals.

Results: The frequency of DC-SIGNR 7/7 genotype was significantly higher in patients infected with HIV-1 and DC-SIGNR 7/5 genotype was significantly higher in injecting drug users. A salient finding of this study was the association of the heterozygous 7/5 DC-SIGNR genotypes with higher percentage of DC and their subsets and higher CD4+ T cell counts and lower viral load compared to the homozygous 7/7 DC-SIGNR genotypes in patients infected with HIV-1. The expression of DC-SIGNR was higher in patients infected with HIV-1 with positive correlation with CD4+ T cells as compared to healthy individuals. Dendritic cells in culture, on infecting with HIV-1 virus (AIIMS 53), showed up regulation of DC-SIGNR and co-stimulatory molecules.

Conclusion: This is the first study to assess the DC subsets, and its association with DC-SIGNR polymorphism in injecting drug users and HIV-1 infected patients and DC-SIGNR expression in HIV-1 infected patients. This study suggests the protective role of 7/5 DC-SIGNR genotype in HIV-1 infection and the higher expression of DC-SIGNR in HIV-1 infection suggest possible role DC-SIGNR in activated dendritic cells during HIV-1 infection and needs to be studied.

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Methods: Blood from 230 seronegative healthy individuals, 200 injecting drug users, and 230 patients infected with HIV-1 was collected. The repeat region polymorphism in DC-SIGNR was performed by PCR. DC-SIGNR expression on PBMCs was determined by real time PCR and flow cytometer. DC-SIGNR expression on PBMCs was determined by real time PCR and flow cytometer in antiretroviral naïve HIV-1 infected patients and healthy individuals and in culture of monocyte derived dendritic cells from healthy individuals.

Results: The frequency of DC-SIGNR 7/7 genotype was significantly higher in patients infected with HIV-1 and DC-SIGNR 7/5 genotype was significantly higher in injecting drug users. A salient finding of this study was the association of the heterozygous 7/5 DC-SIGNR genotypes with higher percentage of DC and their subsets and higher CD4+ T cell counts and lower viral load compared to the homozygous 7/7 DC-SIGNR genotypes in patients infected with HIV-1. The expression of DC-SIGNR was higher in patients infected with HIV-1 with positive correlation with CD4+ T cells as compared to healthy individuals. Dendritic cells in culture, on infecting with HIV-1 virus (AIIMS 53), showed up regulation of DC-SIGNR and co-stimulatory molecules.

Conclusion: This is the first study to assess the DC subsets, and its association with DC-SIGNR polymorphism in injecting drug users and HIV-1 infected patients and DC-SIGNR expression in HIV-1 infected patients. This study suggests the protective role of 7/5 DC-SIGNR genotype in HIV-1 infection and the higher expression of DC-SIGNR in HIV-1 infection suggest possible role DC-SIGNR in activated dendritic cells during HIV-1 infection and needs to be studied.
**P07.07**

**Coculture of HIV-1-Infected Dendritic Cells with Lymphocytes Impairs Host Restriction Factor SAMHD1 and Activates Immune Defenses**


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**Background:** HIV-1 replication in dendritic cells (DCs) is restricted by SAMHD1. This factor is counteracted by the viral protein Vpx of HIV-2 or SIV or SIV, but is absent in HIV-1. We previously observed a stimulation of HIV-1 replication in immature DCs cocultured with primary CD4 T or B lymphocytes. This suggests that HIV-1 restriction in DCs may be overcome in coculture conditions. The aim of this study was to decipher SAMHD1-mediated restriction in DC/lymphocyte coculture.

**Methods:** Primary monocyte-derived immature DCs were incubated with various R5 HIV-1 primary isolates during 2h, and then cocultured with autologous CD4 T and B lymphocytes. After 48h and 72h, the percentages of infected DC-SIGN+/CD3- MoDCs were detected by intracellular p24 antigen staining. Simultaneously, the expression of intracellular SAMHD1 and the maturation phenotype were quantified. IFN-α production was measured in the supernatants. Virus-like particles containing Vpx (VLP-Vpx) and exogenous dNTPs were used as control.

**Results:** SAMHD1 expression in DCs was significantly decreased from 80% to 10% when DCs were cocultured with CD4 T or B lymphocytes for 48h and 72h (n=9 donors). This decreased SAMHD1 was correlated with an increase HIV-1 replication in cocultured DCs. In addition, IFN-α production was detected in the supernatant of infected DC/CD4 T lymphocytes coculture and DCs acquired their maturation status 48h and 72h post-infection. As controls, VLP-Vpx increased HIV-1 replication and IFN-α release, and decreased SAMHD1. Addition of exogenous dNTPs increased HIV-1 replication but without modifying SAMHD1 and IFN-α release.

**Conclusion:** These results demonstrate that coculture with lymphocytes decrease the expression of SAMHD1 in DCs leading to a significant increase of HIV-1 replication in DCs and triggering an antiviral immune response. The innate sensing of HIV-1-infected immature DCs, observed in coculture conditions further suggest that HIV-1 replication and restriction in DCs should be studied in more physiologically relevant models of DC/lymphocyte coculture.

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**P07.08**

**HIV Exposed Seronegative Have Distinct Type I and Type II Interferon Responses to TLR7 and TLR8 Stimulation Compared to Susceptible Women**


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**Background:** Toll-Like Receptor 7 and 8, recognizes the single strand RNA from the HIV virus. A group of commercial sex workers (CSWs) are thought to be repeatedly exposed to the HIV virus in the course of their work yet remain uninfected.

**Methods:** The CSWs studied were grouped into two based on years of enrollement in cohort, HIV negative status and continuous engagement in sex work. The two groups were, HESN who had been enrolled in cohort for more than 7 years, and a second group was called New Negatives (NN) who were in cohort for less than 7 years. Freshly isolated PBMCs were stimulated overnight with ssRNA40 (TLR7/8) and Imiquimod (TLR7). Changes in expression of phenotypic and activation markers was done using flow cytometry and TLR signaling pathways analysis done using PCR arrays. Solubles cytokine were quantified using cytokine bead arrays from culture supernatants.

**Results:** T cells of HESN were activated more and produced high amounts of IFN-γ in response to TLR7 and TLR8 stimulation compared to those of NN women. Overall PBMCs of HESN produced higher quantities of of TNF-α and IL-1β after ssRNA stimulation, but lower quantities of IL-1β and IL-10 after Imiquimod stimulation compared to NN women. PBMCs from HESN women produced higher amounts of IFN-γ in response to ssRNA, but lower amounts of IFN-α2 and CXCL10 in response to Imiquimod a TLR7 ligand, as compared to NN women. An evaluation of the regulation or balance of cytokines in the milieu before and after TLR stimulation revealed a more regulated cytokine environment in HESN culture but a dysregulated milieu in NN women. An analysis of TLR signalling pathway following stimulation revealed differences in activation of intracellular pathways between the two groups of women and between TLR7 and TLR8 ligands.

**Conclusion:** Differences exist in cellular activation, cytokine production and TLR signaling between HESN and NN CSWs.
P 0 7.10

Dynamics of Immune Cells Recruitment and Mobilization in Skin After DNA Vaccination with auxoGTUmultiSIV Plasmid in Non-human Primates

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Background: Because of HIV biology, classical vaccination strategies failed to induce protective responses. For this virus, it is necessary to turn to new strategies. DNA vaccine presents some advantages such as its safety (no viral agent used) and the mimicry of natural virus infection. The DNA auxoGTUmultiSIV vaccine in association with electroporation (EP) induces strong and persistent cellular responses in cynomolgus macaques.

Methods: Cynomolgus macaques were injected with auxoGTUmultiSIV (1mg/ml) or with PBS (control) by intradermal route with EP. Skin biopsies of injection sites were performed at different time points and cells were extracted to study early cellular events involved in the local immune responses.

Results: At steady state, Langerhans cells (LC) (HLADRhiCD1ahiCD207+CD1c-) are the only antigen presenting cells in epidermis (1.6±0.9%). In dermis, a population of resident macrophages (HLADR+CD163+CD11b+CD11clow) (1.98±1.09%) and a population of HLADR+CD11c+CD1a+ dermal DC have been characterized. The latter can be separated in 2 subsets: CD1a+CD1c- and CD1a+CD1c+. In epidermis, 24h after injection, we observed an initial increase of LC with an up-regulation HLADR, suggesting their activation. At 72h, LC decreased to their baseline level. Interestingly, a population of HLADR+CD1aintCD1c+ cells appeared as soon as 24h and reached a peak at 72h. This population seems to up-regulate CD1a and down-regulate CD1c over time and presents an intracellular expression of CD207, an exclusive marker of LC in human. A recruitment of granulocytes (HLADR-CD66+) and inflammatory macrophages (HLADR+CD163midCD11bhi) was observed in epidermis and dermis, mainly at 24h after injection. These observations were rather attributed to EP than to vaccine injection.

Conclusion: Our results suggest that EP, independently of the presence of DNA, is sufficient to induce danger signals and cells recruitment. These early events could facilitate antigen uptake and presentation. This work provides important clues to the local mechanism of inflammation and opens up new possibilities for vaccine strategies.

P 0 7.09

Long-Term Non-Progressors Display Expansion of T Cells Expressing NK Cell Receptors that Associates with CD4 T Cell Count and Viral Load

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Background: Increased expression of inhibitory NK cell receptors (iNKRs), and decreased expression of activating NKRs (aNKRs) has been documented on NK cells in HIV-1 infection. As little is known regarding the expression or role of NKRs on T cells, we analysed the expression of various NKRs on T cells in HIV-1 infected long-term non-progressors (LTNPs).

Methods: The proportions and intensity of expression of killer cell immunoglobulin-like receptor (KIR) (KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1 and KIR2DS4), C-type lectin (NKG2A, NKG2C and NKG2D) and natural cytotoxicity receptors (NCR) (Nkp30 and Nkp46) on CD8 and CD4 T cells were analysed in the peripheral blood of 14 LTNPs (viral load range, 1.59-4.86 log copies/ml and CD4 range, 327-2000 cells/µL) and 14 race, gender and age-matched healthy controls.

Results: LTNPs displayed relative to controls: (i) Increased proportions of KIR2DL1, KIR2DL3, KIR2DS4 and Nkp46 expressing CD8 and CD4 T cells, and increased proportions of KIR2DL4, KIR3DL1, NKG2C and NKG2D expressing CD4 T cells; (ii) Lower fluorescence intensity of KIR2DL3, KIR2DL4, NKG2C and Nkp46 on CD8 and CD4 T cells, as well as Nkp30 on CD8 and KIR2DL1, KIR3DL1, KIR2DS4 and NKG2D on CD4 T cells. Importantly, in the LTNPs, higher CD4 T cell counts associated with lower proportions and higher intensity of Nkp46 expression on CD8 T cells (r=-0.692, P=0.006 and r=0.609, P=0.021, respectively), as well as lower proportions of KIR3DL1 (r=-0.745, P=0.002) and Nkp46 (r=-0.552, P=0.041) expressing CD4 T cells. Furthermore, lower viral loads associated with higher intensity of KIR3DL1 (r=0.645, P=0.013) expression on CD8 T cells and lower proportions of KIR2DL3 (r=0.708, P=0.005) and Nkp46 (r=0.625, P=0.017) expressing CD4 T cells.

Conclusion: We have demonstrated a strong induction of iNKRs and aNKRs on T cells in LTNPs. Lower proportions and higher intensity of NKR expression on CD8 and CD4 T cells associate with higher CD4 T cell counts and lower viral loads among LTNPs, highlighting the importance of their study in HIV-1.
**P07.11**

**HIV Protective KIR/HLA Genotypes Influence NK Cell Mediated Inhibition of HIV Replication in Autologous CD4 T Cells via CC-chemokine Secretion**

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**Background:** Carriage of the genetic combination encoding high expression Natural Killer (NK) cell inhibitory Killer Immunoglobulin-like Receptors (KIR)3DL1 with their ligand HLA-B*57 (‘h/y+B*57) has the strongest effect on slowing time to AIDS and HIV viral load control compared to Bw6 homozygotes (Bw6hmz). NK cell education, which requires inhibitory NK receptor engagement and the integration of signals from NK receptors binding their ligands on target cells determines NK cell anti-viral functional potency. We questioned whether NK cells from ‘h/y+B*57 carriers inhibited HIV replication more potently than those from Bw6hmz, as do those from carriers of another HIV protective genotype, i.e. 3DS1+‘B0L.

**Methods:** We studied 5 HIV seronegative ‘h/y+B*57 carriers, 5 3DS1+‘B0L, and 9 Bw6hmz. Purified NK cells were co-cultured with autologous HIV infected CD4 T cells for 10 days. Cultures were assessed for supernatant p24 levels and frequency of p24 positive CD4 T cells on days, 3, 7 and 10 and for CC-chemokine secretion on days 1, 2 and 3.

**Results:** NK cells from carriers of ‘h/y+B*57 and 3DS1+‘B0L inhibited HIV replication better than those from Bw6hmz (p<0.05 at days 7 and 10). NK and CD4 T cell contact was required for NK cells activation but, once activated, NK cells inhibited viral replication in autologous CD4 T cells in a non-contact dependent fashion through secretion of CC-chemokines CCL3, CCL4 and CCL5. NK cells from carriers of protective genotypes produced higher levels of CC-chemokines than did those from Bw6hmz.

**Conclusion:** NK cells from individuals carrying protective KIR/HLA genotypes inhibit HIV replication in autologous CD4 T cells more effectively than those from a non-protective genotype. The inhibition of viral replication in autologous infected CD4 T cells is partially due to a block at the level of entry mediated by the secretion of CC-chemokines. NK cell education likely influences the anti-HIV potency of NK cells from ‘h/y+B*57.

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**P07.12**

**Therapeutic Vaccination Using HIV-1 gp120/NefTat/AS02A Enhances Responsiveness of NK Cells Through the Induction of gp120-Specific CD4+ T Cell Help**

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**Background:** Recent studies in animal models suggested that antigen-specific CD4+ T cells can provide immunological help not only to CD8+ T cells and B cells, but also to natural killer (NK) cells. As human NK cells can mediate anti-HIV-1 activity, novel approaches harnessing the function of both HIV-1-specific T cells and NK cells represent an attractive option to improve future vaccines. Using samples from a clinical trial employing a therapeutic subunit protein vaccine inducing strong HIV-1-specific IL2+ CD4+ T cell responses, we assessed whether enhancing HIV-1-specific CD4+ T cell function can restore NK cell activity in antiretroviral-treated HIV-1-infected individuals.

**Methods:** Using flow cytometry, we examined the function of CD4+T cells and NK cells following HIV-1 peptides stimulation of PBMCs derived from subjects with untreated chronic HIV-1 infection and from treated HIV-1-infected volunteers participating in a double-blinded, randomized, placebo-controlled clinical trial of GlaxoSmithKline Vaccines’ HIV-1 gp120/NefTat subunit protein vaccine formulated with the AS02A Adjuvant System.

**Results:** In a subset of chronically HIV-1-infected individuals, stimulation of PBMCs with Gag-derived peptides led to the production of IL-2 and IFN-gamma by antigen-specific CD4+ T cells and NK cells, respectively. Reconstitution of cytokine-producing CD4+ T cells by therapeutic immunization led to substantially enhanced NK cell function. In individuals receiving the therapeutic vaccine, CD4+ T cells produced significantly higher levels of IL-2 in response to gp120 compared to controls at 6 weeks post-vaccination, and this enhanced antigen-specific CD4+ T cell response was associated with more than one log increase in IFN-gamma production by NK cells.

**Conclusion:** Our data show that enhanced NK cell activity can be promoted by therapeutic immunization in HIV-1-infected individuals through reconstitution of HIV-1-specific CD4+ T cell function. Further investigations are warranted to examine whether vaccine-induced CD4+ T cells can improve cytotoxic NK cell responses against HIV-1.
**P07.13**

**Inflammatory Biomarkers and Clinical Outcomes in Primary HIV-1 Infection**

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**Background:** Inflammatory biomarkers are associated with increased morbidity/mortality in chronic HIV-1 infection; however, their role in primary HIV-1 infection is poorly understood. We sought to determine if previously described inflammatory biomarkers are associated with the viral load set point (VLSP) in primary HIV-1 infection and their utility in predicting clinical outcomes of disease progression.

**Methods:** A longitudinal retrospective analysis was conducted of 92 patients with untreated primary HIV-1 who were clinically followed for a median of 591 days. VLSP was determined for all study subjects during the first year post-infection and their plasma was tested for TNF-alpha, IL-6, CRP, D-dimer, IL-1-beta, and IFN-gamma. Primary endpoints were VLSP and days to CD4+ T cell count <500 cells/µL, secondary endpoints were days to CD4+ T cell count <350 cells/µL and days to antiretroviral therapy (ART) start. The association of biomarkers with the endpoints was assessed by Spearman-rank, log rank, and Cox proportional hazard models.

**Results:** Only TNF-alpha was highly correlated with VLSP (R=0.39, p<0.0001). Log rank analysis separating the groups by mean TNF-alpha (≥8.9 pg/mL) showed a trend towards differences for predicting days to CD4+ T cell count <500 cells/µL (p=0.07), while days to ART start was significantly different (p=0.03) between groups. In post-hoc analysis, the upper three quartiles for TNF-alpha were significantly associated with fewer days to CD4+ T cell count <500 cells/µL compared to the lowest quartile (p=0.005, log rank) with a Cox proportional hazard ratio 5.0 in univariate analysis (CI 1.5-16, p=0.01), 3.1 when adjusted for concurrent viral load (CI 0.8-11.3, p=0.10) and 4.5 when adjusted for concurrent CD4+ T cell count (CI 1.3-16, p=0.02).

**Conclusion:** Plasma TNF-alpha correlates with the VLSP during primary HIV-1 infection and may be associated with early disease progression. The clinical impact of inflammation due to high TNF-alpha levels independent of viral replication deserves further study.

**P07.14**

**Sex Differences in IRF5 Levels and Implications for pDC IFNα Response to TLR7**

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**Background:** Differential sex-dependent immune activation is a common feature in both HIV-1 and autoimmune diseases. Plasmacytoid dendritic cells (pDCs) derived from females have been shown to produce significantly more IFNα in response to HIV-1-encoded TLR7/8 ligands than pDCs derived from males, resulting in stronger secondary T cell activation. We investigated the impact of the interferon regulatory factors 7 and 5 (IRF7/5) on the observed sex differences given their central role in TLR7 signaling.

**Methods:** Fresh PBMCs were isolated from healthy donors. IRF7 and IRF5 levels were measured by flow cytometry directly ex vivo and after TLR7 stimulation. Translocation of IRF5 to the nucleus of pDCs following TLR7 stimulation was visualized by confocal microscopy and quantified within cell populations among multiple individuals using the TissueFAXS slide scanner at high magnification.

**Results:** Whereas no differences between males and females in baseline levels of IRF7 in pDCs were observed, baseline levels of IRF5 in pDCs were 1.8 times higher in females than in males (p<0.01). Importantly, after TLR7 stimulation, levels of IRF5 were significantly higher in pDCs secreting IFNα compared to the non-secreting pDCs (p<0.05). In addition, we observed a trend towards more IRF5 translocation into the nucleus in females than in males pDCs after 2 hours of TLR7 stimulation. Interestingly, significantly more IRF5 was localized in the nucleus than in the cytoplasm in pDCs already at baseline, in line with recent reports that IRF5 may constitutively bind the IFNA promoter in specific cell types.

**Conclusion:** These results indicate that sex differences in IRF5 levels might contribute to the described higher IFNα production upon TLR7 stimulation in females, providing new insights into the mechanisms underlying higher inflammation observed in HIV-1-infected females and stronger responses to vaccines reported for females compared to males. These studies highlight potentially novel targets for the modulation of inflammation and immune responses.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L2, Room 211 – 212. Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L2, Room 211 – 212.

**P07.15**

**Erythrocytes’ Presence Enhance HIV Production on Monocyte Derived Macrophages (MDM)**

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**Background:** Erythrocytes of HIV+ individuals present p24 Antigen, RNA-HIV and bound-specific antibodies. HIV-erythrocyte association was described to be mediated by immune complexes or complement factors and DARC in the erythrocyte membrane. Given that macrophages are widely known reservoirs established at the first stages of infection and the close contact of erythrocyte and macrophages, this work focuses on the capacity of the HIV produced by macrophages to bind to erythrocytes membrane.

**Methods:** MDM (monocyte derived macrophages) were obtained from healthy donors’ buffy coats and infected with BaL. Erythrocytes were obtained from healthy donors (one Afro-American woman DARC−) and purified with Dextran. After 7 and 10 days post inoculation (pi) MDM were incubated with erythrocytes DARC+, DARC− and RPMI. P24Ag was determined in erythrocytes supernatant. The supernatant of the MDM incubated with RPMI was fractioned in 3, one to measure p24 and other 2 incubated with purified erythrocytes DARC+ and DARC−. P24 was determined.

**Results:** The total amount of virus produced by MDM incubated with erythrocytes was significantly higher than the amount produced when they were incubated with RPMI (p=0.005). The binding capacity of the virus produced by MDM was analyzed with erythrocytes DARC+ and DARC− and no differences were observed between donors. The percentages observed were similar to those obtained when the erythrocytes were incubated with different viral stocks. Similar results were obtained in different pi days.

**Conclusion:** Due to the importance of macrophages and erythrocyte described, and as it was demonstrated here, the presence of erythrocytes may increase viral production of infected MDM, this fraction should be thoroughly considered in the pathogenesis of HIV infection, given that this close contact always occurs in the infected patient during the early stage of infection. So far, the presence or absence of DARC showed no relevance, therefore, other receptors involved in the binding process should be further studied.

**P07.16**

**Frequency, Phenotype and Function of KIR+ and NKG2A+ NK Cell Subsets During Acute and Chronic HIV -1 Clade C Infection**

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**Background:** Mature NK cells are heterogeneous in the expression of activating and inhibitory receptors, and differ in proliferative and functional activity in response to a range of cytokines. Recent studies have suggested that NK cell maturation and function in response to infection is influenced by a stepwise decrease of NKG2A receptor expression and acquisition of KIRs by CD56dim NK cells over time. We investigated the frequency, phenotype and functional capacity of NK cells based on NKG2A and KIR expression during acute and chronic HIV-1 infection.

**Methods:** NK cell phenotypic changes were characterized on PBMCs from individuals with acute (n=12), progressive (n=12) and treated HIV-1 clade C infection (n=12) versus healthy donors (n=12) using: NKG2A, KIR and CD57. The cytolytic potential of individual NK cell subsets was determined by CD107a, MIP1β, IFN-γ and TNF-α expression using multiparameter flow cytometry following stimulation with K562 and 721.221 cells.

**Results:** The overall frequency of NKG2A and KIR-expressing NK cell subsets was similar in all studied groups; the KIR+NKG2Aneg were the most dominant subset while KIR+NKG2A+ was significantly less frequent (P<0.0001). Although alterations were noted in the KIRnegNKG2A+ and KIR+NKG2A+ NK cell subsets in individuals with progressive HIV-1 infection, these differences were not statistically significant. The KIR+ NK cell subsets (KIR+NKG2A+ and KIR+NKG2Aneg) exhibited high CD57 expression across all studied groups compared to subsets lacking KIR. The absence of NKG2A in KIR+NKG2Aneg and KIRnegNKG2Aneg NK cell subsets was accompanied by a lower cytolytic activity of NK cells in all studied groups (P<0.0001). No significant differences were noted in differentiation phenotype and function between the individual groups of HIV-1-infected individuals.

**Conclusion:** In line with previous studies (Beziat et al, 2010), the KIR+NKG2Aneg NK cells were more terminally differentiated as shown by higher expression of CD57, however exhibited lower cytolytic activity compared to other NK cell subsets.
Background: HIV-1 infection induces both T cell activation and inflammatory cytokine production, and inflammation is an important driver of disease progression. We sought to characterize the multivariate patterns of cytokine production associated with viral load and to identify cellular sources of the relevant cytokines.

Methods: Plasma samples from patients with early HIV-1 infection and patients enrolled in a single arm trial of structured treatment interruption (STI) were analyzed for levels of 19 cytokines. To identify cellular sources of these cytokines and specific upstream stimuli, PBMCs from uninfected individuals were incubated with either live HIV-1, adriothiol inactivated HIV-1 (AT-2), the TLR7/8 agonist CL097, or the HIV-derived TLR7/8 agonist ssRNAaGag1166 and analyzed by intracellular cytokine staining or by multiplex analysis of culture supernatants. A TLR7/9 antagonist was added to cultures to assess the role of this pathway in production of relevant cytokines.

Results: Plasma concentrations of IP-10 were most strongly associated with HIV-1 viral load ($p<0.0001$ for STI group, Bonferroni correction threshold of significance $p \leq 0.0026$). Partial least squares regression (PLSR) of all the plasma cytokine levels also identified IP-10. In vitro stimulation and intracellular cytokine staining identified monocytes and mDCs as the dominant source of HIV-1 induced IP-10. Partial least squares discriminant analysis (PLSDA) identified distinct cytokine/chemokine patterns in culture supernatants associated with whole virus versus TLR7/8 stimulation (IP-10, IL-2, IL-4, IL-13 and IL-5). TLR7/9 blockade abrogated HIV-1 induced IP-10 in our in vitro culture system.

Conclusion: Among the inflammatory cytokines induced by HIV-1, the strongest individual predictor of viral load was IP-10, although a model integrating multiple cytokines via PLSR had superior performance. In vitro stimulation with HIV-1 and TLR7/8 ligands induced production of IP-10 predominantly from monocytes and mDCs, and a TLR7/9 antagonist significantly blocked IP-10 production. These data suggest that HIV-1-mediated stimulation through TLR7/9 pathways may drive IP-10 production in viremic infection.
Background: Accumulating evidence suggest an HLA-dependent protective effect of certain Killer-cell immune-globulin like receptors (KIRs) in the course of HIV-1 infection. However, the mechanisms underlying these effects in individuals with protective KIR/HLA haplotypes still remain elusive. Licensing of KIR-expressing NK cells in the presence of their cognate HLA ligands might play a role in the control of viral replication through more rapid and efficient NK cell responses. To investigate this hypothesis, we analyzed the response of KIR2DL1/L3+ NK cell subsets in association with cognate HLA-C haplotypes in HIV-1 infected individuals and HIV-1 negative controls.

Methods: For this study 24 HLA-Bw4(-), KIR2DL1/L3(+) individuals with primary HIV-1 infection and 18 HIV-negative subjects were enrolled. NK cell responses and frequency of KIR expression were measured by flow cytometry. NK cell responses were defined by levels of degranulation and production of IFN-γ and TNF-α (p=0.03 as compared to KIR2DL1(-) NK cells). The proportion of KIR2DL1+ NK cells was not increased in the presence of HLA-C1(+) haplotypes (p=0.48). Of note, within HLA-C group 2(+) individuals levels of degranulation and IFN-γ production of KIR2DL1(+)- NK cells did not differ from KIR2DL1(-) NK cells. However, we observed a significantly increased frequency of polyfunctional KIR2DL1+ NK cells as determined by simultaneous measurement of degranulation and production of IFN-γ and TNF-α (p=0.03 as compared to KIR2DL1(-) NK cells).

Conclusion: Our results indicate a HLA-C group 2 haplotype-dependent preferential expansion of NK cells expressing the inhibitory receptor KIR2DL1 during primary HIV-1 infection. This expansion seems to be accompanied by an increase frequency of polyfunctional KIR2DL1+ NK cells. Both observed effects might be explained by more efficient licensing of KIR2DL1+ NK cells in HLA-C2+ carriers.
Elucidating NK Cell-Mediated Lysis of HIV-1 Primary Infected Cells by Antibodies

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Background: Increasing evidence support a role of inhibitory activities beyond neutralization in the control of HIV-1 infection by antibodies (Abs). Antibody-dependent cellular cytotoxicity (ADCC) has been proposed as a possible mechanism of protection in several vaccination trials. ADCC Abs bridge infected target cells with FcγR bearing cells, such as Natural Killer (NK) cells. This binding results in cell lysis, besides direct lysis as part of innate NK cell-mediated mechanism. Currently, several in vitro assays are available to detect such activity. However, there is no protocol worldwide accepted. In this study, the antiviral contribution of ADCC was investigated using physiologically relevant conditions on primary HIV-1 infected CD4T cells and using autologous primary NK cells.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque centrifugation. NK cells and CD4 T-lymphocytes were purified from PBMC by magnetic positive selection. The contribution of NK cells in elimination of HIV-1 infected cells was detected by intracellular viral p24 staining. NK functional activity was evaluated by flow cytometry by detection of CD107a expression.

Results: We found that effector NK cells display potent reduction of HIV-1 infected target cells in the absence and in the presence of Abs (monoclonal and polyclonal samples). Interestingly, cell lysis did not correlate with NK CD107a expression levels, a commonly used surrogate marker of ADCC activity in vitro.

Conclusion: Detection of ADCC under physiologically relevant conditions with primary cells, support the potential in vivo role of this inhibitory mechanism and highlight the necessity to compare the outcome of currently used ADCC assays.
**P08.01**

Env Binding to the α4β7 Integrin Complex Is Not a General Property of Most HIV-1 Variants

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**Background:** HIV-1 gp120 has been reported to bind and signal through α4β7 by means of a tripeptide motif in the V2 loop that mimics structures present in the natural ligands of α4β7. These findings suggested that α4β7 may facilitate selective infection of CD4+ T cells in the GALT, and could contribute to the massive depletion of T cells from this tissue in HIV-infected individuals. Furthermore, the immune-correlates in the RV144 vaccine trial have generated the hypothesis that V1V2 antibodies to an epitope near the putative α4β7 binding properties of most HIV-1 glycoproteins by flow based methods.

**Results:** The natural α4β7 ligand, MAdCAM-1, bound efficiently to RA-activated human PBMCs and transiently transfected 293T cells expressing the integrin complex. α4β7 binding properties of 12 HIV-1 glycoproteins were analyzed using linear mixed effect models over 2-39 months post infection and compared to viral load and CD4 T cell counts. There were no differences in binding IgG titres between TFV and placebo arms. We hypothesized that preservation of CD4 T cells could augment B cell function resulting in higher levels of HIV-specific IgGs in the blood and genital tract of women in the TFV arm. The correlations between HIV-specific IgGs in blood and cervicovaginal lavage (CVL) for gp70_B.CaseAV1_V2, BioV3.C, gp41 immunodominant epitope, 1086 trimer, C.conEnv03 gp140, Con6 gp120/B, ConS gp140 CFI, p24 & p66 RT) was measured by Luminex. IgG titres in the two compartments were analyzed using linear mixed effect models over 2-39 months post infection and compared to viral load and CD4 T cell counts.

**Results:** There were no differences in binding IgG titres between TFV and placebo in either blood or CVL to any of the 10 antigens. Lower CD4 counts were associated with higher Env-specific IgG titres (all p<0.005). Higher viral loads were associated with higher gp41 and gp41 immunodominant epitope titres regardless of study arm (both p<0.05). Notably, blood titres for 9 of 10 HIV antibody specificities positively predicted the similar specificity in matching CVL for gp70_B.CaseAV1_V2 (β=0.21; R=0.27; p=0.05), p24 (β=0.5169; R=0.63), p66 (β=0.44; R=0.43), 1086 Trimer (β=0.3; R=0.45), gp41 immunodominant epitope (β=0.37; R=0.41), Con5gp140 CFI (β=0.25; R=0.36), C.conEnv03gp140 (β=0.23; R=0.44), Con6 gp120/B (β=0.27; R=0.40) and BioV3.C (β=0.42; R=0.58) (all p<0.001).

**Conclusion:** Preserved CD4 T cell responses consequent to TFV use did not impact on binding IgG responses in women who became infected. In contrast, markers of disease progression were associated with higher systemic HIV-specific IgGs suggesting an immunopathogenic effect. The correlations between HIV-specific IgGs in blood and blood implied plasma IgG mucosal transudation.
**P08.03**

**Potent Neutralizing Antibodies Can Be Recovered from the Genital Tract Using the Non-invasive Softcup® Technique**

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**Background:** The genital tract is an important site to test for HIV-specific responses during natural infection and in vaccine trial settings, as it is the main point of entry during HIV infection. Mucosal sampling techniques continue to be investigated and optimised. Our previous results showed that IgG antibody yields obtained from cervicovaginal lavages (CVL) were very low due to the high dilution factor. Here we tested Softcup® specimens with undiluted genital secretions to determine whether the Softcup® technique could be implemented as a method for collecting antibodies to assess neutralization of HIV.

**Methods:** Softcup® samples were obtained from 9 women with known neutralization breadth in the CAPRISA 002 Acute Infection cohort during routine follow-up visits. The softcup was inserted at the beginning of the visit and was removed after two hours. IgG antibodies were isolated from the genital secretions by Protein G columns. Total and HIV-specific antibodies were quantified by ELISA. Neutralization assays were performed in TZM-bl cells using Env-pseudotyped wild type and mutated viruses for epitope mapping.

**Results:** The average collection time was 150 minutes (range: 95-185 minutes) and fluid phase secretions obtained per Softcup® ranged from 30ul to 135ul. The total IgG yields from the Softcup® collection method were about 3 fold higher than those from 1.8ml of CVL (p=0.0029) enabling us to perform neutralization assays against more viruses than possible with CVL. Purified IgG exhibited potent neutralization of SF162 (Tier 1) and at least three Tier 2 viruses for each participant. Epitope mapping suggested that mucosal IgG specificities mirrored matched plasma samples.

**Conclusion:** The Softcup® method enabled us to isolate high yields of potent HIV-specific neutralizing IgG antibodies from genital secretions. Therefore, the Softcup® technique could be employed as an efficient, non-invasive method for mucosal sampling and facilitate the mapping of neutralizing antibody specificities in vaccine trials.

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**P08.04**

**Adenovirus Vector-Based SIV Vaccines Induce Durable SIV-Specific CD4+ and CD8+ T Cells in the Foreskin in Rhesus Monkeys**

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**Background:** Foreskin is the principal site of HIV infection in men. However, little is known about HIV-specific immune responses in the foreskin. Using the rhesus monkey model, we investigated whether SIV-specific T cells traffic to the foreskin following systemic vaccination and infection, and we compared the phenotype and activation status of foreskin and peripheral blood T cells.

**Methods:** 40 adult male Indian origin rhesus monkeys (Macaca mulatta) were used in the study. Animals were immunized with adenovirus (Ad) serotype 35 and 26 vectors expressing SIV-Gag, Pol and Env immunogens. Other animals were infected with SIVsmE660 or SHIV-SF162P3 or served as non-immunized/non-infected controls. Paired peripheral blood and foreskin specimens were analyzed for SIV-specific CD4+ and CD8+ T cell responses, T cell phenotype, and activation status using intracellular cytokine staining and mult parameter flow cytometry.

**Results:** Immunization with Ad35/Ad26-based SIV vaccines elicited durable SIV-specific CD4+ and CD8+ T cells in the foreskin that were detectable beyond 1 year following vaccination. These SIV-specific T cells in the foreskin were at higher magnitudes than those in peripheral blood (0.21% vs. 0.03% for foreskin vs. peripheral blood CD8+ T cells [n=14], p<0.001), were also detectable in the foreskin of SIV/SHIV infected animals (0.26% vs. 0.08% for foreskin vs. peripheral blood CD8+ T cells [n=22], p<0.01) and secreted both IFN-γ and TNF-α. CD4+ and CD8+ T cells in the foreskin were primarily transitional and effector memory phenotype, expressing high levels of the activation markers CD69, HLA-DR and CCR5. No increased activation of foreskin CD4+ T cells was observed in vaccinated monkeys compared with controls.

**Conclusion:** Ad35/Ad26-based SIV vaccines elicited durable SIV-specific CD4+ and CD8+ T cell responses in the foreskin that were detected for more than 1 year following vaccination. These findings suggest that systemic SIV/HIV vaccination strategies can elicit potentially important SIV/HIV-specific immune responses in foreskin.


**P08.05**

**Inflammation in the Male Genital Tract: CMV and HIV Shedding**

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**Background:** In the male genital tract, reactivated replicating CMV increases HIV shedding in semen, target cell activation and seminal concentrations of pro-inflammatory mediators such as MCP-1, RANTES and IL-8. The impact of HIV and CMV co-infections underscores the need for broadening HIV treatment strategies to include CMV treatment.

We investigated the impact of seminal CMV infection in HIV-infected and uninfected men on inflammatory biomarkers present in semen.

**Methods:** CMV viral loads were measured in the semen of 38 HIV-infected and 20 HIV-uninfected men. Concentrations of 20 inflammatory and regulatory cytokines were measured in seminal plasma by Luminex. The relationship between CMV shedding in the seminal compartment and inflammation was investigated using multivariate regression analysis.

**Results:** We found that 13/25 (52%) of HIV-infected ARV naïve, and 6/20 (30%) of HIV-uninfected men were shedding CMV in their semen. HIV plasma viral loads were significantly correlated with seminal CMV viral loads. In semen, HIV viral loads were significantly higher in semen samples in which CMV was detectable compared to samples in which CMV was not detectable. IL-15 and IL-7 was weakly associated with CMV viral loads in the semen of HIV-infected men, and the IL-7 association was upheld after controlling for the effect of both seminal HIV and plasma viral loads. In semen, HIV viral loads were significantly higher in seminal CMV infection. Therefore, CMV shedding in the semen of HIV-infected men may increase the risk of secondary transmission and in uninfected men, may put men at risk for HIV acquisition.

**Conclusion:**

Increases of seminal IL-15 and IL-7, both drivers of CD4 T cell homeostasis, may elevate levels of HIV target cells in the genital tract of men by contributing to T cell proliferation. This may be due to HIV-induced depleted seminal target cell levels and/or CMV co-infection. Therefore, CMV shedding in the semen of HIV-infected men may increase the risk of secondary transmission and in uninfected men, may put men at risk for HIV acquisition.

**P08.06**

**Levels of HIV gp120-Specific Binding Antibodies in the Female Genital Tract Are Correlated with Genital Inflammation**

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**Background:** Genital inflammation has been implicated in recruitment of B and T cells to the genital mucosa and may play a role in the availability of total and HIV-specific antibodies present in genital secretions.

**Methods:** We investigated the longitudinal relationship between genital inflammation and HIV gp120-specific antibodies present in genital secretions from women in the CAPRISA002 Acute Infection Study. A total of 40 consenting women, recently infected with HIV-1, were included. Cervico-vaginal lavages (CVLs) were available from 24/40 women 7 months post-infection, 26/40 at 12 months post-infection, with 10/40 women having samples available at both time points. Twenty one cytokines were measured in CVLs using a high-sensitivity and human cytokine Milliplex kits including IL-1β, IL-6, Eotaxin, MCP-1, MIPα, RANTES, IL-8, IL-2, IL-15, G-CSF, GM-CSF, TNF-α, sCD40L and IFN-γ. The concentrations of total IgG, total IgA and IgG gp120 were determined by ELISA.

**Results:** In HIV-infected women, the concentration of total IgG in CVL was 80-3000 times higher than IgA. There was a significant positive correlation between the concentration of total IgG in CVL and the amount of HIV-specific gp120 which was seen at both months 7 and 12. Women with the highest antibody titres at one time point tended to have the highest titres at the next time point. Total IgG in CVL was significantly positively correlated with 11/21 genital tract cytokines measured (IL-1β, IL-6, Eotaxin, MCP-1, MIPα, RANTES, IL-8, IL-2, IL-15, G-CSF and GM-CSF). Similarly, concentrations of HIV gp120 binding IgG were correlated with concentrations of 9/21 (IL-6, TNF-α, MCP-1, MIP-1α, RANTES, sCD40L, IFN-γ, IL-15, and GM-CSF).

**Conclusion:** This relationship between inflammatory cytokines and HIV-specific antibody detection in the genital tract needs attention since the production of mucosal antibodies in response to a vaccine is desirable; however these cytokines are also chemo-attractant for HIV target cells and could potentially increase the risk of infection.
P08.07

Prime Boost Mucosal HIV Vaccine Using Recombinant Influenza Virus Vector Stimulated Specific and Mucosal CD8+ T Cell Immune Response in BALB/C Mice

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Background: Most HIV vaccine candidates to date have failed to elicit effective immune responses that are necessary to control HIV infection. The results of a promising phase III trial conducted in Thailand using a recombinant canary pox vector vaccine (ALVAC) expressing HIV Gag in combination with recombinant HIV-Env glycoprotein gp120 (AIDSvAX), showed 31.2% efficacy in humans and raised the prospect of a protective vaccine. The most recommended HIV vaccines are focusing to induce specific CD8+ T as a critical to control progression of HIV virus.

Methods: This study project used influenza viruses as mucosal live vaccine vector to stimulate effective CD8+ T cell immunity. Recombinant influenza A viruses, H3N2 (HK-x31) and H1N1 (A/PR8/8/34) expressing defined mouse HIV-1 CD8+ T cell epitopes (H-2Kd Gag197-205 and H-2Kd Tat17-25) in the neuraminidase (NA) stalk were generated using reverse genetics and administered as a prime boost vaccine within different program of vaccination, intranasal-intranasal, intravaginal-intranasal, intranasal-intravaginal and intravaginal-intranasal vaccination in BALB/C mice. Following those prime-boost vaccinations, tetramer and intracellular cytokine staining assays used for detection of specific CD8+ T cell immune response in addition, mucosal HIV-specific CD8+ T cells were detected using antibodies directed against specific integrins (LPAM-1, CD103 and CD44).

Results: Our result showed comparable HIV and endogenous influenza specific CD8+ T cell responses following intranasally-intranasally prime boost vaccination in harvested lymphoid tissues. Also, intranasally-intranasally prime boost expressed mucosal surface integrins of local and distal lymph nodes higher than the levels observed following intravaginal vaccination.

Conclusion: The route of vaccination using mucosal viral vaccine vector in BALB/C mice has an important role in stimulation both specific and mucosal CD8+ T cells within a high level and these cells may be important for migration of mucosal specific CD8+ T cells given the mucosal acquisition of HIV infection and control of HIV infection.

P08.08

HIV-1 Transfer from Mucosal Dendritic Cells and Macrophages to T Lymphocytes, and Inhibition by Antibodies

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Background: Following sexual transmission, Antigen Presenting Cells (APCs) in mucosal sites are considered as the first cells encountered by HIV. Their crosstalk with CD4 T lymphocytes favor efficient HIV cell-to-cell transfer, leading to rapid viral dissemination. However, most HIV transfer studies involve monocyte derived dendritic cells displaying characteristic distinct from those of mucosa residing dendritic cells (DCs). The aim of this study was to define the respective role in HIV transfer of various DCs subsets and macrophages present at the mucosal site. The potential inhibition of HIV transfer by antibodies was determined.

Methods: Langerhans and Interstitial DCs (LCs/IDCs), purified plasmacytoid DCs (pDCs) or Monocyte Derived Macrophages (MDMs) were incubated with primary HIV-1 during 2 hours before addition of autologous activated CD4-T lymphocytes. Different inhibitors were added in the cocultures: fusion inhibitor T20 to analyze the percentage of virus fused to APCs; Indinavir to define the percentage of trans-infection; and neutralizing antibodies to determine their inhibitory potential. After 48 to 72 hours, the percentage of infected cells was quantified by intracellular p24 staining and flow cytometry analysis.

Results: We found that LCs/IDCs, pDC and MDMs efficiently transferred HIV particles to CD4-T lymphocytes. Interestingly, the kinetic of HIV transfer was more rapid in MDMs compared to DCs subsets. Moreover, MDMs transferred HIV preferentially in trans whereas LCs/IDCs mainly in cis. Anti-gp120 neutralizing antibodies (2G12, VRC01, b12) were able to block viral transfer to T cells. Noteworthy, this inhibition was 1000 fold more efficient in MDMs than in DCs cocultures.

Conclusion: Altogether, these results highlight the important contribution of different HIV mucosal target cells and emphasize the role of macrophages in the rapid and efficient replication and transmission to T-lymphocytes. They demonstrate that the inhibition of cell-to-cell transfer by neutralizing antibodies involves different mechanisms of inhibition depending on the type of APCs targeted.
**P08.09**

**CCL19 and CCL28 Augment Immune Responses to HIV-1 gp140 by Mobilizing Responsive Immunocytes into Secondary Lymph Nodes and Mucosal Tissue**


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**Background:** Induction of broad and potent neutralizing antibodies at the mucosal portals of entry remains a primary goal for most vaccines against mucosally acquired viral infections. Selection of appropriate adjuvants capable of promoting both systemic and mucosal responses will be crucial for the development of effective immunization strategies. Given the central roles of APRIL, CCL19 and CCL28 in B cell maturation or T/DC/IgA-ASC migration; we asked whether plasmid co-delivery of CCL19, CCL28 or APRIL can enhance antigen-induced immune responses to HIV-1.  

**Methods:** Using candidate HIV-1 plasmid DNA (p-gp140) and gp140 recombinant protein (rec-gp140) as immunogens, we investigated the immunomodulatory roles of plasmid co-delivery of pAPRIL, pCCL19 and pCCL28 in mice when administered by intramuscular and intranasal routes of administration.  

**Results:** We demonstrate that pCCL19 and pCCL28, but not pAPRIL, significantly enhance antigen-specific responses. gp140-specific antibodies in serum enhanced by pCCL19 or pCCL28 were broadly distributed across all four IgG subclasses of which IgG1 was predominant. The enhanced systemic and mucosal antibodies showed increased neutralizing activity against both homologous and heterologous HIV-1, where potency correlated with gp140-specific serum IgG and vaginal IgA levels. Measurement of gp140-specific cytokines produced by splenocytes demonstrated that pCCL19 and pCCL28 augmented balanced Th1/Th2 responses. pCCL19 and pCCL28 also elevated IgA+ cells in colorectal mucosal tissue. pCCL19 co-delivery resulted in an increase of CCR7+ CD11c+ cells in mesenteric lymph nodes (MLNs) and both CCR7+ CD11c+ and CCR7+ CD3ε+ cells in spleen, whereas pCCL28 co-delivery resulted in an augment of CCR10+ CD19+ cells in both spleen and MLNs.  

**Conclusion:** Our findings indicate that pCCL19 and pCCL28 can enhance HIV-1 Env-specific systemic and mucosal antibody responses as well as T cell responses. Such enhancements appear to be associated with mobilization of responsive immunocytes into secondary lymphoid organs and mucosal tissues through interactions with corresponding receptors.

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**P08.10**

**Vaccine-Induced Intestinal and Salivary IgA Correlates with Reduced SIV Viremia in Orally-Challenged Neonatal Macaques**


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**Background:** Vertical transmission of HIV in breast milk remains a primary route of pediatric infections. While serum antibodies are critical for elimination of HIV within the host, the importance of mucosal IgA in preventing oral transmission is not known. Neonates produce less IgA than adults and are thus more susceptible to oral pathogens. Here we tested whether an oral pediatric SIV vaccine could induce SIV envelope (Env)-specific salivary and intestinal IgA, and protect against oral SIV acquisition.  

**Methods:** Rhesus macaques were immunized orally at birth with a live attenuated Mycobacterium tuberculosis strain engineered to express SIV Gag and SIV Env, and twice boosted with MVAgpe. Nine weeks after birth, the infants were orally challenged using a weekly regimen of low-dose SIVmac251 (5000 TCID50).  

**Results:** All vaccinated infants had developed SIV-specific T cell responses in blood and tissues at the time of challenge. In addition to the induction of SIV-specific plasma IgG and IgA antibodies in all animals, salivary and intestinal SIV-specific antibodies were detected in 8 of 8 and 3 of 8 vaccinated animals, respectively, prior to challenge. Although vaccination did not prevent infection, vaccinated infants (n=3) with intestinal and salivary IgA at the time of challenge had significantly lower peak and set-point viremia compared to the other vaccinated animals (P=0.0154 and P=0.0357, respectively). In fact, there was an inverse correlation between peak viremia and SIV Env-specific IgA in fecal extracts (P=0.0019) and saliva (P=0.034). Serum IgG and IgA antibodies to SIV Env were also induced by vaccination, but these were not associated with viremia.  

**Conclusion:** Thus, mucosal, but not systemic, antibodies may have contributed to control of virus replication. Vaccine strategies that promote the development of mucosal IgA antibodies may improve the efficacy of pediatric vaccines to prevent oral HIV acquisition.  

Funding by: R01 DE019064, R01DE022287 (KA) and HHSN261200800001E (MP, JL).
Background: The B cell population in the gut, the largest lymphoid organ in human body, has been largely overlooked in previous immunological studies. The objective of our study was to characterize the phenotypes of B cell subsets present in human gut and to compare their frequencies with those found in blood of healthy and HIV-1-infected individuals.

Methods: Peripheral blood mononuclear cells (PBMC) and mucosal mononuclear cells (MMC) were isolated from blood and colon biopsy samples, respectively, from individuals recruited by the UCLA CFAR Mucosal Immunology Core Laboratory (MICL). B cell subsets were analyzed utilizing a 10-color flow cytometry panel. The reliability of the panel was established comparing the measurements obtained from five healthy low-risk individuals in two visits two weeks apart.

Results: B cells were defined as CD3-CD16-CD19+, and the B cells subsets as: naïve (CD27- IgD+, memory (CD27+), resting memory (CD27+CD21high), plasmablasts (CD27highCD38high), plasma cells (CD19+-CD27highCD20-CD21-), and exhausted B cells (CD20+CD21lowCD27+). In healthy individuals, we found that mucosal gut samples had fewer naïve cells, more memory cells, fewer resting memory B cells, more plasmablasts and more plasma cells than blood samples. In HIV infected individuals, we found an increase in the percentages of plasmablasts and plasma cells in the gut as compared to healthy individuals. We also observed an increase in the proportion of exhausted B cells in the both the blood and gut of HIV positive individuals. Resting memory cell subsets in the gut of HIV-1 seropositive subjects were almost nonexistent. HIV infection affected neither the circulating resting memory B cells nor the percentages of naïve B cells in blood and gut.

Conclusion: The observed B cell subset distribution was consistent with the high level of antigen exposure and the pro-inflammatory nature of the gut mucosa. In HIV-1-infected individuals, the gut mucosa was characterized by an increase in activated and terminally differentiated B cells.

B-Cell Subset Phenotypes in Human Blood and Gut Mucosa in HIV-1 Infection

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Background: A major impediment to designing HIV vaccines/microbicides to elicit protective mucosal immunity is a lack of understanding of what immunological patterns predict HIV susceptibility. This is likely a function of the general complexity of the immune system. Approaches to date have studied individual or few components in isolation, thus limiting the ability to make associations or predictions on disease outcome. Therefore new approaches are critically needed. Multivariate computational modeling, utilizing comprehensive high-throughput datasets, is fast emerging as an effective method to unravel complex immunological systems. Partial least squares determinant analysis (PLSDA) is one such technique to determine patterns of features that best distinguish groups. Here we explore the utility of this approach using bacterial vaginosis as a surrogate mucosal condition.

Methods: Cervicovaginal lavage samples from BV+ women (n=9), BV- women (n=33), and trichomoniasis-infected (Trc) controls (n=6) were analyzed by LC-LC-MS/MS. Features (classifiers) were identified by PLSDA using Matlab software.

Results: Over 500 unique proteins were identified, and PLSDA analysis indicated distinct patterns of protein expression associated with BV+, BV-, and Trc+ samples. Latent variable loadings of the model identified a pattern of ~30 specific proteins associated with BV+, and ~10 with Trc+ samples. Multivariate analysis was required to discern these patterns. PLSDA model performed with 92% accuracy in predicting BV+ samples from controls on calibration and 78% accuracy on cross-validation.

Conclusion: This demonstrates that mucosal proteomic datasets, parsed with multivariate analysis can distinguish a mucosal clinical condition with high accuracy. Our hypothesis is that this approach could identify protein expression profiles associated with increased risk of HIV acquisition to contribute to a predictive model. This could be applied to vaccine/microbicide endpoints for efficacy and potential insight into mechanisms of protection.

Utilizing Data-Driven Modeling and Proteomic Approaches for Predicting Mucosal Immunity of the Female Genital Tract

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P08.13

Use of Tissue Explants to Evaluate Mucosal Immune Responses in Non-Human Primates (NHPs) Vaccinated with ALVAC/AIDSVAX B/E

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Background: The STEP and RV144 trials have revealed the need to further improve the NHP model in order to establish better correlation between NHPs and humans studies. This pilot study aimed to use NHP cervicovaginal and colorectal tissue explants to assess B cell responses elicited upon vaccination with the same regimen of ALVAC/AIDSVAX B/E used in RV144.

Methods: Six Rhesus macaques were immunized at weeks 0 and 4 with ALVAC and at weeks 12 and 24 with ALVAC/AIDSVAX B/E. Colorectal and cervicovaginal biopsies were obtained four weeks pre-vaccination, and two weeks after each immunization. Serum was collected at the same time points and one week after each shot. Biopsies were cultured and not after ALVAC alone. Specific IgG/IgA were detected in serum at the baseline, a significant decrease in IL-2, GM-CSF, IL-12, IL-13, IL-15, cytokine profile following each immunization with pre-immunization cytokines were assessed by Luminex at the same time points after ALVAC/AIDSVAX boosts. IgG/IgA in culture supernatant of cervicovaginal and colorectal explants progressively decreased during the 15 days of explant culture. When comparing the cytokine profile following each immunization with pre-immunization baseline, a significant decrease in IL-2, GM-CSF, IL-12, IL-13, IL-15, IL-17, MIP-1α, MIP-1β and TGF-α was observed at both mucosal sites after introduction of the ALVAC/AIDSVAX combination.

Conclusion: Immunization with ALVAC/AIDSVAX B/E elicited specific anti-gp120 HIV-1CM244 IgG and IgA systemically and mucosally. Cytokine secretion was affected at both mucosal sites, which could represent a biosignature of the vaccine combination. Ex vivo mucosal models allow assessment of mucosal B cell responses to vaccination, improving the NHP model and providing key information for subsequent clinical trials.

P08.14

Intestinal Commensal Bacteria Shape the Pre-transmission Antibody Repertoire to HIV-1 Infection


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Background: The initial HIV-1 antibody response in plasma and mucosal sites is non-neutralizing and targeted to Env gp41. The blood plasma cell (PC) response to gp41 derives from a polyreactive memory B cell pool of gut flora-reactive B cells (Liao et al. J. Exp. Med. 208: 2237 (2011)). These data gave rise to the hypothesis that a pre-transmission commensal bacterial antibody response that cross-reacts with HIV-1 antigens may shape the B cell response to HIV-1. Here we study the repertoire of HIV-1 antibodies induced in terminal ileum and ask if HIV-1 antibodies are cross-reactive with commensal bacteria.

Methods: Terminal ileum antibodies from individuals early in HIV-1 infection and chronically HIV-1-infected were isolated by reverse transcriptase/polymerase chain reaction from sorted single plasma and memory B cells, and evaluated for the reactivity to HIV-1 and gut flora whole cell lysates (WCL).

Results: Of 412 mAbs isolated from terminal ileum, 19 (5%) were HIV-1-reactive. Of the 19 HIV-1+ Abs, 14 were gp41-reactive, and of these 11 (79%) were cross-reactive with gut flora WCL. Analysis by 2237 (2011)). These data gave rise to the hypothesis that a pre-existing antibody response that cross-reacts with HIV-1 antigens may shape the B cell response to HIV-1. Here we study the repertoire of HIV-1 antibodies induced in terminal ileum and ask if HIV-1 antibodies are cross-reactive with commensal bacteria.

Conclusion: The isolation of clonal lineages shared by terminal ileum and blood that cross-react with commensal bacterial antigens and Env gp41 is evidence for the hypothesis that the initial non-neutralizing response to gp41 is a result of HIV-1 gp41 triggering of pre-existing gut flora-reactive B cells. These data similarly suggest that pre-existing commensal bacterial-reactive B cells may also be involved in the response to HIV-1 gp41-containing vaccine candidates.
P08.15

The Inner Foreskin of Healthy Men Resembles Inflamed Epithelium and Has Features of a Compromised Skin Barrier


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Background: Circumcision provides partial protection against multiple viral sexually transmitted infections (STIs), but the mechanisms associated with risk are not fully understood. We hypothesized inner and outer foreskin might have distinctly modified epithelium.

Methods: To assess the barrier functions of the foreskin epithelium, we compared the inner and outer foreskin samples collected following elective circumcision using immunofluorescence microscopy, hematoxylin and eosin analysis, as well as quantitation of inflammatory mediators and structural skin proteins using multiple bead array technology.

Results: No evidence of microtrauma was identified in the foreskin of 21 healthy, sexually active men from Lima, Peru (ages 21-29 years) as assessed by signs of keratinocyte activation, fibronectin deposition, or parakeratosis. However, multiple structural differences in the epithelium of the inner and outer foreskin suggested differential permeability and inflammation. The inner foreskin stratum corneum was thinner (median 15.7µm, IQR 12.1-20.9) than the outer (19.2µm, IQR 15.9-23.8) (Wilcoxon, p=0.012), and it thickened as it approached the base of the penis. The prevalence of components of the cornified envelope ( involucrin and keratin 1, 10) was reduced in inner vs outer foreskin suggesting differential barrier functions. Additionally, the distribution of tight junction components claudin 1, claudin 4 and occludin was suggestive of chronic inflammation at the inner foreskin of healthy, sexually active men. Epithelial explant cultures confirmed that the inner foreskin epithelium secreted higher levels of GM-CSF (inner median 2.9ng/ml IQR 0.8-10.4, outer median 0.9ng/ml IQR 0.7-1.6), IP-10 (inner median 9.9ng/ml IQR 2.1-44.0; outer median 2.2ng/ml IQR 1.9-9.7) and RANTES (inner median 16.6ng/ml IQR 6.6-22.9; outer median 7.9ng/ml IQR 4.1-14.4) than the outer foreskin counterparts (p<0.003).

Conclusion: Subclinical changes to the inner foreskin of healthy men might make this site inflamed and more permeable to STIs, potentially explaining the benefits of circumcision for STI prevention.

P08.16

In Men at Risk of HIV Infection, Antibodies Can Reach the Human Foreskin Epidermis at Ratios Similar to Those in Blood

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Background: The characteristics of the humoral response in the human penis have been minimally explored, yet may be critical for protecting insertive men at the sites of sexual exposure to HIV.

Methods: We characterized the humoral responses in foreskin samples collected by circumcision from 17 sexually active 21-29 year old men at risk of HIV infection in Lima, Peru. Dermal and epidermal explants were cultured for 48h and the antibody secretion was quantitated in the supernatants using multiple bead arrays. To compare foreskin to plasma antibody concentrations, we normalized the antibody results highlighting how vaccine design could benefit from characterizing the antibody production at genital sites.

Results: Dermal foreskin IgM (median 0.2), IgG2 (median 0.71), IgA (median 0.42), and IgE (median 0.001), were present at increased concentrations compared to paired blood (median IgM/HSA 0.06, median IgG2/HSA 0.14, median IgA/HSA 0.07, median IgE/HSA 0.00006), suggesting that there is local antibody secretion at the foreskin dermis (Dunn’s test p<0.05). IgG4 and IgE minimally reached the foreskin epidermis (median IgG4/HSA 0.0008, median IgE/HSA 0.000001), suggesting that these isotypes have limited ability to provide protection at this site of HIV exposure (Dunn’s test p<0.01). On the contrary, foreskin epidermis IgM (median 0.04), IgG1 (median 0.45), IgG2 (median 0.31), IgG3 (median 0.02) and IgA (median 0.11) had levels comparable to those of blood (median IgG1/HSA 0.36, median IgG3/HSA 0.02), indicating efficient access of the humoral responses to the area (Dunn’s test p>0.05).

Conclusion: The foreskin harbors antibody-secreting cells at the dermis with efficient antibody penetration to the foreskin epidermis. The results highlight how vaccine design could benefit from characterizing the antibody production at genital sites.
Hormonal Contraception Use and HIV-1 Risk in the Context of Prevention Research: DMPA and NET-EN Dampen Immunity in the Female Genital Tract

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Background: The use of injectable hormone contraceptives (HCs) is common among women who wish to prevent unintended pregnancies, especially in regions with high HIV-1 prevalence such as Southern Africa. Recent observational studies suggest a relationship between the hormonal contraceptive use and increased risk for HIV acquisition. The biological intersection at the mucosa between risk associated with HC use and protection provided by microbicides or vaccines is not understood. Here we investigated the relationship between HC use, genital tract inflammation and tissue inhibitors of metalloproteinases (TIMPs) concentrations in genital fluid collected from HIV-uninfected women at high risk for HIV infection.

Methods: Concentrations of 25 inflammatory, regulatory and hematopoietic cytokines and TIMP-1, TIMP-2, TIMP-3, and TIMP-4 were measured by luminex in cervicovaginal lavage samples (CVLs) from 156 high-risk HIV-uninfected women. Information on HC use was collected by questionnaire and confirmed by high performance liquid chromatography.

Results: Concentrations of 14/25 cytokines measured in CVL (including IL-1α, IL-9, IL-12p40, IL-15, IL-17, MIP-1α, IFNa, Eotaxin, Fractalkine, EGF, FGF2, PDGF-AA and TGF-α and MCP-1) were significantly lower in women using injectable contraceptives compared to those not using HC. While inflammatory cytokines and growth factors appeared to be suppressed in women using HCs, we observed no significant differences in TIMP levels from women using injectable contraceptives compared to non-users.

Conclusion: The data suggest that injectable HCs suppress innate immunity which could potentially result in decreased host resistance to invading pathogens. The immunosuppressive properties of injectable HCs within the female genital tract could modulate the potential efficacy of vaccines and prevention strategies.
**P08.19**

**Intestinal Myeloid DCs Display an Activated Phenotype and Are Less Susceptible to HIV-1 Infection Compared to Blood DCs**

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**Background:** We recently showed that human colonic lamina propria (LP) CD11c+ DC actively shuttle R5 HIV-1 across an intact epithelial barrier and transfer infection to CD4+ T cells. However, the susceptibility of intestinal DC to HIV infection has been poorly investigated, due to difficulties in isolating mucosal DC.

**Methods:** CD11c+ myeloid DC obtained from the colonic LP were further characterized as CD103+ and CX3CR1+ and the expression of HIV-1 receptors analyzed in comparison to blood DCs. Supernatant obtained from an ex vivo culture of healthy human colonic mucosa was used to condition monocyte-derived DC in an in vitro model as to mimic the exposure of DC to intestinal microenvironment. Conditioned-DC (C-DC) were analyzed by flow cytometry for the expression of HIV-1 receptors and activation markers, and incubated in vitro with either R5 or X4 HIV-1 to study their susceptibility to infection.

**Results:** C-DC displayed an activated phenotype, a significant down-regulation of CCR5, CD4 and CX3CR1, an up-regulation of CXCR4 and a moderate modulation of DC-SIGN expression compared to unconditioned DC. No change in the CD103 expression was observed. Interestingly, both R5 and X4 HIV-1 replicated less efficiently in C-DC compared to unconditioned DC. Colonic supernatants contained the CCR5-binding chemokines Mip1α and MCP-1 and the CX3CR1 ligand fractalkine, whereas the CXCR4 ligand SDF-1α was absent. IL-10 and IL-2, described to induce CXCR4 up-regulation on DC, were also detected. Thus, this specific intestinal milieu may determine the observed phenotype. Both CX3CR1+ and CD103+ CD11c+ DCs were detected in human colonic LP. Interestingly CD11c+ DC showed lower CCR5 and higher CXCR4 expression compared to blood DC, and a similar activation profile, which confirmed the results obtained after intestinal conditioning.

**Conclusion:** Thus, the intestinal microenvironment module the expression of HIV receptors on DCs and their capability to replicate the virus.

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**P08.20**

**Assessing Differences in HIV and Nanoparticle Transport in Mucus over the Course of the Female Menstrual Cycle**


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**Background:** Mucus barriers offer the first line of defense against pathogen acquisition in the female reproductive tract (FRT). This study aims to determine how cervicovaginal mucus (CVM) changes over the course of the menstrual cycle in individual study participants.

**Methods:** CVM collected 3 times a week over a 6 week period from individual donors using an Instead Softcup worn for 3 hours. Parameters such as pH and blood hormone levels were recorded throughout the study period. The CVM was immediately isolated from the softcup by centrifugation. Multiple strains of fluorescently labeled HIV (Clades B, C, & AE) were individually mixed with fluorescent pegylated polystyrene 119nm beads and introduced into the CVM sample with minimal dilution. Particle transport was determined using fluorescence at a 150ms frame rate for 1 minute collecting 400 total frames. Particle transport was quantified using computer-assisted particle tracking algorithms. The rate of mean square displacement (MSD) and a, the exponent of the dependence of MSD on time-lag was determined for each particle observed.

**Results:** We find that CVM pH remained relatively stable over the in individual donors, averaging around pH4. A pH increase was observed during the time of menstruation to a pH of 7. Particle mobility generally increased in the luteal phase of the menstrual cycle and during menstruation. The differences in mobility between virus strains was not significant.

**Conclusion:** This study reveals changes in the CVM mucosal barrier over the menstrual cycle. These changes identify potential changes that normal hormonal fluctuations may have on the efficiency of HIV acquisition. A better understanding of how the menstrual cycle may influence HIV acquisition by women serves to inform and facilitate the development of an HIV vaccine to decrease HIV acquisition in women.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L2, Room 211 – 212.
Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L2, Room 211 – 212.

**P08.21**

**Qualification of the Particle Diffusion Assay for Single Particle Tracking**

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**Background:** Fluorescent microscopy allows the direct imaging of virus transport (diffusion) in vitro. Shukair et al., 2012 tracked the movement of fluorescent HIV-1 virions in cervicovaginal mucus (CVM), calculated the mean squared displacement (MSD) of these particles, and found that HIV-1 diffusion was significantly hindered compared to controls. Currently, the virion diffusion assay is being used to study the potential interaction of HIV-1 with antibodies in CVM induced by vaccination in a sub-set of volunteers previously enrolled in the RV144 Thai trial who received canarypox vCP1521 and bivalent gp120 subtype B/CRF01_AE (AIDSVAX B/E), who received additional boosts with AIDSVAX B/E (RV305).

**Methods:** To achieve Good Clinical Laboratory Practice (GCLP) for the laboratory assay, standard operating procedures (SOPs) were developed. Assay qualification was conducted jointly at NWU and AFRIMS to ensure the consistency of assay performance and data validity. Assay qualification included intra-assay variability, inter-assay variability and inter-operator variability. Artificial mucus simulant and 200-nm PEGylated beads were utilized as controls and MSD was derived from tracking particles movement for 400 frames per minute. Statistical analysis of $\alpha$, the exponent of the dependence of MSD on time-lag, was conducted to evaluate assay variability.

**Results:** The % Coefficient of variation (CV) of $\alpha$ values found in the intra-assay variability at NWU and AFRIMS were (3.2%-5.9%) and (1.8%-5.0%), respectively. A slight increase of CV was observed in inter-assay variability at NWU (4.9%-6.0%) and AFRIMS (2.4%-4.1%) compared to intra-assay variability. The inter-operator variability CV of all assays conducted by three performers each at NWU and AFRIMS was 7.3% and 2.4%, respectively.

**Conclusion:** The %CV from both NWU and AFRIMS was low and below 25% that was used as acceptable value. The assay was qualified for a clinical trials setting and can now be applied as a new tool to study mucosal immunity.

**P08.22**

**Pharmacologic Expansion of Colonic Mucosal Th17 Cells in Rhesus Macaques**

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**Background:** We previously found that circulating and gut-resident Th17 cells present at the time of SIV infection are associated with lower peak and set-point viral loads (Hartigan-O’Connor et al., Sci Trans Med 4:136ra69, 2012). By screening for untreated macaques with extraordinarily large or small Th17 populations, we demonstrated that natural variability the size of the pre-existing Th17 population is sufficient to partly account for variability in control of SIV infection. We also showed that IL-2 treatment slows growth of Th17 cells in vitro, reduces the size of the Th17 population in vivo, and is associated with higher viral loads.

**Methods:** We assessed the potential of various pharmacologic treatments to expand Th17 cell populations in macaques and to thereby slow disease progression. Drugs assessed include candidate aryl hydrocarbon receptor ligands (FICZ and tranilast), one antibody affecting IL-2 signaling (basiliximab), and immunoglobulin-linked IL-21 cytokine (IL-21-Ig).

**Results:** Data from the drug treatment groups demonstrate considerable heterogeneity in effects on Th17 cells. Most treatments produced either no effect or small, transient effects. Tranilast, however, caused Th17 compartment expansion that was sustained throughout the treatment period. In three out of four cases, the expansion achieved was more than 3-fold over baseline. Expansion of Th17 cells in blood was accompanied by similar expansion in colon tissue and lymph node.

**Conclusion:** These changes will allow testing of the link between Th17 cells and SIV disease progression in future interventional studies.
**P08.23 LB D**

The Nervous System Can Restrict ‘at a Distance’ Langerhans Cell-Mediated HIV-1 Transmission via the Neuropeptide Calcitonin Gene-Related Peptide

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**Background:** Upon its mucosal entry, human immunodeficiency virus type 1 (HIV-1) is internalized by Langerhans cells (LCs) in stratified epithelia and transferred locally to T-cells. In such epithelia, LCs are in direct contact with peripheral neurons secreting calcitonin gene-related peptide (CGRP). Although CGRP has immunomodulatory effects on LC functions, its potential influence on the interactions between LCs and HIV-1 is unknown.

**Methods:** The impact of CGRP on LCs was evaluated in vitro in monocyte-derived-LCs and ex vivo, in mucosal explant tissues. Furthermore, correlation with infection in vivo was performed by measuring CGRP level in plasma of HIV-1-infected individuals, non-treated and ART-treated in comparison with normal individuals, as well as of rhesus monkeys acutely infected with SHIV.

**Results:** We show that CGRP acts via its receptor expressed by LCs and interferes with multiple steps of LC-mediated HIV-1 transmission. CGRP increases langerin expression, decreases selected integrins, and activates NFkB, resulting in decreased HIV-1 intracellular content, limited formation of LC-T-cell conjugates, and elevated secretion of the CCR5-binding-chemokine CCL3/MIP-1α. These mechanisms cooperate to efficiently inhibit HIV-1 transfer from LCs to T-cells and T-cell infection. In vivo, HIV-1 infection decreases CGRP plasma levels in both vaginally SHIV-challenged macaques and HIV-1-infected individuals. CGRP plasma levels return to baseline following highly active anti-retroviral therapy.

**Conclusion:** Our results reveal a novel path by which a peripheral neuropeptide acts at the molecular and cellular levels to limit mucosal HIV-1 transmission, but also provide for the first time the evidence that the nervous system can restrict ‘at a distance’ the early events of HIV-1 transmission at the mucosal level. More, CGRP receptors agonists might be used therapeutically against HIV-1. Novel CGRP receptor agonists, active especially at mucosal epithelia at targeting LCs, might represent a completely new class of anti-HIV-1 molecules.

Ganor et al; J. Exp. Med, 2013 in press

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**P08.24 LB**

Combined Mucosal and Systemic C.1086 Envelope Immunization of Lactating Monkeys Induces Potent IgA and Functional IgG Antibody Responses in Milk


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**Background:** Vaccination of lactating monkeys with a founder HIV Envelope (Env) in an MVA prime/intramuscular (IM) protein boost strategy induced functional IgG responses but poor Env-specific IgA responses in milk, whereas intranasal (IN) protein boost induced strong IgA but poorly functional IgG milk responses. Therefore, we sought to improve Env-specific antibody responses in milk using a combined IN/IM protein boost strategy.

**Methods:** Adjuvanted T/F clade C HIV Env C.1086 was administered simultaneously IN/IM to four hormone-induced lactating rhesus monkeys previously primed with MVA expressing C.1086 gp140 and boosted twice IN with C.1086 gp120. Plasma and milk samples were assessed for HIV Env-binding antibody and neutralization responses in TZM-bl and A3R5 cells. C.1086 Env-specific B cells were isolated from the blood and milk of one vaccinated animal, immunoglobulin genes were amplified by nested RT-PCR, and antibody reactivity was assessed with HIV Env proteins.

**Results:** Following IN/IM boost, plasma Env-specific IgG responses in plasma (median concentration 123 vs 616 mg/ml) and Env-specific IgA responses in milk (median concentration: 1.7 vs 7.6 mg/ml) increased compared to IN only boost. Importantly, whereas no autologous or tier 2 neutralization was detected in plasma or milk following IN only boost, autologous neutralization was detected in all plasma and two of four milk samples following IN/IM boost. Moreover, heterologous tier 2 neutralization in A3R5 cells was detected in the plasma of all vaccinated animals. Most isolated Env-reactive monoclonal antibodies reacted weakly against gp41 (19 in blood and 16 in milk), but three in blood and in milk reacted strongly against gp120. The majority of Env-reactive antibodies were IgG2 isotype, but one gp120-reactive antibody from blood and two from milk were IgA1 isotype.

**Conclusion:** IN/IM boost induces both functional Env-specific IgG responses and potent Env-specific IgA antibodies in breast milk, which may be advantageous for prevention of infant HIV-1 transmission.
P08.25 LB

No Increase in Activated T Cells and Limited Increase in Rectal Mucosa Following HIV-1 DNA/rAd5 Immunization

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Background: HVTN076 is an exploratory HIV vaccine trial designed to examine mucosal immune responses to the multiclade HIV-1 DNA/rAd5 vaccine developed by the NIH Vaccine Research Center, also used in the HVTN505 test-of-concept study.

Methods: Seventeen healthy Ad5 seronegative HIV-1-uninfected adults were enrolled and 16 completed all vaccinations (3xDNA, rAd5, 0, 4, 8, 24 weeks). Mucosal biopsies were obtained from sigmoid colon and rectum by flexible sigmoidoscopy at baseline and one month following rAd5. Rectal biopsies by anoscopy were obtained 2 weeks following the final DNA and 1 week following rAd5. Single-cell suspensions following collagenase digestion were assayed directly for phenotyping and after overnight rest for intracellular cytokine staining.

Results: Although HIV-specific CD4+ and CD8+ T cells were detected in blood at one month post rAd5 (81% and 69% of participants, respectively), these were not detected in colon/rectum samples at any time point. Ad-specific CD4+ T cells were detected at baseline in blood (77%) and colon (87%). Hexon-specific response rate and magnitude increased after vaccination in blood, but this increase was less common in colon/rectum. There was no change in the proportion of CD4+ T cells expressing CCR5 or in activated CD4+ T cells (Ki-67+Bcl-2lo) in the rectum after rAd5 vaccination.

Conclusion: The HIV-1 DNA/rAd5 vaccine regimen did not induce detectable HIV-specific T cells in colon/rectum, a site of HIV exposure where such cells could contribute to immune protection. These cells could be present, but in vitro expansion may be necessary for detection. Pre-existing mucosal Ad-specific T cells were detected in the majority of these Ad5 seronegatives. Activation of these T cells is a concern related to potential enhanced risk of HIV infection following rAd5 vaccination; however, post-vaccination increase in Ad-specific T cells was detected in only a small proportion of vaccine recipients. Vaccine-induced activation of T cells in bulk, at least as measured by Ki-67/Bcl-2, was not detected.

P08.26 LB

Preliminary Evaluation of Mucosal Immune Responses with Mucosal Explants in Humans Vaccinated with ALVAC/AIDSVAX B/E During the Ongoing RV305 Trial

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Background: Analysis of antibodies elicited during RV144 showed absence of neutralizing antibodies despite partial protection. Furthermore, analysis of B-cell responses in clinical trial participants has been limited to biological fluids and not at the site of transmission in mucosal tissues; emphasizing the absence of models for B-cell responses in the field of anti-HIV vaccine. Ex vivo mucosal tissue explants models could provide a surrogate endpoint that might have been predictive of protection/susceptibility in the RV144 study. The ongoing randomized, double blinded, placebo-controlled RV305 trial of late boost with ALVAC and AIDSVAX B/E used alone or in combination, provides a unique opportunity to test this hypothesis in human cervicovaginal and sigmoidal tissue explants.

Methods: Healthy study participants from RV144 were enrolled in RV305 and received at weeks 0 and 24 a vaccination with ALVAC/AIDSVAX B/E (group I), AIDSVAX B/E (group II), ALVAC (group III) or placebo in each group. Sigmoidal and cervicovaginal biopsies were obtained at week 26. Total and specific anti-gp120 HIV-1CM244 IgG and IgA secreted in tissue explant culture supernatant were measured at days 3, 7, 11 and 15 with quantitative ELISA. Limited unblinding was performed only allowing correlation of samples to groups.

Results: Variable levels of total IgG and IgA were measured among participants in all explant cultures. Specific IgG was detected in certain cervicovaginal and sigmoidal tissue explants culture supernatants from groups I and II; and not in group III. No differences were detected in the levels of specific IgA between the three groups in both tissue explant models.

Conclusion: Specific anti-gp120 HIV-1CM244 IgG are elicited mucosally among some RV305 participants who received late boosting with ALVAC/AIDSVAX B/E or AIDSVAX B/E. Hence, ex-vivo mucosal tissue models could represent a new tool to assess mucosal B-cell responses to vaccination during clinical trials.
Selection and Optimization of a Mucosal Sampling Method for Application in Phase 1 Clinical Trials


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Background: An efficacious preventive HIV vaccine will likely require induction of immune responses at mucosal surfaces. One approach is to deliver vaccines intra-nasally. A pilot study was performed in Rwanda and Kenya to determine if anti-HIV antibodies could be detected in nasal secretions collected from the nasal cavity (NC), naso-pharynx (NP) and oral secretions.

Methods: Nasal samples were collected using FloQ Swabs from 35 HIV-seropositive and 35 HIV-seronegative volunteers. Saliva was collected from parotid glands using Salimetrics Oral Swabs, oral fluid (transudate) was collected into Falcon tubes. Eluted samples were tested for gp140 Env (Clade A UG37) and Gag p24 (Clade B LAI) IgG and IgA antibodies.

Results: Volunteers indicated that the NC collection was preferable to the deeper NP sample, suggesting that NC sampling may result in greater compliance with repeated sampling. Anti-HIV antibodies were detected in nasal secretions of 100% of HIV-seropositive samples with IgG expressed at a higher level than IgA. Anti-gp140 IgG and anti-p24 IgG were detected in 100% and 93.4% of nasal samples, respectively. IgA anti-gp140 and anti-p24 IgA were detected in 94% and 88.5% of nasal samples, respectively. No significant differences were detected between NC and NP samples in magnitude or quantity. All nasal samples from HIV-seronegative volunteers were negative for IgG and IgA anti-gp140/p24 except for 4 Rwandan volunteers with low levels of IgA anti-p24.

Conclusion: NP sampling appeared to have little benefit over NC sampling, and HIV antibodies were detected in all HIV-seropositive individuals. NC sampling may provide a unique and tolerable method to collect antibodies in an HIV vaccine trial following intranasal vaccination.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L2, Room 211 – 212.

Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L2, Room 211 – 212.

**P09.01 D**

**Anti-HIV Activity of 5-Hydroxytyrosol, a Microbicidal Candidate**

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**Background:** 5-Hydroxytyrosol (5-HT) is a natural compound that has previously shown biochemical activity against HIV integrase and gp41. In this work we show that 5-HT is able to diminish viral replication in vitro and prevent infection in vivo of humanized mice without toxic effects.

**Methods:** Recombinant viruses carrying luciferase-renilla reporter with different properties (Wild type and R5 and X4 tropic, VSV-pseudotyped HIV or resistant HIV clones) were used to infect cell lines or primary lymphocytes. In vivo experiments were performed using BLT humanized mice challenged with HIV-1 JR-CSF 15 minutes after 5-HT administration.

**Results:** 5-HT inhibited X4, R5 tropic HIV and NNRTI, NRTI and Integrase inhibitors resistant HIV infections with IC50 values between 10 and 80 µM. 5-HT decreased VSV-pseudotyped HIV replication but did not inhibit replication driven by a full-length HIV-DNA clone transfected in lymphoid cells suggesting a pre-transcriptional target independent of viral entry. When antigen presenting cells (APCs) DCSIGN+ were present in cell culture, 5-HT potency was >10 fold higher, which would be essential for its microbicidal activity, since mucosal infection is highly enhanced by APCs. Lastly, humanized mice were pre-treated with 5-HT (0.03%) and 33% protection was reached.

**Conclusion:** Although 5-HT potency is not in the highest range, it is active against HIV in a wide range of situations including resistant viruses and is devoid of toxicity at doses 100 times higher than IC50. In vivo effect showed a level of protection of 33%, although 5-HT dose used was 30 times lower than that of Tenofovir 1% suggesting that higher doses would improve protection. Altogether these results and easy formulation made 5-HT a good microbicidal candidate either alone or in combination with other antiretroviral drugs.

**P09.02 D**

**Neutralization of Diverse HIV Strains by V3 Specific DARPin with Different Structural Preferences in Epitope Recognition**

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**Background:** We previously utilized the DARPin technology, a novel binding protein scaffold, to derive gp120-reactive agents with inhibitory activity and succeeded in selecting a HIV gp120-V3-loop reactive clone, 5m3_D12, with neutralizing activity and a novel, structure-dependent recognition mechanism which overcomes HIV envelope shielding (Mann A, J Virol 2013). Expanding on our previous finding, we sought here to select DARPin specific for subtype B V3 loop utilizing a structurally arrested V3 loop mimetic peptide for selection.

**Methods:** Three selection strategies were explored using a V3 loop mimetic (MN sequence, IV register) and recombinant JR-FL gp120 protein as panning targets and DARPin DNA libraries encoding either two or three internal ankyrin repeats. Obtained DARPin clones were sequenced, mapped for reactivity with diverse V3 mimetics, wt and mutant gp120 binding, antibody competition binding and probed for inhibitory activity using the TZM-bl pseudotype virus inhibition assay. The interaction of DARPins with V3 loop mimetics was analyzed by x-ray structure determination.

**Results:** The mimetic based selection strategies proved successful, yielding V3-specific DARPins which differed substantially in their binding mode and preference for specific V3 structures. Several of the selected V3 specific DARPins displayed a considerable breadth in inhibiting subtype B isolates. Of note, neutralization profiles differed from DARPin 5m3_D12, highlighting the possibility that different structures of the V3 loop are sampled on different viruses.

**Conclusion:** The structure-dependent recognition of the V3 loop DARPin is of high interest, as it may allow generation of a new type of entry inhibitor, which specifically recognizes the native structure of the V3 loop on the trimer. V3 loop mimetics proved to be valuable panning targets, to focus the selection of DARPin clones to the V3 loop, which opens up multiple opportunities for future DARPin selections by using mimetics of diverse HIV envelope regions.
**P09.03**

**Increasing HIV Testing for Prevention: Acceptability of Rapid HIV Test for Self Testing Among MSM from Buenos Aires, Argentina**

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**Background:** Consistent with the incorporation of rapid HIV test in several clinics in Argentina, the objective of this study was to explore the acceptability of a rapid HIV test available over-the-counter for self testing (ST) among men who have sex with men (MSM).

**Methods:** During 2006-2009, 500 MSM was recruited through Respondent Driven Sampling for an HIV prevalence/incidence study. Attitude toward HST was explored among HIV negative MSM (n=440). Data were weighted prior to analyses.

**Results:** Participants reported they were likely to buy ST (73.1%), test themselves frequently (77.1%), and that it would simplify testing (70.8%). Furthermore, 70.4% reported they would probably use it alone, 67.5% would use it with a stable partner, and 56.4% with a friend/partner. Only a minority (21.3%) felt that they would be embarrassed to purchase it at a pharmacy. While a majority acknowledged that ST use would deprive them of receiving counseling (60.6%), 73.5% declared they would go for help if they tested positive; 16.6% said they would think about killing themselves if they tested positive (25% of which reported a previous suicide attempts). In terms of off-label use of ST, 66% would use it before having sex and 55.4% would do it in order to avoid the use of condoms. Those likely to buy a ST were older than those unlikely to buy it (median age, 29 vs. 24 years old, p=0.014). MSM who reported they would feel embarrassed to buy the test (25 vs. 29 years old, p=0.012) and those who think about killing themselves (22 vs. 30 years old, p<0.001) were younger.

**Conclusion:** The high acceptability of an HIV ST found among MSM should be considered, as it can improve early diagnosis and prevention of future transmissions. Suicidal ideation expressed by some participants in case of positive results requires further exploration.

**P09.04**

**Willingness to Use Rectal Microbicides for HIV Prevention Among HIV-Negative Argentinean MSM Differences According to Formulation**

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**Background:** The objective was to explore the willingness to use rectal microbicides for HIV prevention among MSM reporting HIV-negative status.

**Methods:** During 2006-2009 a sample of 500 MSM was recruited through Respondent Driven Sampling for an HIV prevalence/incidence study. Microbicide acceptability was assessed using three 10-point Likert-type questions inquiring whether the respondent would be willing to use a microbice 1) in general, 2) formulated as a suppository, and 3) formulated as a gel/lubricant. Possible responses ranged from 1 (completely unwilling) to 10 (completely willing). Participants underwent a consent process and responded to a survey that included demographic and behavior information. Data was weighted prior to analyses.

**Results:** Among 411 MSM reporting to be HIV negative, median score for microbicide acceptability was 8.0, with greater willingness to use a gel than a suppository (7.0 vs. 5.0). Comparing men who only have sex with men vs. those who also have sex with women and/or trans partners, the former expressed higher acceptability in general (10 vs. 5, p<0.001), for gel (10 vs. 5, p<0.001) and for suppository (8 vs. 2, p<0.001). Acceptability was significantly higher among men with high school level than among those with lower formal education level (in general: 10 vs. 5, p=0.002; gel: 10 vs. 5, p=0.003 and suppository: 7 vs. 2, p=0.001). A trend toward lower acceptability was found among MSM who reported not using condoms during sexual intercourse (including receptive or insertive anal or vaginal sex with men, trans or women) during the past two months. Acceptability was significantly lower among those who do not consider probable the use of a rapid HIV self-test, in case it became available.

**Conclusion:** Results showed high acceptability of microbicides, especially for gel formulation. MSM not willing to use microbicides were also likely to not accept other prevention strategies, like condoms or using rapid self tests prior to intercourse.
**P09.05**

**An Aptamer Neutralizing Diverse HIV-1 subtype C Isolates, Bind to V1/V2 Region of gp120**

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**Background:** Aptamers, which are artificial nucleic acid ligands akin to antibodies in function, represent a new class of molecules that can prevent HIV-1 infection. We previously isolated a group of RNA aptamers against envelope trimer expressed on the surface of pseudotyped HIV-1 Env, to inhibit virus entry. One of these aptamers, called CSIR1.1, significantly neutralized diverse HIV-1 subtype C isolates and bound to gp120 in a manner that competes with antibodies to the CD4 binding epitope. In this study, we mapped the aptamer binding site (“aptatope”) of CSIR1.1 on gp120.

**Methods:** Site directed mutagenesis was used to generate a panel of alanine mutations on gp120 from HIV-1 Env to systematically map the CSIR1.1 aptatope. We used ELISA and luciferase reporter gene assay in TZM-bl to identify residues important for aptamer binding to monomeric gp120 and virus neutralization. Furthermore, we investigated the importance of N-glycans in forming CSIR1.1 aptatope on gp120 by ELISA.

**Results:** Alanine mutation of four residues (K121A, D167A, Y177A, V182A) located on the V1/V2 region of gp120 abrogated both binding and neutralization activity of CSIR1.1. We observed that CSIR1.1 interacted with monomeric gp120 in a similar manner to CD4-IgG. Also, elimination of N-glycan 276 by site directed mutagenesis reduced neutralization activity of CSIR1.1 by 10-fold but did not affect binding to monomeric gp120 bearing the same mutation and to a deglycosylated protein.

**Conclusion:** These results indicated that, CSIR1.1 interacts with residues on V1/V2 loops of gp120 and occlude access to the CD4 binding site. The results also suggest that the 276 N-glycan is important for virus neutralization by CSIR1.1 but not binding to monomeric gp120. This study, gave an insight on the structural interaction between CSIR1.1 and gp120 thereby helping to elucidate the antiviral mechanism of CSIR1.1.

**P09.06**

**ARV Use in HIV-Positive Pregnant Women Who Attend Couples’ Voluntary Counseling and Testing Services in the Copperbelt Province, Zambia**


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**Background:** One quarter to one third of couples presenting at Couples’ Voluntary HIV Counseling and Testing (CVCT) services in Zambian government clinics have at least one HIV+ partner. 96% of pregnant women in the Copperbelt accessed VCT services at antenatal clinics (ANC) and were offered PMTCT regimens if HIV+. Though 16.4% of pregnant women tested at ANC were HIV+, only 69% received the PMTCT regimen. This abstract examines ARV use in HIV+ pregnant women identified at CVCT services in government clinics in the Copperbelt Province.

**Methods:** Zambia Emory HIV Research Project (ZEHRP) has expanded CVCT services between October 2010 – March 2013 in select clinics in Ndola, Kitwe, Chingola and Luanshya districts in the Copperbelt province. HIV results, pregnancy status, ages and previous HIV testing history are recorded anonymously. Couples attending CVCT are provided appropriate referrals within the government clinics, including PMTCT and ART when applicable.

**Results:** During this period, 68,319 couples were tested: 25% of the couples tested were pregnant; 15% of whom included HIV+ women. 30% of the HIV+ pregnant women were in discordant relationships and 75% were not on ARVs. Of all HIV+ pregnant women, 77% had been previously HIV tested but only 24% of them reported ARV use at the time of CVCT.

**Conclusion:** The proportion of pregnant women who test HIV+ is similar in ANC and CVCT services in the Copperbelt. ARV use is low among pregnant women tested at CVCT. Some may not yet have accessed ANC and PMTCT services due to early gestational age, but there are also gaps in comprehension about PMTCT, problems with ARV access, and concerns about stigma. CVCT can strengthen PMTCT programs in Zambian government clinics through male involvement and referral to PMTCT and ANC services.
**P09.07**

**Multilevel Analysis of HIV Predictors Among Female Sex Workers in Nigeria: Lessons for HIV Vaccine Trial**

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**Background:** Female sex workers (FSW) are important high risk group. Their burden of HIV infection is of strategic importance to the national response since they have the highest HIV prevalence in Nigeria. Lessons learnt from HIV surveillance among them will play vital role in their involvement in HIV vaccine trial. This study examined the predictors of HIV infection and its effects at the state level.

**Methods:** This study involved further analysis of a national survey data targeted at high risk populations. This was 2010 Integrated Biological and Behavioral Surveillance Survey (IBBSS) that involved HIV testing among 4459 FSWs in brothels and non-brothels of 9 Nigerian states. Additionally, information was collected on demographic, and sexual and reproductive health variables. Random-effect logistic regression with a random slope was fitted to ascertain state level variation in predictors of HIV infection among FSW.

**Results:** The mean age was 26.5±5.8years; HIV prevalence was 21.2% with brothel-based FSW having 25.6% and non-brothel based having 16.5%. The mean age at first sex was 17±2.7years; average clients/day was 4; 29.3% of married FSWs were HIV+, 21.7% of HIV+ FSW used condom with their last client while 21.9% of HIV+ FSW used condom consistently. Significant predictors of HIV infection were age 25-34years OR=1.5 95%CI (1.3-1.8); ever married OR=1.5 95%CI (1.1-1.8), and protective factors include received education on HIV/AIDS/STIs OR=0.8 95%CI (0.6-0.9); secondary education OR=0.6 95%CI (0.4-0.9) and tertiary education OR=0.5 95%CI (0.3-0.8). The estimated variance between state was 0.24 with a standard error of 0.13.

**Conclusion:** HIV infection among FSW is dependent on the state they practice their trade. This study provided better understanding of HIV infection among FSW; findings will be useful in designing state-level intervention and strategic document for FSW. As a result of their high HIV prevalence, FSWs are potential candidates of future HIV vaccine study in Nigeria.

**P09.08**

**Aptamers that Inhibits Entry of Diverse HIV-1 Subtype C Is Not Cytotoxic**

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**Background:** HIV-1 subtype C accounts for majority of the global AIDS epidemic yet most antiretroviral (ARV) drugs are developed against subtype B. The long-term cumulative cytotoxicity of ARVs is among the major causes of treatment failure in HIV infected patients. Thus, the objective of this study was to test the cytotoxicity and efficacy of RNA aptamer called B40 and its shortened derivative called UCLA1 against diverse HIV-1 subtype C isolates.

**Methods:** B40 aptamer that was previously shown to have efficacy against HIV-1 was tested for cytotoxicity in human cardiomyocytes and PBMCs by measuring cell viability, caspase 3/7 activity, levels of monoamine oxidases (MAO) A and B and cytochrome P450 3A4 (CYP3A4) metabolic enzymes, and mitochondrial DNA (mtDNA) depletion. It was compared to a panel of non-nucleoside reverse transcriptase inhibitors, nucleoside reverse transcriptase inhibitors, protease inhibitors, and the T20 entry inhibitor in all the assays. B40 and UCLA1 were tested for cytotoxicity in TZM-bl cells and PBMCs. Activity of UCLA1 was tested against a large panel of subtype C viruses in different cell types and its therapeutic index (TI) estimated.

**Results:** B40 and T20 did not affect the viability of cardiomyocytes and PBMCs at any concentration tested. They also did not interfere with the cellular activity of CYP3A4 or MAO A and B. Levels of monoamine oxidases (MAO) A and B and cytochrome P450 3A4 (CYP3A4) metabolic enzymes, and mitochondrial DNA (mtDNA) depletion. It was compared to a panel of non-nucleoside reverse transcriptase inhibitors, nucleoside reverse transcriptase inhibitors, protease inhibitors, and the T20 entry inhibitor in all the assays. B40 and UCLA1 were tested for cytotoxicity in TZM-bl cells and PBMCs. Activity of UCLA1 was tested against a large panel of subtype C viruses in different cell types and its therapeutic index (TI) estimated.

**Conclusion:** Overall, these data support the development of B40 and UCLA-1 aptamers as new entry inhibitor molecules with no cytotoxicity at the estimated potential therapeutic dose, especially against HIV-1 subtype.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L2, Room 211 – 212. Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L2, Room 211 – 212.

**P09.09 LB**

**Seroconversion Incidence Cases Among Cohabiting Discordant Couples While the HIV Index Partner on ARVs in Kigali Rwanda**


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**Background:** Studies in Rwanda demonstrated that 90% of HIV infections acquired are in marriage and couples counseling reduces HIV incidence from 20% to 3% per year and could therefore prevent 78.8% of all new heterosexual infections. In Africa, the introduction of antiretroviral medication (ARVs) resulted in substantial reduction in morbidity and mortality from HIV/AIDS, where ARVs reduced HIV transmission by up to 96% within discordant couple. Thus, it is important to supplement ARVs with Couples HIV Counseling and Testing (CHCT) to encourage adherence and reduce HIV transmission.

**Methods:** The study enrolled 2,555 cohabiting discordant couples from January 2010-June 2013 in Kigali Rwanda. Couples were encouraged to come back for follow-up visits every three months. The overall seroconversion incidence cases were measured. Within the follow-up period, seroconversion incident cases among couples where one partner was on ARV were compared to seroconversion incident cases among couples where the positive partner was not on ARV. Sequencing is used to establish epidemiologic linkage between spouses in seroconverting couples.

**Results:** In this period, 2,555 discordant couples (M+F- 1,336 and M-F+1,219) received CHCT and 1,356 (52%) were on ARVs at baseline and 362 (14%) discordant couples initiated ARVs during the follow up visits. 4,524 follow-up visits were recorded, with more than 50% of discordant couples having one or more follow-up visits. There were total of 33 seroconversion incident cases (23F, 10M). In the 11-seroconversion incident cases, the HIV index partners were on ARVs and in the 22-seroconversion incident cases the HIV Index partners were not on ARVs. Sequencing results are pending to establish which of these seroconvertors acquired HIV from their HIV+ spouse, or from another partner.

**Conclusion:** Many studies demonstrated that antiretroviral therapy reduces the risk of HIV transmission among HIV discordant couples. Scaling up CHCT into the existing ARVs programs can reduce the HIV transmission rate.
Factors Associated with In Vitro Immune Response to Mycobacterial and Tetanus Antigens Following BCG and Tetanus Immunisation in Infancy

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Background: BCG is a widely used vaccine but has a varied efficacy and reasons for this are still not clear. Several hypotheses have been advanced but none has been proven. In contrast tetanus toxoid (TT) usually confers good immunity. Understating factors that influence responses to cCFP of Mycobacterium tuberculosis and to TT might enable us to understand why BCG gives such a variable response and enable development of better vaccines.

Methods: Children aged five years from the Entebbe Mother and Baby Study (ISRCTN32849447) who received BCG or tetanus immunisations in infancy were assessed for in vitro cytokine responses to crude Culture Filtrate Protein of Mycobacterium tuberculosis (cCFP) and TT. Associations between cytokine responses and maternal and infant socio-demographic factors and infections were analysed.

Results: 1191 and 1162 five year olds, respectively, were assessed for response to cCFP and TT. Among those who received BCG in infancy, 86.0%, 48.3%, 71.3% and 89.7% had detectable responses for IFN-γ, IL-5, IL-13 and IL-10 respectively. For those who received tetanus immunisations in infancy 39.1%, 39.2%, 59.7% and 52.2% had detectable responses for IFN-γ, IL-5, IL-13 and IL-10 respectively. The response to cCFP showed associations with BCG vaccine strain, child HIV status and stunting. The response to TT was associated with HIV and asymptomatic malaria.

Conclusion: BCG strain, nutrition and child’s HIV status may contribute to poor BCG efficacy in our setting. At age five years, we found no evidence of an effect of prenatal exposure to maternal helminth infections or malaria. Funding: Wellcome Trust

Effectiveness of Maternal and Infant Prophylaxes as PMTCT Strategies in Rift Valley Province, Kenya

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Background: Prevention of Mother to Child Transmission (PMTCT) of Human Immune Deficiency Virus (HIV) is a national program to prevent HIV-vertical transmission and comprises of exclusive breastfeeding, exclusive alternative feeding, infant and maternal PMTCT prophylaxes. In order to determine the effectiveness of these strategies we received and analyzed samples from HIV exposed infants under the Early Infant Diagnosis Program in the Rift Valley Province of Kenya.

Methods: Samples received in the laboratory between January and December 2012 were analyzed using the Cobas Ampliprep/ Cobas Taqman and Abbott M200sp/rt test methods. Data on infant prophylaxis, maternal prophylaxis and infant HIV status were collected and frequencies, ratios and proportions calculated. The rates of infant HIV infection were compared with each PMTCT strategy.

Results: A total of 6466 samples were tested in of which 556 (8.6%) were HIV positive. One hundred ninety two (34.5%) of the 556 HIV positive infants had their mothers on prophylaxis while 228 (41%) of the infants were on prophylaxis. Of the 5910 HIV negative infants, 4442 (75.2%) had their mothers on prophylaxis whereas 5137 (86%) of the infants were on prophylaxis.

Conclusion: Comparatively, the rate of HIV vertical transmission for infants whose mothers were on prophylaxis was lower than those without prophylaxis (OR= 5.7). Similarly, infants on prophylaxis were significantly less likely to be infected by HIV through vertical transmission (OR=9.5). Adherence to prophylactic medication was self reported and which may lead to under or over estimation of the rates. Maternal and infant prophylaxes are important PMTCT strategies which if strictly rolled out can further help reduce vertical transmission of HIV. The provision of full HAART to HIV positive expectant and breastfeeding mothers is critical in reducing these high vertical transmission rates. A combination of these two modes of prophylaxes provides better protection to infants against vertical transmission.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L2, Room 211 – 212. Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L2, Room 211 – 212.

**P10.03**

**Impact of In Utero HIV-1 Exposure on Chemokine Receptor Expression by Infant CD4+T Cells and Susceptibility to HIV-1**

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**Background:** Uninfected infants born to HIV-1-infected women have lower CD4+ T cell counts in infancy than those born to uninfected mothers. In utero exposure to maternal HIV-1 infection could shape CD4+ T cell responses and chemokine receptor expression affecting T cell homing and numbers in the circulation.

**Methods:** Chemokine receptor expression on cord blood CD4+ T cells of uninfected HIV-1-exposed and healthy infants was assessed with flow cytometry directly and after culture. HIV-1 susceptibility of cord blood CD4+ T cells was assessed with an in vitro infection assay measuring p24. A multiparameter Luminex bead assay was used to assess levels of soluble factors.

**Results:** A small yet significantly higher percentage of CD4+ T cells expressed chemokine receptors associated with inflammatory responses (i.e. CCR3, CCR8, CXCR6) in HIV-1-exposed infants compared to healthy controls. The cytokines IL-1_ and IL-8 were increased in plasma from HIV-1-exposed infants. In vitro culture of CD4+ T cells from HIV-1-exposed infants with i.e. IL1_ or IL-2 resulted in more CD4+ T cells bearing inflammatory-associated response chemokine receptors compared to controls, (CCR3 P<0.01, CCR8 P=0.03), whereas CCR7, largely expressed by naïve cells, was lower than in controls (P=0.01). Only after in vitro stimulation, CD4+ T cells could be infected with HIV-1. CD4+ T cells of HIV-1-exposed infants were more susceptible to HIV-1 after in vitro stimulation than from healthy controls.

**Conclusion:** In sum, CD4+ T cells of HIV-1-exposed infants have an enhanced state of activation, with an increased expression of inflammatory response-associated chemokine receptors. This likely affects the homing of CD4+ T cells and may be the underlying mechanism for reduced numbers in blood. Furthermore, the primed immune system alters susceptibility to HIV-1 and may enhance postnatal HIV-1 transmission in HIV-1-exposed children.
**P11.01 D**

**Getting to Zero with an HIV Vaccine: Modeling the Impact of Introduction of the Current HIV Vaccine Pipeline Under the Investment Framework**

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**Background:** The 2011 UNAIDS Investment Framework showed how scaling up key HIV interventions could dramatically reduce new HIV infections and deaths in low- and middle-income countries by 2015. Even with the reduction in HIV incidence modeled in the Framework there would still be approximately one million new infections per year. In order to further reduce incidence, introduction of new prevention options, including HIV vaccines, is necessary. With follow-on studies to RV144 well underway, DNA, MVA and adenovirus vaccine trials ongoing and promising work in candidates to elicit broadly neutralizing antibodies, it is a matter of time before an HIV vaccine will become part of the combination prevention paradigm.

**Methods:** This study assesses the potential impact of an HIV vaccine in reducing new infections within combination HIV prevention programs and ultimately controlling the epidemic. Different potential candidates in the HIV vaccine pipeline are considered and their potential implementation modeled using assumptions including year of introduction, efficacy, cost and coverage.

**Results:** While the timeframe for introduction of an HIV vaccine is longer than other options, the impact could be quite substantial. Potential vaccine efficacies of 30, 50 and 80 percent were modeled, with each scenario showing impact on incidence. At varying levels of efficacy, early and strategic introduction of a low- and moderate-cost HIV vaccine is shown to be cost-effective and beneficial.

**Conclusion:** Remarkable progress is being made towards the development of a safe and effective HIV vaccine. Along with other new prevention options, including pre-exposure prophylaxis and treatment as prevention, HIV vaccines will have a major role to play in reducing new infections. Existing technologies and other new prevention options may lower new infections substantially, but an effective HIV vaccine could provide the push to achieve the final bend in the curve needed to bring new infections to near zero.

**P11.02 D**

**A Survey of Community Opinions and Preferences on PrEP, Microbicides and Vaccines in 5 Regions and with Key Populations in Kenya**

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**Background:** Recent results of PrEP, Microbicides and Vaccine trials have received mixed reactions at community level. We documented community, key populations (MSM, SW, Youth), and health providers’ preferences on New Prevention Technologies (NPT) in 5 regions of Kenya.

**Methods:** A questionnaire was administered after workshops on NPTs and was followed by FGDs. Quantitative data was analyzed using MS Access 2007 and Stata version 11. FGD tapes and notes were transcribed and analyzed by two independent researchers who identified emerging themes.

**Results:** 42% of respondents (N=158) were involved in HIV/AIDS for more than 5 years and 20% were involved in NPT research and advocacy for more than 5 years. 62% reported having a fairly good knowledge of HIV vaccine research and discourse of the recent results against 51% for microbicides and 50% for PrEP. The average efficacy level that most respondents would be comfortable to advocate for introduction and use in their community was 74% for vaccines, 71% for microbicides and 76% for PreP. Vaccine was selected as preferred NPT across key populations but was equally ranked with microbicides for heterosexual women. Microbicides were ranked second. PrEP was ranked highest for discordant couples, MSM and MSW. Most respondents preferred microbicide gels (78%), injectable vaccines (69%) and injectable PrEP (67%). However, higher preference for microbicides ring was noted with SW and oral vaccine and oral PrEP with MSM. The highest preference for product regimen was every 5 years for a vaccine (64%), before and after sex for microbicides (61%), and twice a week for PrEP (59%) with varied preferences between key populations. Most preferred service delivery modes were health centers followed by family planning/HIV centers. Rationale for choices were guided by product characteristics, expected cost, accessibility, inclusivity, side-effect, efficacy and stigma.

**Conclusion:** Understanding evolving community preferences should be proactive, conducted in sync with trial and demonstration projects to inform R&D.
P11.03 D

The Role of Stakeholders Involvement Towards the Success of Vaccine/ Clinical Trials—The Partners PrEP Study

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Background: Kampala clinical trial site was one of the 9 sites implementing the Partners PrEP Study, a double-blind randomized controlled trial of daily oral antiretroviral pre-exposure prophylaxis (PrEP) to prevent HIV acquisition. Like other clinical trials/vaccine trials, involvement of stakeholders greatly contributed to meeting recruitment targets.

Methods: Before recruitment of participant into the partners PrEP study begun, stakeholders from target communities were sensitized about the study. Collaborating VCT sites, the Community Advisory Board (CAB), Community health workers, Local council and religious leaders were briefed about the objectives of the study to enhance acceptability to participate. Stakeholders engagement at planning and implementation regulated the activities of the study and ensured participants safety.

Results: From 65 collaborating VCT sites, 245 counselors were trained in couple counseling and testing. 1758 couples were referred for screening and 582 couples were enrolled (screening to enrollment ratio 3:1). The VCT sites referred the highest number of couples to the study 1514 and 504 couples were enrolled (86%). The Community Advisory Board (CAB) referred 13 couples for screening, 2 couples were enrolled. From the 145 village health workers trained, 27 couples were referred for screening, 10 were enrolled (1.7 %). Out of the 42 outreaches 121couples were screened 41 enrolled (7%). The media, religious leaders and local council leaders contributed 6%.

Conclusion: Involvement of stakeholders in vaccine/ clinical trials to mobilize participants and to explain the objectives of the study to the community/ policy makers contributed to the success of the partners PrEP study. Stakeholders involvement in recruitment is an effective method of maintaining steady participant recruitment, adherence to study drug and retention.

P11.04

Applying Market Research to Advance Implementation Science for New HIV Prevention Technologies: Two Case Studies with Most-at-Risk-Populations

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Background: Widespread gaps between clinical trial efficacy and real-world effectiveness of existing biomedical interventions highlight the importance of assessing end user preferences and concerns that may influence uptake of new HIV prevention technologies. We present methods and results from two studies to bridge research-to-practice gaps for HIV vaccines and rectal microbicides (RM).

Methods: We conducted community-based mixed-methods investigations using sequential triangulation designs among MSM and transwomen in Thailand and Black women in Canada. Focus groups were implemented using a semi-structured interview guide, analyzed thematically and applied to guide construction of discrete choice modeling (Thailand) and conjoint analysis (Canada). Thailand participants double-ranked 8 sets of 5 cards (best-worst) of hypothetical multi-attribute RMs. Full-rank data were analyzed using logit likelihood functions estimated with custom routines in Gauss matrix programming language. Canada participants rated the acceptability of 8 multi-attribute HIV vaccine scenarios, with conjoint analysis to determine the relative impact of different attributes.

Results: Thailand: Informed by focus groups (n=37 participants; mean age=24.8 years), a pilot survey (n=24; mean age=24.5 years) indicated efficacy had the greatest marginal effect on choice, with participants more than twice as likely to choose a 99% vs. 50% efficacy RM. Pericoital (vs. daily) usage increased probability of choice by >70%; gel (vs. suppository) formulation increased choice by >40%.

Canada: Informed by focus groups (n=26 participants; mean age=32.1 years), the survey (n=206; mean age=35.1 years) indicated efficacy (99% vs. 50%) had the greatest impact (22.6/100) on acceptability, followed by side effects (none vs. minor; 8.6), cost ($10 vs. $250; 7.6) and duration (10 vs. 1 year; 6.5).

Conclusion: Methods traditionally used in marketing research can be applied in culturally appropriate ways to assess end user preferences and concerns about new HIV prevention technologies. Evidence from these analyses may inform product development and support implementation science to translate efficacious products into effective prevention tools.
P11.05

“Sisters, Mothers, Daughters and Aunties”: HIV Vaccine Acceptability Among Black Women in Toronto

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Background: Black women in North America are at disproportionately high risk for HIV infection. In order to anticipate expectable research-to-practice gaps between vaccine availability and uptake, we assessed the acceptability of future HIV vaccines among Black women in Toronto.

Methods: Women were recruited using venue-based sampling in Toronto. Data were collected on sociodemographic characteristics and acceptability of 8 hypothetical HIV vaccine scenarios, each defined by 7 variable attributes. Participants rated acceptability of each vaccine scenario, presented in a set of 8 laminated cards. We used conjoint analysis to quantify the relative impact of each vaccine attribute on acceptability.

Results: Among participants (n=206; mean age=35 years), 52% were of Caribbean and 48% African heritage, 81% born outside Canada. Mean monthly income was $1,683. Forty-percent had completed high school, 42% were single/never married, and 40% employed full-time. Acceptability of the 8 vaccine scenarios ranged from 82.1 (SD=24.6) to 37.1 (SD=25.3); mean vaccine acceptability = 58.8 (SD=17.2; 100-point scale). Efficacy had the greatest impact on acceptability. Vaccine scenarios with 99% efficacy had an average acceptability score 22.56 (95% Confidence interval [CI]=19.30, 25.83) points higher than those with 50% efficacy, followed by side effects (none vs. minor; 8.56, 95% CI=5.85, 11.27), cost ($10 vs. $250; 7.57, 95% CI=5.33, 9.81), duration of protection (10 vs. 1 year; 6.53, 95% CI=4.38, 8.67), and doses (1 vs. 4; 1.75, 95% CI=0.32, 3.18). Women of African (vs. Caribbean) descent, single/never married (vs. married) and working part-time/unemployed (vs. full-time) had lower acceptability scores.

Conclusion: Black women in Toronto indicated a modest level of acceptability for future HIV vaccines. Educational interventions that promote the benefits of partially efficacious vaccines and include clear explanation of potential side effects, in conjunction with vaccine cost subsidies, may increase vaccine uptake. Differences in acceptability within Black communities suggest the need for tailored interventions to address barriers to uptake.

P11.06

Will HIV Vaccination Reshape HIV Risk Behavior Networks? A Social Network Analysis of Drug Users’ Anticipated Risk Compensation

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Background: A successful HIV vaccine could substantially impact the epidemic; however, risk compensation (increased risk behavior after vaccination) is a significant concern. While the impact of vaccine-related risk compensation on individual-level HIV risk has been explored, the potential impact on community-level risk network structure is largely unknown. The purpose of this study was to examine the impact of drug users’ anticipated HIV vaccine-related risk compensation on the overall structure of their risk network.

Methods: Data were collected from 405 rural drug users (75% with history of injection drug use) enrolled in a longitudinal, social network study in the US. HIV risk network ties were those in which partners had unprotected sex and/or shared injection equipment in the past 6 months. Dyad-specific data were collected on self-reported likelihood of increasing/initiating risk behavior in response to HIV vaccination. Through cross-referencing names and demographic information about named network members, a sociometric HIV risk network was constructed.

Results: Anticipated increases in risk behavior and initiation of new risk relationships was reported for 8 dyads, resulting in a 4% increase in the number of ties in the overall HIV risk network (n=131 to n=136) and the inclusion of four additional individuals in the network. The initiation of new relationships resulted in the connection of otherwise disconnected components of the HIV risk network; the largest component of the network doubled in size from five to ten. Interestingly, among all relationships in which increased risk behavior was anticipated, interviewees reported that they were likely to encourage their partners to receive the vaccine.

Conclusion: These preliminary data suggest that HIV vaccine-related risk compensation could possibly impact risk network structure. The potential for network-level changes to mitigate the positive impact of HIV vaccination (particularly with low-efficacy vaccine) should be carefully examined, as should the dynamics and motives of peer-based vaccine promotion.
P11.07

Engaging and Recruiting MSM in HIV Research: Experiences from the Early HIV Capture Cohort Study in Kampala, Uganda

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Background: HIV research among MSM in Uganda is uncommon with two documented studies since the onset of the epidemic. Therefore, a knowledge gap exists on how best to engage and recruit MSM for HIV studies in general and during the debate surrounding the Anti-Homosexuality Bill (AHB). Makerere University Walter Reed Project is conducting the Early HIV Capture Cohort Study, which seeks to enrol HIV uninfected MSM in Kampala. We describe experiences engaging and recruiting this population during a challenging period.

Methods: Using leaders of identified organizations, five members of the MSM community were identified and selected to join a special advisory group to guide recruitment and link MUWRP to the community. Brainstorming sessions with various community members and separate individual conversations with additional knowledgeable members were organized to discuss; most effective recruitment strategies, anticipated challenges and feasibility of certain study procedures including twice weekly clinic visits.

Results: Recommended and adopted recruitment strategies included; Peer led approaches, extemporized approach and community Sundays. There was consensus that twice weekly clinic visits would be acceptable. Contention surrounded use of coupons as a recruitment tool and the impact the AHB would have on study participation. Within a month of recruitment initiation, 67 MSM were booked for screening, 47 were screened, 18/47 enrolled, 15/18 began bi-weekly clinic visits while 29/47 are awaiting results to determine eligibility. Within a month of recruitment initiation, 67 MSM were booked for screening, 47 were screened, 18/47 enrolled, 15/18 began bi-weekly clinic visits while 29/47 are awaiting results to determine eligibility. Only 8/67 did not turn up for screening for reasons not provided. For confidentiality, identification of participants at the site is by use of finger print scanning, which links to participant study number that appears on all study documents.

Conclusion: Although replicating recruitment strategies used in previous studies is helpful, it is critical to undertake a real-time community assessment exercise however rudimental, to gain informed understanding of local or unique study populations as a matter of Good Participatory Practice.

P11.08

Enhancing Capacity of Ethics Review Committees in Developing Countries: The Kenyan Example

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Background: The high increase in HIV vaccine clinical trials taking place in developing countries and the complexity of the protocols for these trials requires that local ethics review committees (ERCs) reviewing them have the required capacity to ensure that they are conducted to the highest ethical standard.

Methods: With a grant from Global Health Research Initiative (GHRI), Kenya AIDS Vaccine Initiative (KAVI) and the National Council for Science and Technology (NCST) embarked on an exercise to enhance the capacity of ERCs in Kenya to review such protocols. The process involved conducting an audit of all accredited and non-accredited ERCs in the country, conducting their training needs assessment to identify the gaps. The information obtained was then used to develop materials used to train the ERC members in several workshops conducted in different parts of the country.

Results: Five accredited and 13 non-accredited ERCs in Kenya were identified. Four of the accredited ERCs were located in the capital city of Nairobi. The most common challenge/need cited by participants during the needs assessment was excess work load, and the lack of coordination/communication between the ERCs. To date, participants from 8 institutional ERCs have been trained as follows: 36 from institutions in the western part of the Kenya and 22 from institutions in the south eastern coastal region of the country. A total of 116 ERC members have attended the ethics training workshops to date. The training workshops are still on course.

Conclusion: KAVI and NCST have developed training modules for training ERC members in Kenya and are in the process of developing a manual for the trainings. The Kenyan experience may be used to enhance capacities of ERCs not only in the East African region, but in other developing countries.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L2, Room 211 – 212. Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L2, Room 211 – 212.

**P11.09**

**Regulatory Capacity Building Under the Canadian HIV Vaccine Initiative: Addressing Challenges of Developing National Regulatory Authorities**

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**Background:** The Canadian HIV Vaccine Initiative (CHVI) is a partnership between the Government of Canada and the Bill and Melinda Gates Foundation with the goal of developing a safe, affordable, effective and globally accessible HIV vaccine. Health Canada, working in collaboration with the World Health Organization, is responsible for CHVI’s regulatory capacity building program which is aimed at strengthening the regulatory capacity of developing national regulatory authorities (NRAs) in order to help protect the ethical and scientific integrity of HIV vaccine clinical trials occurring in these countries.

**Methods:** The regulatory needs of developing NRAs were identified using surveys, face-to-face meetings and teleconferences. Health Canada vaccine and regulatory experts developed and administered training using case studies and interactive group discussions. Training sessions included clinical trial application review, clinical and quality review of vaccines and vaccine lot release.

**Results:** Since 2010, Health Canada has trained over 100 participants from more than 40 countries under the CHVI. This includes the establishment and continued mentorship with regulatory agencies in Nigeria and Malawi and regional training in Southern and Eastern Africa. Health Canada has prepared and delivered regulatory training sessions via vaccine and clinical trial forums, and sponsored NRAs to attend these forums to provide learning opportunities and encourage the exchange of best regulatory practices. This also includes the development and implementation of guidance in the area of HIV vaccine clinical trials.

**Conclusion:** Health Canada has successfully implemented capacity building activities in countries with developing NRAs to help strengthen their capacity in the regulation of vaccines. Moving forward, training efforts will utilize a regional approach in order to maximize training of regulatory staff in countries with similar training needs.

**P11.10**

**HIVACAT’s Community Advisory Board: An Experience on Community Engagement in an HIV Vaccine Research Project in Catalonia**

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**Background:** Aware of the importance of the community involvement, and to allow a fluent communication between researchers and community, the research project HIVACAT set up a Community Advisory Board (CAB). HIV activists, journalists, trials’ participants, researchers and public health professionals compose this CAB, created in July of 2010.

**Methods:** The CAB launched an invitation to different members of the community. The identification took three months. The group meets on a quarterly basis. In each meeting, there are training; updates on the ongoing clinical trials; revision of protocols and material addressed to participants. The CAB uses a mailing list to share documentation, articles and proposals. All documents are sent at least 10 days before the meeting. A chair and a secretary were appointed to facilitate the group work.

**Results:** The CAB has fulfilled their objectives, assessing HIVACAT performance and providing important inputs for the improvement of protocols and communication strategies. These have incorporated the community needs in a more receptive way. At any stage, the CAB members have kept their independence, expressing all their opinions and proposals for the clinical trials design and implementation. During the three years the CAB has met in 10 occasions, has organised four trainings and has reviewed seven protocols, studies and/or materials.

**Conclusion:** Community engagement has shown to have a positive impact on science by facilitating the recruitment and retention of volunteers, through a better communication between researchers and community. That improves the research process and outcomes. However, it is important to evaluate the CAB performance and its impact on the overall goals of HIVACAT. We have identified the need to elaborate a work plan for the CAB, and to define qualitative and quantitative indicators that may help to have a better picture of the impact that community engagement has on the research project.
P11.11

An Integrated Approach to Clinical Trial Capacity Building

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Background: Human resources required to ensure high standards of design and conduct of clinical trials are limited in resource poor settings such as Sub-Saharan Africa. Using funding from the Global Health Research Initiative, we targeted ethicists, laboratory and clinical staff as well as local communities for training, as part of a multi-faceted approach to clinical trial capacity building in the East African region.

Methods: In 2010, we established a clinical research training unit whose mandate was to train clinical research staff in Good Clinical Practice in research organizations in Kenya Uganda and Tanzania. Laboratory staff in the three countries also received training in Good Clinical Laboratory Practice as well as in laboratory quality management systems. In order to build research literacy and acceptance among health care providers in Kenya, nationwide workshops were conducted among lecturers in the Kenya Medical Training Colleges (KMTC) which generate >80% of health care professionals in the country. We also partnered with the National Council for Science and Technology to conduct nationwide ethics training to members of ethics review committees in Kenya.

Results: A total of 171 clinical staff, 248 laboratory staff, 177 KMTC lecturers and 58 ethicists have been trained to date. An inventory of all accredited and non-accredited IRBs has been compiled and the development of a standardized training manual for IRBs is underway.

Conclusion: This program creates an opportunity to nurture South-to-South collaborations and to develop and cascade training programs that are based on culturally relevant experiences. Sustainability of the program is a major challenge and in all cases, the demand for training has far outstripped the available resources.

P11.12

HIV Vaccine R&D Investment Amid a Changing Global Health and Development Landscape

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Background: Since 2004, the HIV Vaccines and Microbicides Resource Tracking Working Group has employed a comprehensive methodology to track trends in research and development (R&D) investments and expenditures for biomedical HIV prevention options, including HIV vaccines, microbicides, pre-exposure prophylaxis (PrEP), treatment as prevention, voluntary medical male circumcision, female condoms, HSV-2 prevention and vertical transmission prevention. Such monitoring is critical to identify investment trends and provide a fact base for policy advocacy on R&D spending.

Methods: To estimate annual investment in HIV vaccine R&D, data were collected from government agencies, nonprofit research organizations, foundations and pharmaceutical/biotechnology companies on annual disbursements for product development, clinical trials and trial preparation and policy advocacy.

Results: Preliminary results suggest that funding was nearly flat in 2012 as the global health and development landscape shifted to accommodate budget and economic pressures in the US and Europe. The HIV vaccine field responded to increased focus by donors on country ownership, capacity sharing and a reduced priority for disease-specific projects by formalizing relationships with research groups in HIV endemic countries and product development efforts outside the field. Public sector funding remained the main source of investment, with a slight increase in estimated industry spending and a slight decrease in philanthropic donations.

Conclusion: 2012 was a year of sustained effort and steady progress. With promising discoveries in broadly neutralizing antibodies, follow-on trials to RV144 underway and new vaccine strategies entering clinical trials, development of a safe and effective HIV vaccine continues to advance. Pursuit of promising candidates and continued exploration of groundbreaking basic research to replenish the candidate pipeline will require sustained investment over the long timeline of HIV vaccine development. Tracking funding trends enables prioritization of promising research and the assessment of the impact of the economic environment, development trends and public policy decisions on HIV vaccine R&D investment.
P11.13

The Role of Technical Assistance in Establishing Couples’ Testing as Standard of Care for HIV Vaccine Trial Participants

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Background: Most African adults are in a cohabiting sexual union and these couples represent the largest risk group for HIV transmission. Couples voluntary HIV counseling and testing (CVCT) is associated with a two-thirds reduction in HIV transmission among HIV serodiscordant couples. WHO guidelines recommend CVCT. Through CVCT, couples experience mutual disclosure of HIV results and receive targeted counseling which affects sexual risk behaviors and hence vaccine trial outcomes. CVCT must be provided as standard of care for vaccine trial participants across countries and sites. RZHRG provides technical assistance (TA) to countries and partners wishing to implement CVCT. TA is useful in ensuring that CVCT services are effectively adapted and implemented across the region.

Methods: International TA events from 2009-2012 are described. TA events were classified as high-level advocacy, training for service providers and promotional agents, as well as training of trainers. Monitoring and evaluation activities are also presented to illustrate country-adapted CVCT models, as well as changes in uptake of CVCT and subsequent identification of discordant couples.

Results: From 2009-2012, RZHRG provided TA to over 1,785 people from 146 partners in 18 countries. Available data from 2012 indicated significant increases in provision and uptake of CVCT in 12 of 18 recipient countries either for research or programmatic purposes. Increased uptake of CVCT led to increased identification of discordant couples. As yet, only two countries offer follow up for discordant couples in government facilities. Workshops held in Rwanda and Zambia in 2012 attracted participants from 23 countries, confirming growing interest in CVCT.

Conclusion: Through TA from an organization with experience in CVCT for vaccine trials and routine health services, recipient countries were able to acquire a multitude of skills which enabled them to more effectively implement CVCT. Countries with capacity to provide quality CVCT services should be considered in site selection for future vaccine trials.

P11.14

Is Omitting Couple’s Voluntary HIV Counseling and Testing (CVCT) in HIV Vaccine Trials Unethical?

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Background: WHO has issued guidelines endorsing CVCT and assisted mutual disclosure for HIV prevention. Training materials are available on the CDC web site.

Methods: The Rwanda Zambia HIV Research Group (RZHRG) has pioneered CVCT since 1986: >90% of pregnant Rwandan women now test with spouses and >100,000 Zambian couples have been tested in RZHRG partner clinics. RZHRG has also provided CVCT technical assistance to 20 other African countries. In 2011, based on the results of HPTN 052, research sponsors instructed RZHRG to discharge large cohorts of discordant couples and refer them to government clinics for Treatment as Prevention (TAP). Follow-up confirms that few have initiated TAP.

Results: International guidelines endorse CVCT and functional models have been developed but neither Global Fund nor PEPFAR include required indicators or provide dedicated funding. More than 90% of African discordant couples have yet to be identified, and follow-up services are not available in government clinics. In one southern African country that includes TAP for all discordant couples in the national guidelines, a prevention trial is underway that will identify but not counsel or treat discordant couples unless CD4 counts are 350 cells/mm3. Paradoxically, because of this same guideline, vaccine trial sponsors no longer consider discordant couples eligible for trials in that country and have withdrawn funding for CVCT.

Conclusion: Clinical trial participants in Africa should be HIV-tested with their primary sexual partners whenever possible. Interpretation of TAP guidelines for prevention trials should be consistent and based not on aspirational goals but on what is actually the ‘locally achievable standard of care’. CVCT is cost-effective, feasible, and affordable. Studies and trials that ignore stable partners and do not provide CVCT have to be considered unethical, and vaccine trials should include discordant couples where choice or lack of TAP provide an ethical basis.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L2, Room 211 – 212.

Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L2, Room 211 – 212.

**Topic 11: Social/Ethical/Access/Regulatory Issues**

**P11.15 LB**

**Vaccine-Induced Antibodies: Impact for HIV Diagnosis, Clinical Trial Participants and Public Health**

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**Background:** The evaluation of vaccine candidates in volunteers is an essential component of HIV vaccine research and development. Vaccine trial participants may develop vaccine-induced antibodies to HIV without being actually infected by the virus. These antibodies are frequently detected by commonly used serological diagnostic tests and may be misinterpreted as a marker of HIV infection. This phenomenon is currently referred to as Vaccine-Induced Sero-Positivity (VISP) or Vaccine-Induced Sero-Reactivity (VISR). As vaccine candidates become more immunogenic and effective and trials more diverse, the number of post-study volunteers with durable VISP/R will rise with consequences for global healthcare systems.

**Methods:** The Global HIV Vaccine Enterprise recently hosted an international convening co-sponsored by NIAID and attended by representatives from academia, government, private institutions, and biotechnology companies to review the challenges presented by VISP/R and to articulate paths to resolution. A number of issues were considered.

**Results:** Clinical vaccine trial participants with durable VISP/R may encounter a range of social harm and require long-term support in countries where vaccine trials are being conducted. Trial volunteers should be informed in advance of the possibility and implications of developing VISP/R. Differentiation of VISP/R from HIV infection poses technical challenges. Solutions must be advanced for vaccine trials and post-study, especially for participants at ongoing risk for HIV, and ultimately for the licensure and deployment of an effective vaccine. For trial sponsors, provision of specialized testing and other services to volunteers with durable VISP/R creates a logistical and financial burden. Review of current VISP/R management practices at a number of organizations indicated diverse approaches and technical solutions, some that could be adopted field-wide.

**Conclusion:** Addressing issues related to vaccine-induced antibodies in HIV vaccine recipients requires commitment and concerted action from the field as a whole in collaboration with local and global agencies.
**P12.01 D**

**Host Immune Environment Impact the Level of CD4 Central Memory Reconstitution in HIV Subjects Receiving ZFN CCR5 Modified CD4 T Cells (SB-728-T)**


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**Background:** Therapeutic vaccine modalities have yet to generate successful outcome; here we have used adoptive transfer of ZFN-modified CD4 T cells to provide HIV-resistant cells to the host immune’s system. Long-term improvement in CD4 count was observed in all SB-728-T treated subjects. Here, we performed phenotypic and transcriptional analyses to elucidate the role of inflammation and persistent immune activation on CD4 reconstitution and SB-728-T persistence.

**Methods:** Nine aviremic HIV+ subjects on ART with CD4 counts btw 200-500 cells/µL received 10-30E9 SB-728-T. Analyses of survival and activation markers were performed on PBMCs collected pre-infusion and at late (M9-12) time points post-infusion.

**Results:** At 12 months post-infusion, 5 of 9 subjects showed greater improved CD4 count (high CD4 responders; median increase= 224 cells/µL) than 4 other subjects (suboptimal responders; 44 cells/µL), which was primarily driven by the central memory T cell subset (TCM). Persistence of CCR5 modified cells does not fully explain the CD4 increases observed in high CD4 responders. High CD4 responders showed decreased PD-1 expression in monocytes post-infusion that correlated with lower PD-1 expression in TCM (r=−.73, p=.03) and higher expression of HLA-DR, CD80 and CD40 in monocytes and LAG-3 in TCM, and expression inversely correlated with reconstitution of TCM 12 months post-infusion (r=−.73, p=.03).

**Conclusion:** Reduction of PD-1/PD-L1 mediated suppression in TCM in high CD4 responders suggests that decreased levels of inflammation may provide a survival advantage of TCM post-infusion. Identification of an inflammatory signature prior to treatment as an important predictor of reconstitution of TCM will help future study design of SB-728-T and other modalities aimed at immune reconstitution.

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**P12.02 D**

**HIV-1 Protease Inhibitor Alter Cellular Protease Activity, Epitope Processing and Presentation to CD8 T Cells**

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**Background:** Epitopes displayed by MHC-I are generated from the intracellular degradation of proteins by proteasome and aminopeptidases. We hypothesize that due to structural homologies HIV protease-inhibitors (PIs) used in antiretroviral-therapies may affect activities of cellular peptides involved in epitope processing and alter epitope presentation to immune cells.

**Methods:** Using a fluorogenic assay the effect of 7 HIV-1 PIs (Ritonavir, Saquinavir, Nelfinavir, Indinavir, Atazanavir, Darunavir and Kaletra) on proteasome and aminopeptidase activities was tested in PBMCs from at least 6 healthy donors. Using PBMC cytosol as a source of peptides the effect of HIV PIs on HIV epitope production kinetics and HIV epitope cytosolic half-life was assessed by HPLC and LC-MS-MS analysis. Finally we assessed the impact of PIs on the endogenous processing and presentation of epitopes by HIV infected cells to CD8 T cells using a fluorescence-based cytotoxicity assay.

**Results:** HIV PIs variably altered proteasome and aminopeptidase activities. Depending on the PI, some activities were inhibited (up to 5-folds, p<0.001), enhanced (up to 9-folds, p<0.001), and others not changed. These PI-induced changes in protease activities were sequence dependent leading to enhanced or reduced cleavage of specific residues and resulting in alteration of HIV epitope production kinetics and intracellular half-life prior to MHC-I loading. For instance Ritonavir increased by 4.6-fold the intracellular B57-KF11 epitope production whereas Saquinavir reduced it by 3.3-fold. Depending on the PI and the epitope the half-life was increased 1.5-fold (p<0.01) or decreased 1.3-fold (p<0.05). Furthermore HIV PI altered (from 2.2-fold decrease to 1.3-fold increase, p<0.01) the presentation of HIV epitopes and recognition by epitope-specific CD8 T cells.

**Conclusion:** These findings suggest that in HIV-infected patients an antiretroviral therapy including PIs might by altering host proteases function modify the pattern of epitope presentation, leading to the elicitation of additional CTL responses against HIV and potentially against other pathogens co-infecting HIV+ persons.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L2, Room 211 – 212.
Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L2, Room 211 – 212.

**P12.03 D**

**Targeting HIV-1 Where It Hurts**

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**Background:** To address HIV-1 variability and escape, our group has assembled the vaccine immunogen HIVconsv from the most conserved functional regions (as opposed to epitopes) of the HIV-1 proteome with the underlying working hypothesis that early focus of vaccine-elicited immune responses on these regions will lead to a better control of both transmitting/ founder and escaping viruses.

**Methods:** In the HIV-CORE002 trial, a gene coding for the HIVconsv immunogen was delivered by a combination of plasmid DNA (D), modified vaccinia virus Ankara (MVA; M) and non-replicating adenovirus of a chimpanzee origin ChAdV-63 (C) to healthy, HIV-1-negative adults in Oxford.

**Results:** The two most immunogenic regimens, CM and DDDCM, induced total frequencies of HIV-1-specific; IFN-g-producing T cells that were up to 10-fold higher than the Merck STEP trial candidate. We examined the ability of vaccine-elicited CD8+ T cells to inhibit HIV-1 replication in vitro using a panel of 8 viruses comprised of multiple viral clades. The CM regimen induced the strongest magnitude and breadth of inhibition; with a group median of 2.8 log10 reductions in viral p24 protein being detected in the IIIb virus isolate one week post MVA. HIVconsv boost. Breadth of inhibition following CM administration showed that all vaccinees inhibited 2/8 viruses from clades A and B, with one individual demonstrating superior control of all eight viruses. In addition, HIV-1 inhibitory responses to multiple viral clades were preserved in several vaccinees from the CM group for up to 20 weeks post vaccination. Expanded CD8+ T cell IFN-g ELISpot responses against Gag and Pol peptides present within the immunogen were also examined and correlation of the data showed that the strongest association was between the magnitude of viral inhibition and Pol-specific expanded CD8+ T cell responses.

**Conclusion:** As a result of the promising IFN-g-ELISpot and viral inhibition results, further studies are planned with the HIVconsv immunogen.

**P12.04 D**

**Local Cellular Immune Responses Precede Systemic Responses Following Intravaginal SIV Infection in Rhesus Monkeys**

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**Background:** Defining the earliest immunologic events following virus exposure will be critical for the design of an effective HIV-1 vaccine. In this study, we investigated the anatomic localization of mucosal and systemic SIV-specific cellular immune responses in rhesus monkeys following intravaginal infection with SIVmac251.

**Methods:** Ten juvenile and four adult naïve, unvaccinated rhesus monkeys were inoculated by the intravaginal route with a single high-dose of SIVmac251 without depot pre-treatment. To evaluate local and systemic cellular immune responses early following infection, we sacrificed monkeys at day 7 and day 10, and we harvested multiple tissues to evaluate SIV gag specific T cell responses by multiparameter intracellular cytokine staining.

**Results:** SIV gag-specific CD8 and CD4 T cell responses were detected first in the female genital mucosa (cervix, vagina, uterus) and bone marrow by day 7, and were detected in gastrointestinal mucosa (duodenum, jejunum, and colon) by day 10. At day 7 and 10, systemic responses in PBMC, lymph nodes, and spleen were still negative. No clear differences in responses were detected between juveniles and adults.

**Conclusion:** These data show that the earliest cellular immune responses following intravaginal SIV infection in rhesus monkeys occur locally in the female genital tract and bone marrow.
P12.05 D

Immune-Correlates Analysis of the Step HIV Vaccine Efficacy Trial—A Post-Hoc Analysis of HIV-Specific and Non-specific Cellular Immune Responses

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Background: As the first proof-of-concept efficacy trial of an HIV vaccine designed to elicit cell-mediated immunity, the Step study provides unique data on the role of cellular responses in predicting trial outcomes.

Methods: We assessed IFN-γ-ELISpot responses at 4 weeks after the second Ad5 immunization in the male per-protocol (PP) cohort. We accommodated post-hoc sampling into Cox proportional hazards models to estimate hazard ratios (HRs) of HIV-1 infection per one-log increase of ELISpot responses, as well as interactions between treatment and ELISpot responses in predicting HIV infection.

Results: We analyzed 112 PP infected cases (Vaccine: n=84, Placebo: n=28), and 962 PP uninfected non-cases (Vaccine: n=729, Placebo: n=233) with available ELISpot data. HIV non-specific background ELISpot responses (i.e.; IFN-γ-secretion in the absence of antigen-specific stimulation) were directly correlated with risk of HIV infection among vaccine recipients (HR=1.61, 95% CI: (1.31, 1.97), p <0.001), but not among placebo recipients. This association among vaccine recipients persisted after controlling for ELISpot responses against Gag, Pol, Nef and baseline covariates. None of the HIV-antigen-stimulated responses were significantly associated with risk of HIV infection. Overall, there was no significant interaction between treatment assignment and background ELISpot in predicting risk of HIV infection, although such an interaction was more pronounced within both uncircumcised and Ad5 seropositive subgroups. Although the difference is small in magnitude, HIV non-specific ELISpot responses were higher in Ad5 seropositive men (p < 0.001) and circumcised men (p=0.02), but lower in vaccine recipients (p<0.001) in multivariate regression models.

Conclusion: The proportion of cells producing IFN-γ without stimulation by exogenous antigen appears to carry information about a subject’s immune status beyond baseline covariates that predict risk of HIV infection among Step vaccine recipients. These results motivate additional research to understand the potential link between IFN-γ secretion and underlying causes of vaccine-associated enhanced infection risk in Step study.

P12.06

Mechanism of a Novel PD1-Based DNA Vaccine in Amplifying HIV-1 GAG-Specific CD8+ T Cells in Mice

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Background: An effective vaccine to elicit a high frequency of protective CD8+ T cell immunity against HIV-1 remains a continuous challenge. Significant progress has been made to target vaccine antigen to dendritic cells (DCs) via DC surface proteins (e.g. anti-DEC205 antibody) but only a low frequency of antigen-specific CD8+ T cell immunity was achieved. To date, it remains elusive which DC receptor targeting would intensify antigen specific CD8+ T cell immunity.

Methods: In this study, we report what we believed to be a novel antigen-targeting DNA vaccine method that exploits the binding of programmed death-1 (PD1) to its ligands expressed on DCs by fusing soluble PD1 with HIV-1 Gag p24 antigen. For mechanistic investigation, we further conducted a parallel study with an anti-DEC205-based DNA vaccine.

Results: As compared with non-DC targeting vaccines, intramuscular immunization via electroporation (EP) of the fusion DNA vaccine in mice elicited consistently high frequencies of Gag-specific, broadly reactive, polyfunctional, long-lived and cytotoxic CD8+ T cells, besides robust anti-Gag antibody titers, which conferred significant protection against mucosal challenge with vaccinia-Gag viruses. Mechanistically, soluble PD1-based vaccination potentiated CD8+ T cell responses by enhancing DCs in antigen binding and uptake, and DC activation in draining lymph nodes. It also uniquely increased the frequency of IL-12 producing DCs in vivo and engaged the intracellular pathway of antigen cross-presentation when compared with the anti-DEC205-based DNA vaccine.

Conclusion: The high frequency of durable and protective Gag-specific CD8+ T cell immunity induced by soluble PD1-based vaccination has important implications for vaccine development and immunotherapy against HIV-1 infection.
P12.07
CD4+ Th17 and Treg Subsets During Acute/Early HIV Infection Are Associated with Distinct Patterns of Disease Progression and Anti-HIV CD8+ Responses

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Background: Th17 and Treg subsets have been related to HIV/SIV disease progression. Their role and direct relation with specific HIV-adaptive T cell responses during primary HIV-infection (PHI) are largely unexplored topics. Aim: To analyze Th17 and Treg subsets, their balance and correlation with clinical parameters and specific anti-HIV responses during acute/early infection.

Methods: PBMCs were obtained from 6 healthy donors (HDs) and 22 HIV infected-subjects: 6 elite controllers (ECs), 4 chronics, and 12 PHIs (within 6 months post-infection (mpi)). PHIs were classified as “PHI350” if CD4-counts dropped below 350 cells/ul within 12 mpi. Th17 and Treg cells (baseline and 12 mpi samples) were identified as CD3+CD4+IL-17+ or CD25+FoxP3+ (respectively). Data was compared inter/intra-groups and correlated to viral load (VL) and CD4-counts (baseline, set-point and 12 mpi), and specific anti-HIV CD8+ responses, using parametric and non-parametric statistics.

Results: Baseline CD4 (p=0.0669, r=0.3885) and set-point (p=0.0486, r=0.5) counts were directly correlated with baseline Th17 absolute counts, which were found to be higher in PHI350 vs. PHI350. Moreover, VL levels at 12 mpi inversely correlated with baseline (p=0.0406, r=0.5965) and 12 mpi (p=0.0208, r=0.6309) %Th17. Th17/Tregs ratio was higher for HDs compared to any HIV+ group (p<0.01), and within PHIs Th17/Tregs was directly associated with CD4-counts at 12 mpi (p=0.0167, r=0.7928). Significantly, Th17 and quality of anti-HIV CD8+ responses, using parametric and non-parametric statistics.

Conclusion: Results suggest that higher counts and frequency of Th17 and Th17/Tregs ratio at early stages after HIV-infection are associated with slower disease progression in terms of clinical parameters. Importantly, preservation of Th17 compartment associates with the capacity to exert specific anti-HIV CD8+ responses at later times post-infection.

P12.08
ADA Reduces the HIV-1-Specific Tregs and Enhances HIV-1-Specific CD4+ and CD8+ Effector and Memory T Cell Responses

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Background: The use of adjuvants to increase the immune response in therapeutic and prophylactic vaccines are gaining relevance today. In this regard, we previously reported that adenosine deaminase (ADA), a key enzyme in the adenosine pathway, acts as a costimulatory molecule, enhancing T cell activation and proliferation acting as a bridge between A2B adenosine receptors on dendritic cells (DCs) and CD26 on T cells. Here we investigated the costimulatory role of ADA by determining the ADA effect on HIV-specific memory and T regulatory cells.

Methods: DCs from HIV+ patients pulsed with heat-inactivated HIV-1 were cocultured with autologous lymphocytes and HIV-1 specific T cell responses were determined as: proliferation (CFSE), cytokine secretion, memory T cells (CD4+CD25-CD45RO+ and CD8+CD25-CD45RO+) and Tregs immunophenotype (CD4+CD25+FOXP3+) expression. Suppression assays were performed to assess the activity of Tregs in the presence of ADA in healthy individuals.

Results: In cocultures, ADA significantly increased HIV-1-specific CD4+ and CD8+ T cell response, specifically induced HIV-1 memory T cells and enhanced secretion of IFN-γ, IL-1β, IL-6 (11-fold), IL-15 (5-fold), RANTES/CCL5, MIP-1α/CCL3 and MIP-1β/CCL4. Interestingly, ADA significantly decreased the HIV-specific Tregs population and suppressive assays indicated that ADA has a dual role depending on the subpopulation of CD4 T cells: ADA decreases in a half the Tregs, but increases the T effector cells (p<0.001). Addition of 5’-N-ethylcarboxamidoadenosine (NECA), an adenosine analog unable to be ADA-deaminated, increased Tregs 2-fold. This NECA-effect was highly reduced (75%) when ADA was added (p<0.001), suggesting a potent role of ADA interfering with NECA signaling, by a mechanism independent of its enzymatic activity.

Conclusion: These findings demonstrate the role of ADA enhancing both the HIV-1-specific CD4+ and CD8+ memory T cell response against HIV-1 while reducing the suppression mediated by HIV-1 specific Tregs indicating that ADA could be an excellent immunomodulator for HIV prophylactic and therapeutic vaccines.
P12.09

Soluble CD40 Ligand Plays an Immunosuppressive Role in HIV Infection via IDO-Induced Tryptophan Catabolism, Treg Expansion and Microbial Translocation

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Background: CD40/CD40-ligand (CD40L) signaling is a key pathway which triggers the tryptophan (Trp) catabolizing enzyme IDO in dendritic cells and is immunosuppressive in cancer. We reported a higher IDO enzymatic activity and Trp catabolism in ART-Naïve patients resulting in an imbalance of Th17/Treg and microbial translocation. Here we assessed the link between sCD40L, Tregs and IDO activity in HIV-infected patients with different clinical outcomes.

Methods: Plasmatic levels of soluble CD40L (sCD40L) and inflammatory cytokines were measured by Beadlyte Multiplex in ART-naive, ART-successfully treated (ST), elite controllers (EC) and ART-naive, ART-successfully treated (ST), elite controllers (EC) and healthy controls (HS) (n=14 per group). Plasmatic levels of Trp and its metabolite Kynurenine (Kyn) were measured by isotope dilution tandem mass spectrometry and sCD14, a microbial translocation marker, was assessed by ELISA. IDO-mRNA expression was quantified by RT-PCR. The in vitro functional assay of sCD40L on Tregs induction and T cell activation (CD38/HLADR/PD-1) were assessed on PBMCs by RT-PCR. Caspase-9, bcl-2 and bax were analyzed by WB. The in vitro functional assay of sCD40L on Tregs induction and T cell activation (CD38/HLADR/PD-1) were assessed on PBMCs by RT-PCR. Caspase-9, bcl-2 and bax were analyzed by WB.

Results: sCD40L levels in ART-Naïve subjects were significantly higher compared to ST and HS (1305±876 vs 503±626 and 442±270 pg/mL; P<0.002) whereas EC showed a minor increase (969±944 pg/mL, P>0.05). In ART-Naïve, sCD40L was negatively correlated with CD4 T cell counts but not with viral load. sCD40L was positively correlated with IDO enzymatic activity (Kyn/Trp ratio) (P=0.004), Treg frequency (P=0.009), plasma sCD14 (P=0.04) and inflammatory cytokines IL-1β, IL-6, IL-10 and IL-15 in all HIV-infected patients. Such correlation with IDO quantity (IDO-mRNA expression) was observed only in ART-Naïve (P=0.02). In vitro functional sCD40L stimulation induced Tregs expansion (P=0.04) and favored Treg differentiation by reducing central memory (P=0.025) and increasing terminal effector Tregs (P=0.006) without impacting T cell activation defined by combination of CD38/HLADR/PD-1.

Conclusion: These results indicate that elevated sCD40L induce immuno-suppression in HIV infection by mediating IDO-induced Trp catabolism, Treg expansion and microbial translocation.

P12.10

Development of Apoptosis Resistance During Acute HIV Infection

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Background: Reservoir cells protection is clue during HIV infection. However, in spite of the relevance of these reservoir cells in the control of viral persistence, how and when apoptosis resistance of persistently-infected cells occurs is not well understood. Persistently infected cell lines showed to be apoptosis resistant under stress conditions, involving the intrinsic pathway (Fernandez Larrosa et al, Retrovirology, 2008 6 19). The goal of this work was to evaluate the dynamics of the achievement of the resistant phenotype in HIV acute infected cells.

Methods: Jurkat cells were infected with HIV (HXB2 strain) at moi=1 TCD/cell, treated with H2O2 as apoptosis-inducer, at different times postinfection (pi), and compared with uninfected (control) cells. Cell viability, p24+ production and apoptosis were evaluated by annexin/IP staining, PMM, and caspase-3 activation by cytometry analysis. Caspase-9, bcl-2 and bax were analyzed by WB.

Results: In Jurkat cells, infection didn’t induce an increase in apoptosis levels (~10% AV+/IP- cells) during the first 5 days pi. H2O2 treatment was associated to 15% apoptosis level in control cells, 12% in infected cells from day 3pi, with 9% from day 5pi (P<0.05). Mechanistic analysis in H2O2 treated cells revealed a significant decrease in caspase-3 activity (12% in control cells & 5% in day 5pi in infected cells, p<0.05) and a clear tendency in reduction in PMM (12% & 7%), caspase-9 activation ratio (2.5 % 1.5) and an increase in bcl-2/bax ratio (1.8 & 4). The percentage of HIV infected cells (p24+ cells) was the same at day 3 or 5 pi.

Conclusion: These results show that during a very early phase of acute infection, HIV infected cells show apoptosis protection to stress condition, and that this resistance involves modulation of the intrinsic pathway, similar to persistently infected cells. This in vitro resistance could help to understand the dynamics of HIV reservoir.
**P12.11**

**Defining Efficacious HIV-Specific Cytotoxic T Lymphocyte Responses Using Saporin-Conjugated Peptide-MHC Tetramers**

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**Background:** In vitro killing of HIV-infected cells by CD8+ cytotoxic T lymphocytes (CTL) is a surrogate marker of CTL antiviral efficacy. Tetrameric peptide-MHC complexes (tetramers) enable identification of antigen-specific CD8+ T cells in a mixed population. Due to the rapid internalization of tetramers by cognate T cells, tetramers are an attractive delivery vehicle to target specific CTL. Conjugating tetramers to saporin, a potent cytotoxin that inactivates ribosomal activity, we have optimized an assay to selectively eliminate CTL of different specificities. This approach facilitates rapid comparison of the antiviral potency of several distinct CTL specificities and identification of the most efficacious anti-HIV responses.

**Methods:** Human peripheral blood mononuclear cells (PBMC) were incubated with HLA-matched or mismatched saporin-conjugated tetramers (tet-SAP) or free saporin for 2h, washed and further cultured for up to 96h before cell loss was quantified by flow cytometry after labeling with corresponding phycoerythrin-coupled tetramers (tet-SAP) or free saporin for 2h, washed and further cultured for up to 96h before cell loss was quantified by flow cytometry after labeling with corresponding phycoerythrin-coupled tetramers (tet-PE). We then superinfected human CD4-enriched PBMC from long-term non-progressors with NL4-3 and cultured alone, with whole autologous PBMC, or with ‘tetramer-zapped’ autologous PBMC. Viral inhibition was assessed by p24 intracellular staining and ELISA over a 10-day time course.

**Results:** Tet-SAP but not free saporin binds to the surface of antigen-specific CD8+ T cells and is internalized by these cells. By 48h post-tet-SAP treatment, up to 97% of tetramer-specific cells are eliminated from the diverse CTL population. Preliminary viral inhibition assays indicate that selective elimination of different CTL specificities has a significant impact on antiviral activity.

**Conclusion:** We have optimized a novel method to eliminate CTL of different specificities in vitro and thereby measure their individual contribution to viral suppression. This rapid and cost-efficient method will enable us to discriminate between the most efficacious and futile CTL responses in HIV infection—knowledge that may increase our chances of developing a therapeutic vaccine.

**P12.12**

**Structure Prediction of Peptide-MHC Complexes for Identifying Immunogens Inducing Broad T Cell Reactivity**

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**Background:** Designing an optimal HIV-1 vaccine faces the challenge of identifying antigens that induce a broad immune capacity. The surface morphology of peptide-MHC complex is one of the key factors controlling the breadth of reactive T cell population. Therefore, accurately determining the structure of pMHC can help to predict robust T cell reactivity.

**Methods:** Here, we present an in silico protocol for predicting peptide-MHC structure. For the 17 test peptide-MHC complexes, a starting configuration of each complex was chosen by homology modeling. We then performed all-atom MD simulations on the ensemble of peptide-MHC conformations obtained by simulated annealing techniques. A robust signature of a conformational transition was identified during all-atom molecular dynamics, which results in a model with high accuracy.

**Results:** Our protocol showed that the average all atom root mean square deviation for the 17 test peptides to each X-ray structure was 1.58 Å. This protocol was used for a blind prediction test with a wild-type peptide (KVAELVHFL) and its two highly immunogenic mutants (KVAEIVHFL and KVAELVWFL). The blind test predicted that the center residues at position five of the analogs were configured to be accessible to solvent, forming a prominent surface, while the residue of the wild-type peptide was to point laterally towards the side of the binding cleft. The high resolution X-ray crystallography data verified these substantial conformational changes in both mutants.

**Conclusion:** Our observation strongly supports a positive association of the surface morphology of a peptide-MHC complex to its immunogenicity, offering the prospect of enhancing immunogenicity of vaccines by characterizing the conformation of MHC binding peptides. Our protocol is applicable for screening antigens to optimize vaccines for not only HIV infection but also other pathogenic infections and cancer where the breadth of T cell responses is pivotal for the outcome of the diseases.
Adaptation to HLA-I Is a Primary Correlate of HIV-1 Disease Progression

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Background: The extent to which cellular immune escape mutations impact HIV disease is unknown. We developed an adaptation score based on a novel multistate, phylogenetically-corrected logistic regression model that estimates the probability of an HIV sequence given an HLA class I repertoire.

Methods: An HLA allele-specific adaptation score was derived by comparing the probability of the sequence with and without the allele, conditioned on the other alleles expressed by the individual, and was averaged to produce a score for the individual’s class I repertoire. Models were trained on 1,888 full-proteome clade B and 2,083 Gag/Pol/NeF clade C sequences. Associations with VL and CD4 were assessed out of sample using cross-validation and in independent cohorts of elite controllers and linked transmission pairs. All associations were conditioned on HLAs using stepwise regression.

Results: Adaptation was a more significant predictor of both VL (pCladeC=6E-12, NCladeC=1461; pCladeB=9E-6, NCladeB=1025) and CD4 (pCladeC=4E-13, NCladeC=1260; pCladeB=1E-11, NCladeB=1025) than any HLA allele. Adaptation score correlated with VL and CD4 independently, and remained significant in the context of CTL responses to sets of overlapping peptides (pVL<0.002, pCD4<0.0001, N=435). Importantly, adaptation neutralizes the protective effects of all known protective alleles except A*74. Adaptation was significantly lower among 23 elite controllers than 78 progressors (p=0.0006, B57+ p=0.02, B57/- B27- p=0.008). Among transmission pairs, adaptation of the donor sequence to HLA alleles expressed by the recipient predicted higher VL 24 months post transmission (n=0.34, p=0.002, N=81) and shorter time to CD4<250 (HR=3.45, p=0.01); adaptation at 3, 6, 12 and 18 months post transmission predicted subsequent changes in VL.

Conclusion: HLA-mediated adaptation is a primary correlate of disease progression in chronic HIV-1 infection and predicts subsequent VL increases and rate of CD4 decline in a longitudinal transmission pair cohort. Vaccine strategies that reduce the incidence of escape may be imperative.
**P12.15**

**PHPC T Cell Responses in Macaques Vaccinated with a Single Dose of a Novel Non-integrative One Cycle Limited SHIV-Based DNA Vaccine**

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**Background:** Innovative strategies/vectors to induce immune responses that are capable of containing persistent infectious pathogens such as HIV-1 are hardly needed. Variety of vectors has been used in animal models of HIV vaccine but they fail to recapitulate the efficacy of Live-attenuated vaccines. These latter are unsafe to be used in humans. In this work, we developed a novel lentiviral-based DNA vector non-integrating and undergoing a single cycle of replication: CAL-SHIV-IN

**Methods:** Macaques were immunized with a single dose of CAL-SHIV-IN DNA by intramuscular and intradermal routes of injection plus electroporation. We assessed the vaccine induced T cells responses on PBMCs directly ex vivo or after culture of 12 days in vitro under antigenic and homeostatic driven proliferation conditions. We used conventional INF-γ ELISPOT assay to measure immediate cytokine secretion from antigen specific effector cells and from memory Precursor with High Proliferative Capacity (PHPC). We further identified vaccine-specific T cells and determined their memory phenotype and functions (proliferation, cytokines expression) with multiparametric FACS based assays.

**Results:** We found that all immunized macaques developed and maintained peripheral CD8+ and CD4+ T cells responses mainly against Gag and Nef antigens. After an extensive time following initial immunization (week 40 to 47), in absence of persistent antigen stimulation, all vaccinated macaques showed strong PHPC responses containing vaccine specific central memory and effector memory T cells that have expanded and acquired immediate effector functions. Importantly, PHPC responses have been detected in HIV Elite controllers and found to be associated with central memory responses. Thus, the evaluation of T cell responses by PHPC assay might be an attractive tool for the testing of prophylactic vaccines.

**Conclusion:** We demonstrated that a single dose delivery of a one cycle replication SHIV-based DNA lentivector allowed efficient generation and maintenance of PHPC T cell responses with both memory and effector cell characteristics. Importantly, PHPC responses have been detected in HIV Elite controllers and found to be associated with central memory responses. The CD8+ T cell compartment, the same significant trends were observed among groups. Moreover, PHI>350 had two-fold higher proportion of T naive-like cells than PHI<350 (17.14% vs 9.2%). Spearman’s correlation within PHI group showed that the proportion of total T naive was directly associated with baseline CD4 count (r=0.007) and inversely with immune activation (p=0.009). Conversely, percentage of T CM inversely correlated with baseline CD4 count (p=0.0035), immune set-point (p=0.006) and directly with viral load (VL). 

**Conclusion:** Results suggest that a less differentiated memory profile at early stages post-infection is associated with a slower disease progression, in terms of CD4 count and VL, indicating that not only CD8+ function but also phenotype is associated with disease control.

**P12.16**

**Distribution of HIV-Specific and Total CD8+ T Cell Memory Phenotypes During Acute/Early HIV Infection Is Associated with Disease Progression**

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**Background:** Memory CD8 + T cells are important components of protective immunity. Understanding their development during primary HIV-infection (PHI) would contribute to optimal vaccine design. Aims: To analyze total and HIV-specific CD8 + T cell differentiation phenotypes, during acute/early infection, and their correlation with clinical parameters.

**Methods:** Twelve HIV + seroconverters (PHI group), 6 elite controllers (EC) and 4 Chronics were enrolled. Seroconverters were classified as “PHI<350” if CD4 count dropped below 350 cells/ul within 12 months post-infection. Phenotypic (CD45RO, CCR7) and functional markers (cytokines) were used to identify total and HIV-specific CD8 + memory populations. Data was compared inter-groups and correlated to viral load (VL), CD4 counts and both virological and immunological set-points, using non-parametric statistics.

**Results:** The CD8 + T cell differentiation hierarchy was terminal effectors (T E)>naïve (T naive)>effector memory (T EM)>central memory (T CM) in all groups. However, EC showed higher proportion of T naive (38.3%) compared to Chronics (17.44%, p=0.0027) and PHI subjects (29.63%, p>0.05). EC had the lowest proportion of T naive cells (53.18%). T naive was higher in Chronics (19.09%) compared to EC (4.71%, p=0.0046) and PHI (4.59%, p=0.0001). Regarding HIV-specific CD8 + T cell compartment, the same significant trends were observed among groups. Moreover, PHI>350 had two-fold higher proportion of T naive-like cells than PHI<350 (1714% vs 9.2%). Spearman’s correlation within PHI group showed that the proportion of total T naive was directly associated with baseline CD4 count (p=0.007) and inversely with immune activation (p=0.009). Conversely, percentage of T CM was inversely co-related with baseline CD4 count (p=0.0035), immune set-point (p=0.0006) and directly with immune activation (p=0.0022). The same significant associations were observed within the HIV-specific compartment. Additionally, HIV-specific T CM directly correlated with viral set-point (p=0.0269).

**Conclusion:** Results suggest that a less differentiated memory profile at early stages post-infection is associated with a slower disease progression, in terms of CD4 count and VL, indicating that not only CD8 + function but also phenotype is associated with disease control.
**P12.17**

**Mapping and Characterization of HIV-1 Specific Immune Responses Present in Highly HIV Exposed Uninfected Individuals**

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**Background:** To evaluate the specific T cell immune response in highly HIV exposed seronegative individuals (HESN). Previously reported data indicate that virus-specific responses are rarely detected by conventional approaches. We report here the reproducible detection of cellular immune responses in HESN by two novel and improved assays that could inform further development of a preventive HIV vaccine.

**Methods:** HIV-specific T cell responses were analyzed by ELISPOT in PBMC from HESN after a 48h-co-culture with “accelerated” dendritic cells (acDC) in the presence of IL4 and GM-CSF and maturation cocktail. Additionally, a “boosted” flow cytometry approach was employed to more comprehensively capture the anti-viral T cell response to HIV by combining the detection of several effector functions into the same fluorescence channel.

**Results:** Of 29 HESN individuals tested, 11 (38%) showed at least one response to peptide pools covering HIV Gag and Nef. Additionally, using VSV-pseudotyped HIV RT-defective vector as antigen source, positive responses were seen in almost 20% of the individuals tested. A positive correlation was observed between the intensity (p=0.0007) and magnitude (p=0.0137) of the response detected in the HESN and the viral load of the HIV+ partner. Moreover, the result from the boosted flow and cytometry analyses showed a dominant “Th1”-like response pattern against HIV antigens in both, CD8 and CD4 T cell populations.

**Conclusion:** The combined use of our improved acDC technique with a boosted flow cytometric approach allows to detect specific HIV positive responses in a higher percentage of individuals than generally seen with standard assays and to define specific effector function profiles in responses from HESN. The validity of the data is further validated by the positive correlation between the strength of the elicited response and the viral load of the sexual partners.

**P12.18**

**The Use of HLA Class II Associated HIV Polymorphisms in Predicting Novel CD4 T Cell Responses and Viral Escape**

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**Background:** CD4 T cells are the primary targets of HIV infection; however, their role in controlling viral replication remains unclear. We hypothesized that HIV adapts in response to CD4 T cell mediated immune pressure.

**Methods:** Using HIV sequence data and HLA-II alleles, we applied a novel computational approach to identify HIV-1 polymorphisms disproportionately associated with HLA-II alleles in a large African cohort of chronically HIV-infected individuals. Using this approach, we identified 17 HLA class-II associated adaptations. A panel of 34 predicted adapted and nonadapted CD4 T cell epitopes (15-20mer) encompassing the identified polymorphisms were synthesized and evaluated for immunogenicity. Peripheral Blood Mononuclear Cells (PBMCs) from 10 uninfected controls, 20 chronically infected individuals on ART, 11 HIV-controllers (ART naive, viral load <2000) and 8 HIV non-controllers (ART naive, viral load >10,000) were depleted of CD8+ cells and CD4+ T cell responses were quantified using interferon-γ ELISpot assay.

**Results:** The median magnitude (SFC/10^6 cells) of HIV-specific CD4 T cell responses seen in controllers (451; Range 70-2123) was higher compared to non-controllers (223; Range 60-997) p=0.004, and tended to be higher compared with ART treated individuals (343; Range 55-1303). Positive pool responses were mapped to the individual peptide. Some, but not all individuals encoded the HLA-II alleles associated with the predicted HIV polymorphism biologically confirming the CD4 T cell epitope predictions. The magnitude of responses for the adapted epitopes was lower compared to non-adapted epitopes (P=0.0003).

**Conclusion:** We have applied a novel computational approach to identify novel HLA class-II epitopes enabling us to correlate the magnitude and breadth of CD4 T cell responses with HIV progression. The finding that adapted peptides had significantly lower responses also lend credence to the use of HLA-II associated HIV polymorphism analysis in predicting CD4-mediated viral evolution. Our findings will help identify novel CD4 T cell targets relevant to future HIV vaccine design.
P12.19

Cross-Clade CD8+ T Cell Responses in Chronically HIV-1 Clade A/E-Infected Individuals

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Background: Cytotoxic T lymphocytes (CTLs) that can recognize HIV-1 variants are one of key factors for the development of a widely applicable AIDS vaccine. Although CTL responses to HIV-1 clade B and C were well studied, there are only a limited number of studies about the cross-clade reactivity of CTLs. In the present study, we investigated cross-clade CD8+ T cell responses between clade B and A/E viruses in chronically HIV-1 clade A/E-infected Japanese individuals.

Methods: We analyzed cross-clade CTL responses by using 11-mer overlapping HIV-1 peptides derived from the clade B consensus sequence spanning Nef, Gag, and Pol regions, and identified cross-clade epitopes. We analyzed cross-clade CTL recognitions for cells infected with clade B or A/E viruses by using an intracellular cytokine staining assay and finally confirmed cross-clade CD8+ T cells in 28 clade A/E-infected individuals by using an ELISPOT assay.

Results: CD8+ T cell responses to 11-mer overlapping peptides derived from clade B were at a similar level to those to the same peptides found in 401 clade B-infected individuals. Fifteen cross-clade CTL epitopes were identified from 13 regions where the frequency of responders was high in the clade A/E-infected individuals. The sequences of 6 epitopes were conserved between clade B and A/E viruses, whereas 9 epitopes had different amino acid sequences between the 2 viruses. CD8+ T cells specific for 8 diverse epitopes were recognized by both the clade A/E virus-infected and clade B-infected cells. All of the cross-clade CD8+ T cells specific for conserved and diverse epitopes were detected in chronically HIV-1 clade A/E-infected individuals.

Conclusion: These results showed that cross-clade CD8+ T cells were frequently elicited in HIV-1-infected individuals and that in addition to conserved regions polymorphic ones across the clades can be targets for cross-clade CTLs. The present study highlights cross-clade epitopes for potential use in widely applicable CTL vaccines.

P12.20

HLA-Specific Intracellular Epitope Processing Mechanisms Shape an Immunodominance Pattern for HLA-B*57 that Is Distinct from HLA-B*58:01

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Background: HLA-B*57 is strongly associated with immune control of HIV and delayed AIDS progression. The closely-related, but less protective, HLA-B*58:01 presents similar epitopes, but HLA-B*58:01 positive individuals do not generate CD8+ T cells targeting the KF11-Gag epitope linked with low viraemia.

Methods: We tested KF11 peptide binding affinity, off-rate, tetramer staining and presentation from HLA-B*57:01, B*57:03 and B*58:01 along with KF11 epitope processing from HIV-infected CD4+ T cells to KF11 specific CD8+ T cells.

Results: Here we show that HLA-B*58:01 binds and presents KF11 peptide, but fails to process KF11-Gag epitope linked with low viraemia. HIV infected individuals heterozygous for HLA-B*57:03/B*58:01 do make KF11 specific CD8+ T cells, suggesting that HLA-B*58:01 does not abrogate KF11 epitope processing in vivo.

Conclusion: This unexpected finding demonstrates that immunodominance patterns can be influenced by intracellular events independent of HLA binding motifs.
**P12.21**

**Increased Cytotoxic Capacity of Immunodominant HIV-Specific CD8+ T Cell Responses Is Associated with Immune Control Regardless of HLA Type**

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**Background:** HIV-specific CD8+ T cell cytotoxic responses have been implicated in the control of HIV replication in B*27/57+ long-term nonprogressors / elite controllers (LTNP/EC). To determine whether most LTNP/EC regardless of HLA type control HIV replication by a similar mechanism, we measured the immunodominant HIV-specific CD8+ T cell responses and assessed their cytotoxic capacity in B*27/57-negative and B*27/57+ LTNP/EC.

**Methods:** Peripheral blood mononuclear cells from 23 B*27/57-negative and 23 B*27/57+ LTNP/EC were stimulated with overlapping 15-mer peptides spanning Nef, Gag, and Pol and analyzed for intracellular IFN-γ accumulation by flow cytometry. Determination of optimal epitopes targeted by dominant responses was performed with peptide truncations. HIV-specific CD8+ T cell-cytotoxic responses to autologous HIV-infected CD4+ T cell targets were measured by flow cytometric detection of granzyme B delivery to live targets and infected CD4 elimination (ICE) in 17 B*27/57-negative and 18 B*27/57+ LTNP/EC. HLA restriction was assessed with heterologous targets matched at a single HLA allele.

**Results:** Although the magnitude of the total HIV-specific CD8+ T cell response following 6-hour stimulation was equivalent between B*27/57-negative and B*27/57+ LTNP/EC (p>0.05), the optimal epitopes targeted by the immunodominant responses were variable. Following 6-day stimulation, bulk cytotoxic responses of B*27/57-negative and B*27/57+ LTNP/EC were similar based on GrB target cell activity (p>0.5) and ICE (p>0.05). The constituent dominant cytotoxic responses differed between B*27/57-negative and B*27/57+ LTNP/EC and were largely mediated by the immunodominant specificities determined in the 6-hour assays.

**Conclusion:** Even though the epitopes targeted by immunodominant HIV-specific CD8+ T cell responses differed between B*27/57-negative and B*27/57+ LTNP/EC, the increased cytotoxic capacity of these responses was comparable between the groups, suggesting a common mechanism of immune control. These results provide reason for optimism regarding the T cell component potentially stimulated by HIV vaccines by showing that robust HIV-specific CD8+ T cell cytotoxic responses can also be induced in individuals who are not B*27/57+.

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**P12.22**

**Cryptic Epitopes Restricted by Protective HLAs (B*57, B*5801 and B*27) Are Frequently Targeted in Chronic HIV Infection**

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**Background:** Cryptic Epitopes (CE) are peptides derived from the translation of one or more of the five alternative reading frames (ARFs; 2 sense and 3 antisense) of existing genes and are commonly targeted during HIV/SIV infection. In this study, we aimed to understand the role of HIV specific CE predicted to be restricted by protective alleles in HIV-1 pathogenesis.

**Methods:** Peptides (9-11mer) were formulated based on binding algorithms for HLA-B*27, B*57 or B*5801 (protective alleles or PA) and HLA-B*5301 (non-protective allele or NPA). Peptides encompassing all five ARFs of each of the nine HIV-1 encoded proteins and with >50% probability of being an epitope were synthesized and tested for immunogenicity; B*27 (N=30), B*57 (N=39), B*5801 (N=90) and 72 (B*5301). Majority of these peptides were encoded by antisense ARFs (p=0.005 X10^{-49}). PBMC from HIV seronegative donors (N=42) and chronic HIV clade B infected patients (CHI, N=127 total) were used to evaluate T cell responses in an IFN-g ELISpot and ICS assay.

**Results:** Among the PA group (N=87), a majority of patients were B*57 (57%) and were off ART (56%). Overall, 22% and 2% of CHI patients had CE responses in the PA and NPA group respectively (p=0.006, Fischer’s exact). Seronegative donors had a 2% response to CE. In PA group, 21 novel CE specific responses were mapped (median magnitude of 95 SFC/10^6 PBMC). CE specific responses also showed proliferative capacity in a 5 day CFSE based assay. The median VL off ART for responders and non-responders in the PA group was not statistically different.

**Conclusion:** These data underscore the importance of CE targeting especially those that are presented by the so-called “protective alleles” in HIV-1 infection and hence have implications for vaccine design.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L2, Room 211 – 212. Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L2, Room 211 – 212.

### P12.23

**Vif-Derived Epitopes Can Be Efficiently Recognized by Cytotoxic T Lymphocytes**

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**Background:** Determination of viral antigen targets for effective cytotoxic T lymphocytes (CTLs) could be important for development of a T cell based AIDS vaccine. Recent studies have suggested a great contribution of CTL responses specific for some Vif epitopes to simian immunodeficiency virus (SIV) control in rhesus macaques (AIDS 24:2777, 2010; Nature 491:129, 2012). Here, in order to evaluate whether epitopes in the context of Vif can be efficiently recognized by specific CTLs.

**Methods:** We investigated efficiency of target killing by CTLs specific for Mamu-A1*065:01-restricted SW9 (Gag241-249) epitope. Mamu-A1*065:01-positive B-lymphoblastoid cell lines (BLCLs) infected with Sendai virus vectors expressing EGFP (SeV-EGFP), SW9-EGFP fusion protein (SeV-SW9EGFP), and Vif-SW9 fusion protein plus EGFP (SeV-VifSW9-EGFP), respectively, were used for the target cells. After 4-hr coculture of these target cells with SW9-specific CTLs, GFP-positive cell frequencies were measured to assess killing efficiency.

**Results:** Negative control target BLCLs infected with SeV-EGFP showed no killing. SeV-SW9EGFP-infected and SeV-VifSW9-EGFP-infected BLCLs both showed specific killing by SW9-specific CTLs, but the killing efficiency in the latter expressing SW9 in the context of Vif (58%) was significantly higher than the former (32%).

**Conclusion:** Our results suggest that Vif-derived epitopes can be efficiently recognized by specific CTLs, implying that Vif is a promising immunogen for T cell based AIDS vaccines.

### P12.24

**Dual Recognition of Cells Infected with Wild-Type and Escape Mutant Viruses by Cytotoxic T Cells Recognizing Overlapping 8-Mer and 10-Mer Nef Peptides**

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**Background:** It is assumed that presentation of overlapping peptides induces different CTL responses to these peptides which may contribute to effective control of HIV-1 replication and selection of escape. However, the recognition of HIV-1-infected cells infected with wild-type and escape mutant viruses by these CTLs has not been well studied. We here investigated immunological and structural aspects of recognition of HIV-1-infected cells by HLA-A 24:02-restricted CTLs for 2 overlapping Nef epitopes (Nef138-8: RYPLTFGW and Nef138-10: RYPLTFGWCF).

**Methods:** Abilities of CTLs to suppress HIV-1 replication and to select escape mutation were investigated by HIV-1 replication assay and competitive one, respectively. The frequency of specific T cells was measured by tetramer staining. Crystals of HLA-A *24:02 molecules with the peptides were grown using hanging drop method and the structures were determined by X-ray diffraction.

**Results:** Tetramers binding analysis revealed no cross-recognition between RW8 and RF10 by the 8mer- and the 10mer-specific T cells. This was confirmed by crystallographic analyses of 2 HLA-A *24:02 crystals with RW8 and with RF10 that demonstrated a structural similarity between the former structures but difference between the latter ones.

**Conclusion:** The present study demonstrated that patients infected with WT virus can elicit cross-reactive 8-mer-specific and WT 10-mer specific CTLs and then they can effectively select the 2F mutant.
P12.25

Selection of Pol283-8V Mutant by Pol283-8-Specific CTLs in HLA-B*51:01+Long-Term Non Progressors

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Background: We previously showed that 8V mutation at an Pol283-8(TI8) epitope, which was found in only long-term non progressors (LTNPs) of HLA-B*51:01 + Japanese hemophiliacs, weakly reduced TI8-specific CTL recognition, suggesting that selection of the 8V mutant was a critical factor for the long-term control of HIV-1. However, the mechanism underlying selection of the 8V remains unclear. We here investigated how the 8V was selected by TI8-specific CTLs. To clarify the mechanism of the long-term control of HIV-1 in HLA-B*51:01 + LTNPs having the 8V, we further analyzed the function of the CTLs elicited after selection of the 8V.

Methods: We measured the ability of TI8-specific CTL clones to suppress the replication of the 8V mutant virus and to select this mutation in CD4 + T cells infected with the 8V and WT viruses. Furthermore, we investigated the affinity of TCR on the CTL clones by using tetramers of HLA-B*51:01-Pol283 or -Pol283-8V peptide complex.

Results: TI8-specific CTL clones, which were established from PBMCs collected at the time that WT virus was found, had a little weaker ability to suppress the replication of the 8V mutant compared to that of WT virus. They could select the 8V in vitro. In contrast, the CTL clones, which were established from PBMCs after selection of the 8V, had similar ability to suppress the replication of the 8V as that of WT virus and failed to select the 8V in vitro. The former CTLs carried TCR with stronger affinity to the WT tetramer than that to the 8V one, whereas the latter ones carried TCR with similar affinity to both tetramers.

Conclusion: These results suggest that Pol283-8V is an escape mutant selected by TI8-specific CTLs carrying TCR with weak affinity to the 8V. This mutant can elicit the CTLs evenly recognizing WT and 8V mutant so that they can control HIV-1 after the emergence of the 8V in HLA-B*51:01 + LTNPs.

P12.26

Expression of NKG2A on CD8+ T Cells Correlates with Markers of Disease Progression in Chronic HIV-1 Clade C Infection

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Background: HIV-1 specific CD8+ T cells have been strongly implicated in the control of viral replication. However, during chronic HIV-1 infection, HIV-1-specific CD8+ T cells progressively lose critical effector functions. Inhibitory Natural Killer Receptors (NKRs) such as NKG2A/CD94 are up regulated and on antiviral CD8+ T cells during acute polyoma infection in mice and are responsible for down-regulating antigen-specific cytotoxicity during both viral infection and virus-induced oncogenesis. The up regulation of inhibitory NKRs may serve to restrain antiviral CD8+ T cells following antigen clearance as a mechanism to limit immunopathology. The effect of inhibitory NKR receptor expression on HIV-specific CD8+ T cell function and HIV-1 clinical progression has not been characterized.

Methods: We used multi-parametric flow cytometry to compare the frequencies of NKG2A CD8+ T cells from 24 chronically infected HIV-1 patients and 12 HIV-negative controls from Durban, South Africa.

Results: Our data show elevated frequencies of CD8+NKG2A+ T cells (p=0.01) and as well as increased expression of NKG2A receptors (p=0.03) on CD8+ T cells from HIV-1 positive patients in comparison to HIV-negative controls. There was significant inverse correlation between frequencies of CD8+NKG2A+ T cells and absolute (abs) CD4 count (p=0.03 Spearman r=-0.44) and significant positive correlation between frequencies of CD8+NKG2A+ and HIV-1 log viral load (VL) (p=0.03; Spearman r=0.45). Interestingly, the frequencies of CD8+NKG2A+ T cells also positively correlated with frequencies of the T cell exhaustion marker PD-1 on CD8+ T cells (p=0.05; Spearman r=0.44) and the senescence marker CD57 on CD8+ T cells (p<0.001; Spearman correlation 0.64).

Conclusion: Our data suggest that NKG2A expression may contribute to the functional impairment of HIV-specific CD8+ T cells during chronic HIV-1 infection and NKG2A+CD8+ T cells may represent an important population of exhausted T cells. Understanding how NKG2A and other NKRs regulate of antiviral CD8+ T cell responses may provide novel insights into potential therapeutic targets aimed at harnessing T cell immunity for prophylactic HIV vaccine strategies.
HIV-1 Infection Impairs Regulatory T Cell Function

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Background: Understanding disease progression and the nature of the immune response needed for protection are essential to continue the pursuit of an effective HIV-1 vaccine. The impact of Tregs on HIV-1 immune pathogenesis, including potential benefits through suppression of HIV-1-associated generalized immune activation and control of HIV replication, versus possible deleterious effects via suppression of HIV-1-specific immunity, remains incompletely understood. While it has been shown that Tregs can be infected by HIV-1 in vivo and in vitro, the consequences of HIV-1 infection on a per cell basis have not been investigated to date.

Methods: CD4+CD25+CD127- Tregs were isolated from healthy donors and infected with a VSV-pseudotyped HIV-1-GFP virus. GFP-positive (infected) and GFP-negative (non-infected) Tregs were isolated using flow-based cell sorting and their capacity to suppress the proliferation of T cells was tested in vitro. RNA from infected- and non-infected Tregs was isolated and expression of 511 genes was compared using the NanoString technology.

Results: Expanded Tregs were readily infectable by HIV-1-GFP in vitro. We found that HIV-1-infected Tregs were significantly less suppressive than non-infected Tregs, suggesting that HIV-1 infection impairs Treg function on a per-cell basis. Gene expression analysis showed 23 genes differentially expressed after HIV-1 infection. Several genes involved in the Treg lineage and function were down regulated after infection and impaired suppressive function.

Conclusion: Our data suggest that HIV-1 infection alters suppressive function at a single cell level. HIV-1 Infection of Tregs may thereby impair their capacity to limit detrimental generalized immune activation and promote disease progression by providing additional targets. We believe this study provides important insights on the role of regulatory T cells in the context of HIV-1 infection and may lead to new approaches for developing an effective HIV-1 vaccine.

Extensive Yet Ineffective Gag Epitope Variant Recognition Observed in HIV-Progressors

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Background: Efficacy of future HIV vaccines will likely depend on the capacity of the induced immune responses to cope with the extensive sequence diversity found in circulating strains. Vaccination strategies shown to induce T cell responses with improved epitope variant recognition in NHP models are moving into human phase I clinical trials. In order to fully evaluate the protection potential of these vaccines, a better understanding of variant recognition and its role in HIV control is imperative.

Methods: We comprehensively evaluated Gag epitope variant recognition in an untreated cohort of 15 HIV-controllers (viral load [VL] <2,000 RNA copies/ml) and 15 HIV-progressors (VL>10,000 RNA copies/ml). A Gag peptide set of 11-mers overlapping by 10 amino acids (AA) was generated to reflect all variants found in ≥5% of clade B sequences in the LANL HIV Sequence Database. This set includes 1300 peptides and covers all AA in Gag. All subjects were screened for responses by IFN-γ/IL-2 FluoroSpot. For each epitope, variant recognition was calculated as the proportion of variants eliciting a response among all variants tested.

Results: Progressors trended towards recognizing a greater proportion of naturally occurring variants per epitope than controllers (median 56% per epitope vs. 49%, p=0.06). Variant recognition was significantly correlated with VL in individuals with detectable VL (r=0.61, p=0.001). Gag sequencing showed that viral diversity within epitopic regions was higher in progressors than controllers (p=0.0005). Interestingly, viral populations in progressors more often matched the recognized epitope variants than observed in controllers (p=0.13).

Conclusion: These data suggest that diverse viral populations drive epitope variant recognition in progressive infection, but that responses to variants were largely ineffective in either controlling or applying pressure on HIV. Future investigations are necessary to identify immune correlates of effective variant recognition.
P12.29

Ad5 but Not Ad26 Induces an IFN-\(\gamma\)+IL-10+ CD4+ T Cell Population Following Immunization

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**Background:** Adenovirus-based vaccines have arisen as an attractive method to elicit robust cellular and humoral immune responses. Multiple vector serotypes have been developed, but the CD4 T cell responses generated by these vectors have not previously been compared. We therefore, compared the functionality of antigen-specific CD4 T cells induced following Ad5 or Ad26 immunization.

**Methods:** C57BL/6 mice were immunized intramuscularly with 10\(^{10}\)vp of Ad5-SIVgag or Ad26-SIVgag. In another set of experiments, mice were immunized with increasing concentrations of Ad5-SIVgag (10\(^{7}\) vp to 10\(^{10}\) vp). For both experiments, spleen cells were harvested at day 10, and SIVgag-specific CD4 + T cell responses were evaluated by flow cytometry.

**Results:** Immunization with 10 \(^{10}\)vp of Ad5-SIVgag induced a population of IL-10 + producing cells that comprised 30% of gag-specific IFN-\(\gamma\)+ CD4+ T cells. In contrast, the number of CD4+ T cells that co-produced IFN-\(\gamma\) and IL-10 was 8 times lower following Ad26 immunization (p<0.0001). These cells were T-bet + and FoxP3 -. In addition, Ad5 immunization induced a substantially larger fraction of IL-10 + cells that co-expressed PD-1 than Ad26 immunization (85% versus 23%). Moreover, a larger fraction of IFN-\(\gamma\)+ CD4+ T cells were Tim-3 + following Ad5 versus Ad26 immunization (23.7% versus 6%). We also observed increasing frequency of IFN-\(\gamma\)+IL-10+ CD4+ T cells with increasing doses of Ad5.

**Conclusion:** Ad5 vectors, but not Ad26 vectors, induce a large population of CD4 + T cells that produce IFN-\(\gamma\) + and IL-10, an immunosuppressive cytokine that may potentially limit vaccine efficacy. Both the dose and the serotype of the vaccine vector impacted the generation of this population of regulatory CD4 + T cells.

P12.30

Limited HIV Infection of Central Memory CD4+ T Cells Is Associated with Lack of Progression in Viremic Individuals

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**Background:** A rare subset of HIV-infected individuals, viremic non-progressors (VNP), remain asymptomatic and maintain preserved CD4+ T cells despite persistently high viremia. While these individuals have been characterized, mechanisms underlying protection from AIDS progression are unclear.

**Methods:** To identify mechanisms potentially responsible for delayed disease progression in VNPs, we compared VNPs (average >9 years of HIV infection) to HIV-infected individuals with similar CD4+ T cell counts and viral load, but analyzed within 2 years from infection (“putative progressors”, PP), thus avoiding the confounding effect of immunological defects present in chronically HIV-infected subjects.

**Results:** We found that VNP, compared to PP, expressed similar levels of T cell activation and a comparable profile of gene expression, but higher levels of the cell cycling marker Ki67 on CD4+ T cells, a finding suggestive of preserved CD4+ T cell homeostasis. Importantly, VNP showed lower levels of HIV infection of central memory CD4+ T cells (P<0.0272), but not effector memory, compared to PP.

**Conclusion:** Our results suggest that decreased levels of central memory CD4+ T cell infection in HIV-infected VNP may be a key contributor to preservation of CD4+ T cell homeostasis and lack of disease progression despite high viremia. These data suggest that targeting prophylactic vaccines to prevent HIV infection of central memory CD4+ T cells, or therapeutic vaccines to decrease levels of HIV infection in central memory CD4+ T cells, may be essential in the development of novel vaccination strategies.
**P12.31**

**Polyfunctional T Cell Responses to Gag p24 Is Sustained During Effective Anti-retroviral Therapy**

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**Background:** The goal of many therapeutic HIV-1 vaccines is to induce effective CD8 T cell responses in order to control HIV-1 disease progression, often in combination with anti-retroviral therapy. Three qualities of the HIV-specific CD8 T cells could be involved in this control: frequency, breadth of epitope recognition and functional quality. Knowledge about the residual immune response during effective treatment and suppressed viral load is therefore important for the development of an HIV-1 vaccine.

**Methods:** We determined the magnitude, breadth and quality of the HIV-1 specific T cell response in HIV-1 infected viremic individuals (n=19) and individuals on highly active anti-retroviral treatment (HAART) (n=14) following stimulation with peptide pools covering the entire HIV-1 genome (clade B). Cytokine response (IFN-gamma, TNF-alpha, IL-2, MIP-1b and CD107a) was measured by flow cytometry.

**Results:** We found that magnitude and breadth of the CD8 T cell response was significantly higher in viremic individuals than individuals on HAART (p<0.0001 and p<0.0001, respectively) and that the functionality of the overall HIV-1 specific response was significantly different in individuals on HAART and viremic individuals (p=0.0020). In individuals on HAART, the remaining responses were primarily detected upon stimulation with overlapping peptides from Gag p24, integrase and Nef. The Gag p24 response was more polyfunctional than corresponding responses observed in viremic individuals.

**Conclusion:** Identification of highly immunogenic regions also recognized by individuals on HAART may be important for HIV-1 vaccine development. Irrespective of HLA haplotype, we have identified specific regions within the HIV-1 genome that is targeted more frequently in individuals on HAART. These particular regions could be interesting for a future vaccine that might limit the time and opportunity for escape mutations.

**P12.32**

**Synergistic Effect of Multiple Ctls on Control of HIV-1 in Japanese Individuals Carrying HLA-B*35:01 Allele Associated with Rapid Disease Progression**

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**Background:** Our recent study have demonstrated that HLA-B*35:01 is associated with high viral load (VL) in chronically HIV-1 clade B-infected Japanese individuals (Naruto T. et al. J. Virol. 2012). However, some HIV-1 clade B-infected HLA-B*35:01-positive Japanese individuals showed low VL. But it still remains unclear how HIV-1 can be controlled in these individuals.

**Methods:** To investigate the effect of HIV-1 specific CTL responses on the control of HIV-1 infection in HLA-B*35:01-positive Japanese individuals, IFN-gamma Elispot assay was performed using 15 HLA-B*35:01-restricted epitopes in 64 HLA-B*35:01-positive Japanese individuals chronically infected with HIV-1.

**Results:** We found significant associations between the individual response to Nef YY9 or p17 NY9 epitope and lower VL (p=0.0039, or p=0.0233, respectively). To investigate synergistic effect of CD8+ T cell responses on the control of HIV-1 infection in HLA-B*35:01-positive clade B infected individuals, we first performed statistical analysis of the correlation between the total responses to the 15 epitopes and VL. Breadth of them was significantly associated with VL (p=0.0125). Furthermore, in the comparison of each group, the VL of responders to 3 or 4 epitopes (median 14,000 copies/ml) was significantly lower than that of non-responders (median 375,000 copies/ml, p < 0.001).

**Conclusion:** These results suggest that the multiple CD8+ T cell responses to RT VY10, p17 NY9, Nef RY11, and Nef YY9 epitopes were critical in the control of viraemia even in HIV-1 clade B-infected HLA-B*35:01-positive individuals.
**P12.33**

**HIV- Toggle Peptides Identify Super-agonistic Minor Sequence Variants in HIV Gag and Broad T Cell Reactivity to Nef Variants**

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**Background:** Minor sequence variants have been suggested to be of potentially increased immunogenicity and thus being selected against in natural HIV infection. Toggle peptides (TP), representing an approach where sequence variability is directly incorporated into the peptide synthesis, has the potential to identify such superagonistic minor variants with relative ease. Their inclusion in future T cell immunogen sequences could help broaden and functionally improve vaccine induced responses.

**Methods:** 50 HIV-1 clade B infected subjects (28 HIV controllers, 22 no-controllers) were assessed for HIV-specific T cell responses against 14 Toggle peptides and their corresponding consensus overlapping peptide (OLP) sequence and individual variants. Nine TP were located in Gag, the remaining 5 in Nef. Ex-vivo responses were detected by IFNg ELISpot.

**Results:** In agreement with earlier reports, HIV controllers recognized overall more variant peptides and mounted stronger responses than non-controllers (3790 SFC [259-11752] vs 1166 SFC [98-2451] for magnitude (p=0.002) and 6.7 [2.1-13.6] vs 3.06 [0.62-5.1] for number of targeted variants (<0.0001), respectively. In two of the Gag TP, two minor variant sequences were identified, each augmented the frequency of reactivity 2.5-fold and increased the magnitude of the response up to 20-fold compared to the consensus-sequence based OLP. Surprisingly, the frequency of variant recognition and the magnitude of variant-specific responses was higher for Nef TP compared to TP covering Gag (p=0.007 and p=0.059, respectively).

**Conclusion:** Our results establish the differential screening with Toggle and consensus OLP as a rapid approach to identify minor epitope variants with potentially superior immunogenicity than consensus sequences. Further analyses are needed to discriminate between broad Nef variant recognition and the presence of T cell populations targeting individual variant Nef peptides and to determine whether the identified superagonistic variants in Gag can induce responses with stronger anti-viral activity.

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**P12.34**

**Analysis of the Relationship Between Founder Virus Fitness, Primary T Cell Response Breadth and Viral Control in Acute HIV-1 Infection**


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**Background:** Understanding determinants of the efficiency of HIV control by CD8 T cell responses during acute infection is important to inform T cell-based vaccine design. In a pilot study of two HIV clade A-infected subjects with different infection outcomes we found that good viral control was associated with establishment of infection by a less fit founder virus and expansion of a broad primary HIV-specific CD8 T cell response, some components of which were nonetheless rapidly escaped. The current study extends similar analyses to additional subjects to determine the generality of these observations.

**Methods:** 10 HIV clade A-infected subjects sampled at serial timepoints from acute infection, 5 establishing low and 5 high persisting viral loads, were studied. Longitudinal single genome amplification and sequencing of viral RNA was performed, the founder virus sequence deduced, and longitudinal mutations identified. The T cell epitopes recognised in the autologous founder virus were mapped; the kinetics of epitope-specific responses determined and effects of putative escape mutations verified using ELISPOT assays.

**Results:** The HIV-specific CD8 T cell response expanded during acute infection in subjects with good viral control was of greater epitope breadth than that in subjects with poor viral control; nonetheless, viral escape from initially immunodominant CD8 T cell responses was observed in both subject groups. The fitness of the virus initiating infection in these subjects and its reduction by escape mutations is currently under investigation.

**Conclusion:** The results obtained to date suggest that contrasting infection outcomes may be shaped by interplay between viral fitness and the host T cell response, with establishment of infection by a less fit virus enabling expansion of a broader primary HIV-specific T cell response, escape from which may drive further reduction of viral fitness. Vaccines should therefore be designed to induce responses that constrain acute viral replication and drive costly escape mutations.
P12.35

SIV Infection of the Macaque Testis—An Immune Privileged Site?

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**Background:** The rodent testis is a site of immune privilege where adaptive and innate immune responses are suppressed. Evidence for immune privilege in the primate testis, however, is inconclusive. The testes of humans and macaques become infected with HIV and SIV, respectively, so a successful vaccine must prevent testicular infection. We have used the pigtail macaque model to characterize the leukocytes present in the testis, SIV infection of the testis and the strength of immune responses in this region.

**Methods:** Testes from 14 pigtail macaques were studied. Two macaques were infected with SIVmac239. ‘Tru-cut’ core biopsy (2 weeks) and unilateral orchidectomy (10 weeks) were used to sample testes. Interstitial cells were isolated from uninfected (n=12) and infected testes by collagenase digestion and phenotyped using flow cytometry. Nested RT-PCR reactions were used to amplify the SIV env gene from testis samples. T cell responses to mitogen stimulation were measured by intracellular cytokine staining.

**Results:** The leukocyte population of the uninfected macaque testis consisted of >45% monocytes and macrophages, 15% CD4+ and 16% CD8+ T cells (central and effector memory, but few naïve) and smaller populations (<10%) of dendritic, NK and NKT cells. T cell responses to mitogens and super-antigens were highly suppressed in the testis compared to blood (p=0.028). SIV was detected by PCR at 2 and 10 weeks after infection. SIV Infection led to a decrease in the proportion of CD4+ T cells, increase in CD8+ T cells and a shift away from central memory and towards effector memory T cells.

**Conclusion:** Cells within the macaque testis becomes infected with SIV within 2 weeks, resulting in CD4 T cell depletion and CD8 T cell expansion. However, T cell responses are dramatically suppressed in the priimate testis. This raises important questions about whether “non-sterilizing” immunity-inducing HIV vaccines will seed HIV into this immune-privileged site and lead to eventual breakthrough infections.

P12.36

Specificity of T Cell Response, Not Protective HLA Restrictions, Determines Control of HIV Replication in Thai Viraemic Controllers

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**Background:** Previous studies have demonstrated that protective HLA alleles such as HLA-B*27 and HLA-B*57/58 alleles are related to HIV control. However, development of T cell vaccine based on this finding may be problematic due to diversity of HLA alleles in different ethnic backgrounds. We hypothesized that T cell targets were more essential than HLA-restrictions.

**Methods:** Sixty-eight HIV-infected volunteers were classified into viraemic controllers (VC) and non-viraemic controllers (NC) according to their HIV-RNA loads (those with HIV-RNA < 2,000 copies/ml were categorized into VC group). CBC, CD4 count and HIV loads (VL) were determined in ISO-15189-accredited laboratories. T cell responses were analyzed by ELISpot, polychromatic ICS and, in selected cases, by HIV suppression assays.

**Results:** The VC had a median VL of 682 and NC had 17,313 copies/ml, whilst the CD4 counts were similar. Duration of Infection and other clinical parameters were not different. All volunteers were CCR5 wt/wt genotype. Volunteers with HLA-B*27 and HLA-58 supertype did not have lower VL. VC and NC had similar p24-specific T cell response by ELISpot assay. VL in HLA-B27-KK10 responders were not statistically different from those in the non-responders. All HLA-B57/58 epitope responses, except LW9, were not associated with low VL or controller status. Our observation that only LW9-specific T cells had full functional response by ICS and the CTL line suppressed in vitro HIV replication to under detection limit might help explain efficacy of this epitope response. By plasma HIV-RNA sequencing, escape of T cell response did not explain absence of low VL associations in other epitopes. Surprisingly, immunodominant response to AK11 epitope was related to low HIV-RNA load, though this epitope was restricted through non-protective HLA-A*11.

**Conclusion:** In conclusion, targets of T cell response are important for HIV control, and may not be relevant to the epitope restrictions. The observation helps alleviate concerns over HLA restriction in T cell-based HIV vaccine.
P12.37

IL-10 Receptor Blockade Enhances CD4 and CD8 T Cell Responses to a Simian Adenovirus-Vectored HIV-1 Vaccine, ChAdV63.HIVconsv

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Background: HIV-specific CD8 T cells limit viral replication in both acute and chronic infection, but T cell-inducing vaccines tested to date have failed to reduce viral load in clinical trials. Adenoviral vectors stimulate potent T cell responses, but also induce the immunoregulatory cytokine interleukin-10 (IL-10), which may limit their efficacy. We investigated whether blockade of IL-10 signalling could enhance priming of T cell responses to the candidate HIV-1 vaccine ChAdV63.HIVconsv.

Methods: Female BALB/c mice were injected intraperitoneally with 200µg anti-IL-10 receptor (IL-10R) antibody or isotype control antibody 24 hours prior to a single intramuscular immunisation with 5 x 106 infectious units ChAdV63.HIVconsv (day 0). Cytokine production and costimulatory molecule upregulation in splenic dendritic cells was examined 24 hours post immunisation and HIV-specific cytokine production by CD4 and CD8 T cells was analysed on days 14 and 21.

Results: Blockade of IL-10 signalling resulted in a significant upregulation of CD86 on CD8α-CD11c+ dendritic cells 24 hours post immunisation. Blockade did not enhance peak HIV-specific CD4 or CD8 T cell responses (IL-2, IL-4 and IFN-γ production) at day 14 but by day 21 there was a significant increase in the frequency of HIV-specific IL-2-producing CD4 T cells and IFN-γ-producing CD8 T cells from mice treated with the IL-10R blocking antibody. The breadth of HIV-specific CD4 T cell responses (in terms of IL-2 production) was also increased at day 21 in mice treated with the IL-10R blocking antibody.

Conclusion: IL-10R blockade resulted in improved maintenance of CD4 T helper and CD8 effector T cell responses to the HIVconsv immunogen at day 21. This may be due to enhanced priming by dendritic cells. Blockade of IL-10 signalling is a potential strategy to increase the immunogenicity of viral vector vaccines for HIV-1 that warrants further investigation.

P12.38

The Antiviral Efficacy of CD8+ T Cells in Early HIV-1 Infection Is Dependent on Targeting of Low Entropy Regions of the Viral Proteome

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Background: Reliable correlates of immunological control are critical for HIV vaccine development. We and others have shown the capacity of CD8+ T cells to inhibit HIV-1 replication in vitro is highly predictive of their antiviral efficacy in vivo. The design of immunogens that could elicit effective CD8+ T cell responses is a major challenge.

Methods: Ex vivo CD8+ T cell mediated antiviral inhibitory activity was measured in 22 HIV-1-infected Step and Phambili trial participants using PBMC sampled within one year of diagnosis. Investigators were blind to vaccine/placebo receipt. Several CD8+/CD4+ T cell ratios were tested with a panel of ≤5 viruses representing clades A, B and C. In parallel, ex vivo IFN-γ Elispot assays were used to quantify responses to overlapping Gag, Pol, Nef, and Vif peptides (OLPs) that were previously defined as targets of protective T cell responses by association with low viral load in chronically infected responders.

Results: Three individuals showed potent inhibition (>90%) of a clade-matched viral isolate at an optimal CD8+/CD4+ T cell ratio (1:1). This remained detectable at a 1:10 CD8+/CD4+ T cell ratio and cross-clade inhibition was also observed. In contrast, the majority of subjects showed weaker antiviral activity at a 1:1 CD8+/CD4+ T cell ratio (median - 42%). Consistent with this, only 4 had beneficial HLA class I alleles. Frequencies of IFN-γ-producing T cells targeting the ‘protective’ OLPs were modest overall (median 120 SFU/million PBMC, range 0-1880 SFU/million PBMC). Critically, there was a strong correlation between IFN-γ responses to these protective regions and CD8+ T cell mediated virus inhibitory activity ($r^2 = 0.47, p = 0.003$).

Conclusion: Potent CD8+ T cell antiviral inhibitory responses were rare in this cohort but were strongly associated with targeting of low entropy regions in HIV-1 Gag and Pol, supporting their inclusion in HIV-1 immunogens. Analysis of unblinded data to ascertain any vaccine impact on CD8+ T cell responses is planned.
**P12.39**

**A Synthetic Multi-Env DNA Vaccine Induces Functional In Vivo T Cell Immunity Against Diverse HIV Env Immunogens by Novel In Vivo Assay**

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**Background:** In designing new immunogens for the induction of T cells against HIV, testing the effectiveness of the resulting T cell responses against a diverse set of viral antigens is challenging. Primary tools include ex vivo assays and highly animal specific in vivo assays usually limited to single epitope analysis. Recent improvements in DNA technology allowed for the development of minimally invasive local intradermal gene delivery array. We have adapted this technology for visual in vivo determination of viral gene clearance induced by host immunity.

**Methods:** Following immunization, guinea pigs (GP) are injected and electroporated in specific dermal sites with plasmids expressing a HIV gene and GFP. Two days later, sites are assessed for GFP signal. If cells express epitopes specific for the induced CD8+T cells, they are targeted for killing, leading to reduced GFP signal. Naive animals do not drive this in vivo clearance.

**Results:** When assessing killing responses against the vaccinated gene, we show a decrease in GFP signal, supporting a strong CD8+T cell response directed against that specific gene. By expressing alternate genes from those used during vaccination, the breadth of the killing response is determined. GP vaccinated with consensus C vs consensus A envelope develop comparable breadth and robustness of killing against genetically similar envelopes. GP vaccinated with both plasmids have increased ability to kill more diverse unrelated primary envelopes, previously unaffected by either plasmid alone. Immunization with a combination of consensus A, C, D envelope plasmids further broadens clearance against highly diverse primary envelopes, supporting better induction of cross clade CD8+ T cells without negative competition by these immunogens.

**Conclusion:** This novel technology allows for increased in vivo assessment of vaccine induced viral gene clearance with no ex vivo manipulations required. It allows for both linear and conformational epitopes to be tested in a simple model system.

**P12.40**

**Longitudinal Analysis of the Human Immunodeficiency Virus-1 Specific T Cell Response During Primary HIV-1 Infection and Association with Viral Control**

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**Background:** HIV-1-specific CD8+ T cells play a significant role in the control of HIV-1 infection but there is great heterogeneity and progressive diminution in their antiviral effectiveness. Longitudinal analysis of CD8+ T cell responses following acute HIV-1 infection may help distinguish effective versus ineffective CD8+ T cell responses and elucidate mechanisms that lead to CD8+ T cell dysfunction.

**Methods:** We performed longitudinal characterization of CD8+ T cell responses to consensus peptides spanning the full viral proteome in 20 HIV-1C acutely infected, antiretroviral naïve subjects using both the ex vivo and the cultured IFN-γ ELISPOT assay from 28 days post infection and up to one year. Viruses from plasma were also sequenced within defined CD8+ T cell epitopes.

**Results:** There was an inverse correlation between the breadth of Gag responses and viral set point recorded from 25 weeks post infection (P= 0.0003; r= -0.70). A number of responses which were no longer detectable by the ex vivo ELISPOT at one year, were detected when the cultured ELISPOT assay was used. At the earliest time point evaluated, T cell responses were only mounted against 14% of the wild type epitopes and 7% of epitopes with variant sequences. However, analysis at 52 weeks showed that a significantly higher number of both wild type (P=0.006) and variant epitopes (P=0.01) were targeted at 32% and 23%, respectively.

**Conclusion:** These data suggest an important role of Gag-specific T cell responses in the control of viremia during early HIV-1 infection. Some effector T cell responses, which disappear and are considered lost, may still be present in circulation, albeit below the limit of detection by ex vivo ELISPOT. Our data also implies that there is both a defect in the ability of T cells to expand to a cognate stimulus and weak immune selection pressure mediated through some responses during primary infection.
Background: Human leukocyte antigen (HLA) class I variation has been consistently linked to differential disease outcome in HIV infection. HLA-B*27:05 and HLA-B*57:01 are the two HLA alleles most strongly associated with viral control and slow disease progression in Caucasian subjects. Establishing the mechanisms responsible for loss of viral control in individuals that express both these favourable alleles provides a unique opportunity to define the key immune responses mediating immune control.

Methods: We studied a total of 12 HIV-infected HLA-B*27:05/B*57:01-positive adult subjects including: (i) a B Clade infected transmission pair and a CRF1_AE Clade infected transmission pair, where the recipient carried HLA-B*27:05/B*57:01 in each case; (ii) 10 B clade infected subjects comprising 2 progressors, 3 elite controllers and 5 viraemic controllers.

Results: Loss of immune control in a B*27:05/B*57:01 B clade infected subject was associated with acute HCV infection and acquisition of an HIV virus that differed substantially in Pol and Gag (by 13.1% in the amino acid sequence), but not in Nef (by 3.4%), suggesting that progression resulted from HIV superinfection and recombination. Escape mutations in critical HLA-B*27:05 and HLA-B*57:01-restricted epitopes within the superinfecting viral sequence may account for disease progression in this subject. The alternative hypothesis, that viral escape in subjects expressing HLA-B*27:05/B*57:01 arises through selection of multiple escape mutations that are acquired sequentially, despite considerable fitness cost to the virus, is currently being tested in the additional case studies. Disease progression in the CRF1_AE Clade infected B*27:05/B*57:01-positive subject was associated with escape mutations in key HLA-B*57:01-restricted Gag epitopes, but not with superinfection.

Conclusion: Studying loss of immune control of HIV that occurs in individuals despite expression of HLA alleles strongly associated with immune control may provide important evidence to define factors contributing to disease regulation. These features of efficient immune control may be important targets for T cell vaccine design.
P12.43

HIV Specific T Cell Responses in HIV-1/S. Mansoni Coinfection


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Background: HIV and Schistosomiasis coinfection are a common occurrence in Sub-Saharan Africa. It is unclear how schistosomiasis might influence the course of HIV infection. We hypothesized that coinfection with S. mansoni and HIV might downmodulate the T cell response to HIV, regulatory T cell proportions and negative and positive regulatory markers.

Methods: Fifty HIV infected cases, 26 of which were co-infected with S. mansoni were recruited. PBMC were stimulated with pools of HIV PTE peptides, SEB or unstimulated. PBMC stained for viability, CD3, CD4, CD8, IFN-γ, IL-2, TNF-α, CD25, CD45RO, CD127, Foxp3, GARP, CD150, CD244, CD160 and CD279, CD38 and HLA-DR in three staining panels were acquired on a BD LSR II analyzer. Background subtracted cytokine responses and FMO gated marker expression were analysed in FlowJo, Stata 10 and Qlucore.

Results: The mean frequency of T cells secreting IFN-γ, IL-2 and TNF-α in response to HIV peptides was not significantly different between HIV only infected and coinfected individuals, with mean response differences ranging between -0.19 and 0.3% and p values between 0.052 and 0.9 for any cytokine combinations using two tailed student t test. There was a trend towards higher mean frequency of HIV specific cells producing IFN-γ or IFN-γ & TNF-α and higher frequencies of regulatory T cells in the coinfected participants, who also had lower frequencies of CD8+CD38+CD150+CD160CD244+CD279-HLA-DR+ and CD8+CD38+CD150+CD160+CD244+CD279+ HLA-DR+ cells compared to those infected with HIV alone (p=0.046; q=0.145). Other patterns of regulator markers were also found to be different in these contrasts.

Conclusion: HIV specific cytokine secretion did not appear downregulated in co-infection compared with HIV alone, although different patterns of expression of regulatory markers were observed in the two groups, suggesting that complex regulatory interactions might occur without a major effect on the chosen functional outcome measure. The trend suggests enhanced anti HIV specific responses in HIV S.mansoni coinfection.

P12.44

Recombinant Pox-, Adeno- and Herpesviral Vectors Differ in Their Capacities to Induce Antigen Presentation on Dendritic Cells

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Background: Cytotoxic T cell (CTL) responses were found to efficiently control disease progression in some HIV-positive elite controllers and long term non-progressors. Thus, HIV-1 vaccines eliciting broad CTL responses may provide control of HIV replication. Nevertheless, previous vaccination approaches failed to induce protection. Not only improved immunogens but also effective delivery systems for elicitation of strong immunogen expression and protective immune responses are required for the development of vaccines. For the induction of CTL responses, the vectors have to activate dendritic cells (DCs) and induce antigen presentation.

Methods: Here, we compare the recombinant viral vectors NYVAC (poxvirus), Ad5 (adenovirus) and EHV-1 (herpesvirus), all coding for the same artificial polyprotein GPN, for their immunogenicity in a human ex vivo system.

Results: We could show that only NYVAC and EHV-1 induced efficient GPN-expression in DCs, while Ad5 demanded high multiplicity of infection (MOI) and long incubation periods. Due to the low immunogen expression in DCs induced by the Ad5 vector, only remote direct-presentation by infected cells was observed. In contrast NYVAC and EHV-1 induced strong direct-presentation with EHV-1-induced presentation being stronger and longer lasting. In a cross-presentation assay, vaccine-infected HeLa cells were cocultivated with DCs. Only Ad5- and NYVAC-infected cells were able to induce cross-presentation on DCs whereas EHV-1 infection could not elicit cross-presentation. These results are surprising, as EHV-1 induces extensive GPN expression in HeLa cells.

Conclusion: Thus, we could show that the vector choice dramatically influences antigen presentation, and therefore the delivery system for vaccination purposes has to be chosen carefully.
**P12.45**

**Epitope Mapping of the Response to Vaccination with Dendritic Cells Loaded with HIV Lipopeptides**


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**Background:** We have reported that vaccination with ex vivo generated Dendritic-Cell (DC) loaded with HIV-lipopeptides (Gag17-35, Gag253-284, Nef66-97, Nef116-145 and Pol325-335) in patients (n=19) under ART was well tolerated and immunogenic (CROI 2012 PB440). Here we have performed an epitope mapping of HIV responses using multiplex cytokine secretion assay.

**Methods:** Aphereses were performed at w16 (4 weeks after the last vaccination). PBMCs were stimulated for 48 hours with pools (n=36) of 15-mer overlapping peptides spanning vaccine epitopes. Measurements of cytokine secretion (IL-2, IL-10, IL-13, IL-17, IL-21, IFN-γ and IP10) was performed using Luminex® multiplex. Fluorescence intensity (FI) has been analysed on continuous scale and as binary response defined by > Q90 background and > 3 fold median FI among negative controls. Predictions of binding of peptides to HLA class II molecules according to patient’s HLA alleles were performed using netMHCII-2.2 and netMHCIIpan-2.0. Wilcoxon rank-sum tests were used for statistical analyses with estimation of False Discovery Rates (FDR) for controlling test multiplicity.

**Results:** Patients’ HLA class II alleles (19 HLA-DRB1 and 12 HLA-DQB1 molecules represented) predicted a high frequency of responses against Gag253 and Pol325 and to a lesser frequency against Nef116 epitopes. At w16, vaccinations elicited a significant increase of HIV responses as assessed by production of IL-2, IFN-γ, IL-21, IL-13 (dFDR<0.05). Among the 587 responses against vaccine peptides, 415 concerned weak (n=201) and strong (n=214) binders to HLA class II molecules. 37% and 23% responses were directed against Gag253 and Pol325 epitopes, respectively. Profile of cytokine responses was: IFN-γ (28%), IL-2 (18%), IP-10 (15%) and less than 32% for Th2 cytokines (IL-10 and IL-13).

**Conclusion:** DC Vaccination elicited broad T cell responses to 15-mers that matched with HLA class II allele predictions of responses to vaccine epitopes. The majority of them were directed against Gag and Pol epitopes and exhibit a Th1/Th2 profile.

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**P12.46**

**Pre-infection MHC Class I Allele Expression Correlates with SIV Control**

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**Background:** CD8 T cells contribute to controlling human immunodeficiency virus and simian immunodeficiency virus replication. These responses are restricted by MHC class I proteins which present endogenously derived peptides on the cell surface. MHC class I protein expression is regulated at multiple levels and can change depending on an individuals MHC class I repertoire.

**Methods:** We explored whether Mafa-A1*063 expression on the surface of CD4+ T cells is associated with control of SIV replication in a cohort of MHC-defined Mauritian cynomolgus macaques. The expression of Mafa-A1*063 on the surface of cells was determined by incubating cells with a saturating amount of FITC-conjugated peptides known to bind Mafa-A1*063 and measuring the geometric mean fluorescent intensity of the bound peptides by flow cytometry.

**Results:** We found that higher levels of pre-infection Mafa-A1*063 expression on the surface of CD4+ T cells correlated with reduced viral loads while total pre-infection MHC class I expression did not.

**Conclusion:** These results suggest that differences in expression of MHC class I alleles can impact viral loads. Ultimately, this may help explain why some individuals that have MHC class I alleles associated with control of viral replication are unable to maintain low viral loads.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L2, Room 211 – 212.
Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L2, Room 211 – 212.

**P12.47**

Direct Identification of Vaccine-Induced HIV-1-Specific MHC Class I Associated Epitopes by Mass Spectrometry

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**Background:** The challenge of vaccine development for HIV-1 is the high mutation rate and resulting diversity of the virus that allows for the escape from an established adaptive immune response in infected or vaccinated individuals. The immunogen HIVconsv was designed as fusion protein of the 14 most conserved regions across the HIV-1 genome in order to limit viral escape (Letourneau et al., 2007). This vaccine is currently evaluated in clinical trials, inter alia, using Modified Vaccinia Ankara as delivery vector (MVA:HIVconsv).

**Methods:** In order to investigate the potential of this vaccine to induce HIV-1 specific epitope presentation, we have purified the MHC class I associated peptidome of MVA:HIVconsv infected cells followed by identification of the obtained epitope sequences by LC-MS/MS. Furthermore, we monitored changes in epitope presentation during the first hours following MVA:HIVconsv infection by MS based relative quantitation of peptide abundances.

**Results:** In addition to identification of several thousand self-epitopes and over 100 MVA-derived sequences, we also identified 4 HIVconsv-derived epitopes. In the analysed time range, individual vaccine-derived epitope presentation increased with time of infection and correlated with the increase of HIVconsv protein expression in the cytoplasm.

**Conclusion:** This experimental workflow is a powerful new tool for direct identification of epitope sequences and will contribute extensively to the understanding of immunological processes and future vaccine design.

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**P12.48**

PD-1hi CD4 T Cells Accumulate in Lymphoid Tissue and Support Viral Replication During Chronic SIV Infection

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**Background:** The inhibitory receptor PD-1 has been shown to regulate both CD8 T cell and B cell function during chronic SIV infection, but the effects of PD-1 on CD4 T cell function are less well understood. Here, we investigated the role of PD-1 on CD4 T cells in regulating their function and its contribution to viral persistence during chronic SIV infection.

**Methods:** Lymphocytes isolated from the blood, lymph node (LN), and rectum of SIV naïve and SIV infected rhesus macaques (RM) were separated into PD-1 neg, PD-1 lo, and PD-1 hi memory CD4 T cell subsets and characterized by flow cytometry. Sorted cells were used for measuring cell associated viral DNA and RNA by qPCR.

**Results:** In SIV naïve RM, PD-1 lo and PD-1 neg subsets were found in all three compartments, whereas PD-1 hi cells were restricted mainly to the LN and rectum. Following TCR independent (PMA/ionomycin) stimulation, PD-1 hi cells predominantly produced high levels of IL-2 and IL-21. However, they failed to produce any cytokines following stimulation through the TCR. Following SIV infection, despite a global depletion of CD4 T cells, there was a significant increase in PD-1 hi CD4 T cells in both the rectum and LN, with frequencies of PD-1+ memory CD4 T cells correlating directly with viral load. Furthermore, PD-1 hi cells retained cytokine production following PMA/ionomycin stimulation. Interestingly, the PD-1 hi CD4 T cells were found to contain the highest concentration of cell associated viral RNA and DNA. The majority of these cells co-expressed CXCR5 suggesting that these may be localized to germinal centers (GC) where the anti-viral CD8 T cell response is limited.

**Conclusion:** These data suggest that PD-1 hi cells may localize to GC to escape anti-viral CD8 T cell killing, serving as an important source of virus production and promoting viral persistence, at sites of preferential SIV replication.
P12.49

The N-Terminal Domain of HIV-1 Nef Regulates Its Ability to Inhibit or Stimulate T Cell Receptor-Mediated Signaling Events

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Background: Productive HIV-1 replication depends on host CD4+ T cell activation state. The Nef protein modulates T cell activation through interactions with various signaling proteins; however, literature on this topic is equivocal. Some reports suggest that Nef enhances TCR signaling, while others describe an inhibitory effect. To examine this question, we measured transcription factor activation following TCR stimulation in the presence of wild-type (SF2) or mutant Nef.

Methods: We constructed a panel of SF2-Nef mutants in motifs known to affect myristoylation (G2A), CD4 (LLAA) or HLA class I (M20A) down-regulation, SH3 (AxxA) or PACS-1 (AAAA) binding, as well as truncated versions of the protein expressing N-terminal (1-57aa) or C-terminal ‘core’ (58-206aa) domains. Jurkat cells were co-transfected with Nef and NF-κB- or AP-1-driven luciferase plasmids. At 20h, cells were stimulated with anti-CD3 antibody for 6-8 hours, and TCR-mediated transcription factor activity was measured by luminescence.

Results: WT SF2-Nef inhibited TCR-mediated NF-κB and AP-1 activity up to 5- and 10-fold, respectively. CD4 and HLA down-regulation and PACS-1 binding functions of Nef were not required for inhibition, since LLAA, M20A, and AAAA mutants inhibited NF-κB to similar levels as WT. Myristoylation and SH3 binding mutants, as well as the N-terminal domain alone, failed to inhibit NF-κB activity. In contrast, NF-κB activity was enhanced 2-fold relative to negative control in the presence of the C-terminal domain alone.

Conclusion: Results indicate that full-length Nef inhibits TCR signaling through a process that requires myristoylation and the proline-rich motif. Notably, the core domain, which mimics the Nef product generated by protease cleavage, enhanced TCR signaling. The capacity of Nef to carry out opposing functions might reconcile contradictory reports in the literature. Our data suggest that Nef may stimulate T cell activation at early stages following viral entry and suppress similar events at later stages of viral replication.

P12.50

Cytotoxic Capacity During a Recall Response Parallels Increases in Lytic Granule Contents of Virus-Specific CD8+ T Cells

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Background: Although HIV-specific CD8+ T cell cytotoxicity is a clear correlate of immune control in LTNP/EC, the kinetics of lytic granule loading and cytotoxicity remain incompletely understood. A better understanding of the pathways and timing of expression of lytic granule contents is critical for the study of these responses and ultimately their induction.

Methods: Perforin (delta G9 and B-D48 clones) and GrB expression were measured by flow cytometry in HLA B57-KF11 tetramer+ cells at times 0, 6 hours and daily for 6 days following stimulation with HIV-infected CD4+ T cell targets. In parallel, HIV-specific CD8+ T cell proliferative and cytotoxic responses to the same targets were measured by flow cytometric detection of CFSE dilution, GrB delivery to live targets and infected CD4 elimination (ICE).

Results: Perforin and GrB expression were lowest in un-stimulated tetramer+ cells, increased significantly by day 2 and peaked between days 4-6. Perforin and GrB expression were measured by flow cytometry in HLA B57-KF11 tetramer+ cells at times 0, 6 hours and daily for 6 days following stimulation with HIV-infected CD4+ T cell targets. In parallel, HIV-specific CD8+ T cell proliferative and cytotoxic responses to the same targets were measured by flow cytometric detection of CFSE dilution, GrB delivery to live targets and infected CD4 elimination (ICE).

Conclusion: Results indicate that full-length Nef inhibits TCR signaling through a process that requires myristoylation and the proline-rich motif. Notably, the core domain, which mimics the Nef product generated by protease cleavage, enhanced TCR signaling. The capacity of Nef to carry out opposing functions might reconcile contradictory reports in the literature. Our data suggest that Nef may stimulate T cell activation at early stages following viral entry and suppress similar events at later stages of viral replication.
P12.51

Sensitive Detection of Vaccine-Induced T Cell Responses by Intracellular Cytokine Staining Using Automated Gating and Comparison to Baseline

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Background: Intracellular cytokine staining (ICS) using ex vivo stimulation of cells with antigens included in vaccines is a common method to determine T cell immunogenicity. Positivity is determined by comparison between the antigen-stimulated and unstimulated conditions or by comparison between pre- and post-vaccination.

Methods: To assess the feasibility of automated gating to accurately distinguish pre- and post-vaccination samples, HVTN trial 065 with a range of responses to vaccine-encoded proteins (to 80%) was selected. To test for sensitivity in detecting low-level responses, a peptide pool that demonstrated few positive responses by the standard HVTN positivity criteria based on the Fisher’s exact test for IFN-γ and/or IL2 was chosen, PTE-G-Pol-3 (1 responder). Data were from 74 vaccinees (37 training set and 37 test set) at day 0 and 2 weeks after the last vaccination. OpenCyto was used to gate the data following the gating hierarchy determined manually accurately includes cytokine-producing T cells post-vaccination. The sensitivity of detecting vaccine responses was far greater than that determined by standard methods, suggesting previous underestimation of immunogenicity. Major factors resulting in increased sensitivity may include the use of baseline samples (a feature not included in the standard method), automated gating, or statistical methods taking into account single cytokines or co-expression of cytokines.

Results: Four features predictive of vaccination status were selected by the model. All were cytokine-producing CD4+ T cell subsets: IL2+IFN-γ+TNF-α, IL2+IFN-γ+TNF-α+, IL2-IFN-γ+TNF-α, and IL2-IFN-γ+TNF-α-. Overall, 33 of the 37 test samples (90%) were correctly classified as pre- or post-vaccination. Prediction accuracy in placebo recipients (not part of the training set) was 52%, no better than logistic regression was used for classification.

Conclusion: This study demonstrates that automated 2-dimensional gating applied in the hierarchy determined manually accurately identifies cytokine-producing T cells post-vaccination. The sensitivity of detecting vaccine responses was far greater than that determined by standard methods, suggesting previous underestimation of immunogenicity. Major factors resulting in increased sensitivity may include the use of baseline samples (a feature not included in the standard method), automated gating, or statistical methods taking into account single cytokines or co-expression of cytokines.

P12.52 LB

Impact of HLA-Selection Pressure on HIV Replication Capacity at a Population Level

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Background: Previous studies have suggested that effective CTL responses drive selection of escape mutations that reduce viral replication capacity (RC). There is also evidence that certain escape mutations can be transmitted from one host to another, allowing for its accumulation in a population. Here, we hypothesize that high frequency of protective alleles in a population can drive the accumulation of escape mutants with a significant fitness cost at the population level.

Methods: The study compared two ART-naïve HIV B-clade infected cohorts, in Mexico (n=976) and Barbados (n=190), in which protective HLA alleles (HLA-B*27/57/5801) were expressed at 10% and 35%, respectively. Gag genes were amplified from plasma virus and gag-pro chimeric viruses for RC analysis were created as previously described.

Results: Viral loads (VL) were significantly higher in Mexico compared to Barbados (median x versus y; p=<0.0001), and absolute CD4+T-cell counts significantly lower (median x versus y, p=0.007). RC was analysed in a subset of subjects in each cohort matched by CD4+T-cell counts between 300-500 cells/µl that did not differ between the two cohorts (p=0.8). The VL was significantly higher in the Mexican subset (p=0.03). A significantly higher median RC (1.11 versus 1.01, p=0.01) was observed in the Mexican cohort. This RC difference was associated with accumulations in Barbados of four previously described Gag escape mutation where fitness cost has previously been implicated: B57-A163X (p=0.0021), B57/B58-T242X (p=<0.0001), B57/147X (p=0.0001) and B58-T310X (p=0.0006).

Conclusion: RC measured by chimeric virus constructed from CD4-matched cohorts was significantly higher in Mexico compared to Barbados. Our data suggest that this may be related to the higher frequency of protective alleles in Barbados and a higher frequency of Gag escape mutants that reduce viral fitness. Thus viral loads and disease progression rates may differ between distinct populations as a result of distinct frequency of protective alleles.
**P12.53 LB**

**Evaluating CTL-Based “Flush and Kill” HIV Eradication Strategies Against Primary Cell Models of Latency and Natural Viral Reservoirs**

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**Background:** Purging of HIV-infected resting CD4+ T-cells will likely require the coordinated action of latency-reversing agents (LRAs) and cytotoxic T-lymphocytes (CTL). We have developed an assay that incorporates HIV-specific CTL as sensors of latency reversal, and used this to assess LRAs in primary cell models. Based on our findings, we prioritized “flush and kill” strategies and tested these for eradication of HIV from natural reservoirs in vitro.

**Methods:** CD4+ T-cells were depleted of activated cells and infected with HIV. Autologous HIV-specific CTL clones were co-cultured with these targets in the presence or absence of LRAs. IFN-γ was quantified as a measure of CTL recognition. Optimal combinations of CTL and LRAs were co-cultured with autologous CD4 (natural HIV reservoirs) for 96-117 hours. CTL and dead cells were depleted and the residual reservoir was measured by qPCR and viral outgrowth assays.

**Results:** While HDAC inhibitors (HDACi) and common gamma-chain cytokines both reversed HIV latency, HDAC is suppressed CTL function and thus performed poorly in integrated “flush and kill” assays. In contrast, IL-15 superagonist (IL-15SA) both reversed HIV latency and enhanced CTL function. Treating patient CD4+ T-cells with a romidepsin pulse/wash followed by co-culture with HIV-specific CTL and IL-15SA resulted in a 5-fold reduction in proviral DNA and in the elimination of infectious virus as measured by viral outgrowth assays.

**Conclusion:** There are advantages to incorporating CTL into early screening of LRAs for use in eradication strategies. First, CTL can be used as sensors to detect latency reversal above an expression threshold that is intrinsically relevant to CTL-mediated clearance. Second, this allows consideration of the effects of LRAs on CTL function. We show that an optimal LRA/CTL combination approach is capable of depleting the reservoir in vitro. Further study will determine the minimal elements required for this depletion, and the nature of residual proviral DNA.

**P12.54 LB**

**Adenovirus 5 Hexon-Specific CD4 T Cells Are More Susceptible to HIV and Preferentially Depleted During Infection Compared to CMV-Specific CD4 T Cells**

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**Background:** Different antigen-specific CD4 T cells manifest different susceptibilities to HIV with CMV-specific CD4 T cells being particularly resistant. It remains unclear whether such HIV resistance is unique to CMV or can also be found for CD4 T cells specific to other viruses. Like CMV, adenovirus 5 (Ad5) is an important human viral pathogen and has been commonly used as vector for HIV/SIV vaccine delivery. We investigate the susceptibilities of Ad5- and CMV-specific CD4 T cells to HIV.

**Methods:** ELISPOT was used to monitor antigen-specific T cell responses in HIV-infected patients and un-infected volunteers. An in vitro assay was established to assess HIV infection of antigen-specific CD4 T cells. PBMC from HIV-negative individuals were CFSE-labeled, stimulated with Ad5 hexon and CMV antigens, and exposed to HIV. HIV infection of Ad5- and CMV-specific CD4 T cells was determined by intracellular p24 in CFSE-low, CD4 T cells.

**Results:** We observed that Ad5-specific T cell response is preferentially lost compared to CMV-specific response in a chronic HIV infection cohort. Alternatively, the responses specific to both viruses are present at high magnitudes in the HIV-negative volunteers. In vitro studies show that compared to CMV, Ad5-specific CD4 T cells are more susceptible to both R5 and X4 HIV infection. Ad5-specific CD4 T cells demonstrate a mucosal homing phenotype (expression of α4β7 and CCR9), and produced IL-17 in addition to IL-2 and IFN-γ, whereas IL-17 was not detected in CMV-specific CD4 T cells. Analyses of cytokine and intracellular p24 co-expression show that HIV preferentially infects IL-17 and IL-2-producing antigen-specific CD4 T cells.

**Conclusion:** Our in vitro and in vivo data demonstrate that human Ad5-specific CD4 T cells are more susceptible to HIV and preferentially depleted during infection compared to CMV-specific CD4 T cells. The results may have important implications for HIV vaccine design regarding selection of viral vectors.
P12.56 LB

Evaluation of Peripheral and Mucosal Cellular Immune Responses Induced by Late Boost Strategies in HIV-Negative Participants Prior Enrolled in RV144

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Background: To determine cellular immune responses in periphery and sigmoid mucosa in participants enrolled in a randomized, double-blind evaluation of late boost strategies to define relative contribution of AIDSVAX® B/E, ALVAC-HIV or ALVAC-HIV/AIDSVAX® B/E 6-8 years after receiving the original RV144 prime/boost

Methods: Sixteen participants receiving 2 injections of ALVAC-HIV/AIDSVAX® B/E (5), AIDSVAX® B/E (3), ALVAC-HIV (5) or placebo (3) at baseline and week24, underwent sigmoid biopsies at peak-immunogenicity (week26). Mucosal mononuclear cells and paired PBMC were isolated and HIV-specific responses were measured using IFNγ/IL-2 Intracellular Cytokine Staining. Assays were conducted to the following HIV-1 peptide pools: Env, EnvΔV2 (V2 loop depleted) and V2 loop only. Positive responses were defined as >0.05% corrected and at least 3 times background. In PBMC activation of CD4 and CD8 T cells was determined by the expression of HLA-DR, CD38, Ki67 and CD69.

Results: Peripheral responses were largely CD4-mediated and Env-specific. No positive CD4-responses were observed at baseline in the active vaccination arms and in placebo recipients at any time point. 4/5 participants receiving ALVAC-HIV/AIDSVAX® B/E showed Env-specific CD4-responses at week26, while none were seen with AIDSVAX® B/E or ALVAC-HIV alone. In the ALVAC-HIV/AIDSVAX® B/E arm V2-specific and non-V2 responses were seen in 2/5 and 3/5 participants, respectively. Peripheral Env-specific CD8-responses were only observed with ALVAC-HIV (2/5). HIV-nonspecific activation of CD4 T cells indicated by HLA-DR and CD69 expression increased significantly after administration of ALVAC-HIV/AIDSVAX® B/E (p=0.005 and p=0.01, respectively), with no significant changes in CD8 T cell activation regardless of the vaccination arm. In the sigmoid mucosa no Env-specific CD4-responses were observed, whereas modest CD8-responses were seen in participants receiving ALVAC-HIV/AIDSVAX® B/E (2/5) and AIDSVAX® B/E (1/3).

Conclusion: A late boost with ALVAC-HIV/AIDSVAX® B/E induced a strong Env-specific recall CD4-response in the periphery accompanied by CD4 activation. In the sigmoid mucosa only Env-specific CD8-responses were observed, possibly suggesting differences in HIV-specific lymphocyte trafficking between these compartments.
The Essential Role of Epigenetic Regulation for CD4+ T Cell Dysfunction During Chronic HIV-1 Infection

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Background: The molecular mechanisms of CD4+ T cell dysfunction during chronic HIV-1 infection remains still unknown.

Methods: Expression of IL2, IFNγ, and TNFα after T cell stimulation was measured in mRNA and protein levels in HIV-1 infected subjects with different clinical status. DNA methylation status of individual CpG site in the promoter region of these 3 genes was analyzed in CD4+ and CD8+ T cells. Differentiation status and CD57 expression in CD4+ T cells were also assessed.

Results: Expression level of IL-2 after brief T cell stimulation was significantly lower in non-controllers (NC, HIV-VL >25,000 copies/ml) than viremic controllers (VC, HIV-VL <1,000 copies/ml), and there was no significant difference in the expression levels of IFN-γ and TNF-α between the groups. Although DNA methylation status in IFNG and TNF genes were indistinguishable between the groups, frequency of methylated CpG sites in the IL2 promoter region in CD4+ T cells, not in CD8+ T cells, was significantly higher in NC compared to VC. Especially, difference in the methylation status at CpG site 1 located between the NFAT- and Oct-1-binding sites was remarkable between the groups, and the methylation status was inversely correlated to the IL-2 expression level. Although there was no significant difference in differentiation status between the groups, CD57 expression in memory (CD45RA-) CD4+ T cells, a marker as senescence of T cells, was higher in NC than VC. CD57+ memory CD4+ T cells hardly expressed IL-2 even after PMA/ionomycin stimulation, and the CpG site 1 in IL2 gene was hyper-methylated compared to CD57- memory CD4+ T cells.

Conclusion: Our data indicate that DNA hyper-methylation in IL2 gene, which is associated with immunological senescence, contributes to the dysfunction in CD4+ T cells during chronic HIV-1 infection.
P13.01 D

Cross-Neutralizing Antibodies Are Elicited in Macaques Immunized with DNA and Protein Env Vaccines Derived from Subjects During Development of Breadth

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Background: During infection, the dynamic interaction between HIV-1 and the host immune responses drives the selection of Envelope (Env) to evade antibody pressure. Over time the changing virus influences the evolving responses, including the maturing neutralizing antibodies (NAbs). We have SGA cloned and studied the phylogeny of Env sequences that evolved during infection in two broad neutralizer human subjects and characterized the epitope targets of the NAbs. In preceding studies, we developed immunization strategies in rabbits using rationally selected Envs from these two individuals. Here, we vaccinate macaques with the most successful vaccine designs from the rabbit studies.

Methods: Twelve macaques were divided into two groups of six animals and co-immunized with motif-optimized gp160 Env DNA and gp140 trimeric protein at week 0, 4, 12 and 20. Serological samples were collected including blood, bone marrow, and lymph nodes for serum antibody and neutralization monitoring and for avidity measurements and B and T cell-associated analysis.

Results: After one immunization, all macaques rapidly developed >10 4 binding antibody titers to autologous Env. Autologous NAbs developed after two immunizations that were boosted with subsequent immunizations. Heterologous SFG162 NAbs developed after a single immunization and increased following the second vaccination (P=0.022 and P=0.0087 for each group), with titers eventually reaching 10 6. Neutralization breadth was detected at week 5 after two immunizations against Tier 1 and Tier 2 viruses and increased with further immunizations. Fine mapping of macaque NAbs responses provided evidence of recapitulation of epitope specificities in the human subjects.

Conclusion: The non-human primate vaccine model has great value for defining the correlates of protection for humans during exposure events. In this study we show that it is possible to recapitulate in macaques NAB immune responses that developed in human subjects by presenting Envs in a programmed manner, based on specific molecular characteristics uncovered by probing the human quasispecies.

P13.02 D

Synthetic Enhanced EP Delivered Ig DNA Vector Drives Biologically Relevant Anti-HIV-1 Envelope Responses In Vivo

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Background: Monoclonal Ab’s have demonstrated therapeutic utility against several malignancies and infectious diseases. A drawback of this strategy is the time-consuming and expensive process requiring purification and scale up production of the Ab’s for clinical use. A method to produce antibodies in vivo would be significant improvement for this platform. It would be important if these Ab’s could be administered with out induction of vector serology allowing repeated administrations. Furthermore, delivery in a non-permanent fashion would also have advantages.

Methods: Here we report development of new synthetic optimized plasmid vector/improved EP encoding Abs genes for delivery in vivo. This strategy allows for in vivo synthesis and serum expression of such ex vivo developed antibodies. The antibodies were found to be expressed in the blood as well as in other compartments and were functional and at protective levels for model systems.

Results: An “enhanced and optimized” DNA plasmid generates immunoglobulin heavy and light chains (Fab) of an established neutralizing anti-HIV monoclonal antibody (VRC01). We demonstrate that the serum of transfected animals exhibited the ability to bind to HIV envelopes in ELISA and FACS analysis against diverse isolates and this serum possessed HIV neutralizing activity equivalent to the “native” VRC01 antibody in vivo. In vivo delivery seroconverted the animals with in a few hours and neutralizing activity lasted for weeks. This technology has important advantages for in vivo antibody production which could compliment or circumvent the need for standard antigen based vaccination, particularly in situations where there is difficulty in generation of protective antibody responses by immunization.

Conclusion: This is the first study we are aware of using synthetic DNA EP delivery to produce circulating bioactive antibody responses in a living animal. The study has implications for prophylactic and therapeutic strategies for HIV and other important diseases.
Increased Neutralizing Antibody Responses Elicited by a Bivalent HIV-1 Env Protein Vaccine Comprising Stable Mosaic and Clade C HIV-1 gp140 Trimmers

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Background: The generation of globally relevant HIV-1 immunogens mimicking the native, trimeric Envelope (Env) spike, remains a major challenge for HIV-1 vaccine development. We identified a mosaic Env sequence which was capable of forming biochemically stable trimers. We assessed the mosaic Env gp140 trimer in guinea pig immunogenicity studies either alone or with the clade C C97 trimer.

Methods: Mosaic and clade C gp140 trimers were stabilized with a T4-fibritin C-terminal trimerization tag and produced in 293T cells. Guinea pigs were immunized three times at 4-week intervals with 100 µg of mosaic (M), 100 µg of clade C (C) or 100 µg total of an equal mixture of C+M gp140 protein trimers in CpG/Emulsigen adjuvant. Antibody responses were determined by ELISA and TZM.bl neutralizing antibody (NAb) assays.

Results: M, C and C+M gp140 trimer vaccination regimens elicited high-titer, cross reactive binding antibodies in guinea pigs by ELISA. In TZM.bl NAb assays, the C gp140 elicited significantly higher NAb titers than the M gp140 against clade A virus DJ263.8 (P<0.05; NAb titer range 200-950 versus 35-125) and clade C virus MW965.26 (P>0.05; NAb titer range 11,900-35,200 versus 330-630). In contrast, the M gp140 elicited higher NAB titers than the C gp140 against clade B viruses SF162.LS (P<0.05; NAB titer range 800-2300 versus 125-925) and Bal.26 (P<0.05; NAB titer range 730-1800 versus 25-65). NAB responses elicited by the C+M gp140 combination (MW965.26 = 9270-43,750, DJ263.8 = 240-1185, Clade B SF162 = 240-1570 and clade B Bal.26 = 400-1380) proved additive and were superior to either M or C gp140 alone.

Conclusion: The combination of C+M gp140 trimers elicited NABs with increased breadth compared with either trimer alone in guinea pigs. These data suggest that relatively small numbers of immunologically complementing Env trimers may be a feasible strategy to improve the immunogenicity of antibody-based HIV vaccines.
**P13.05**

**An Inactivated Viral Vaccine that Displays Conformationally Intact HIV-1 Clade B Envelope Trimers Induces Neutralizing Antibodies in Rabbits**

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**Background:** An ideal HIV vaccine should induce broadly neutralizing antibodies (bNAb); however, the generation of native, trimeric Env has proven to be a difficult task. To meet this need, we have developed recombinant vesicular stomatitis virus (VSV) vectors that display functional, trimeric HIV-1 clade B (JRFL) Envelope (Env).

**Methods:** JRFL Env was cloned onto the VSV Glycoprotein (G) transmembrane/cytoplasmic tail domain to generate a hybrid EnvG surface protein in the context of a ∆G VSV (VSV-EnvG-∆G). EnvG antigenicity was analyzed by flow cytometry. Female New Zealand rabbits were immunized IM with 3x10⁶, 3x10⁷ or 3x10⁸ PFU formalin inactivated VSV (iVSV-EnvG-∆G) in a dose optimization study. An immunization of 10µg JRFL gp140 foldon trimer (FT) served as control. Immunizations were given at weeks 0, 4 and 18 and all vaccines were adsorbed to 100µg Adju-Phos® (Brenntag, Denmark) and mixed with 75 ISCOM TM units of ISCOMATRIX™ adjuvant (CSL, Australia). Serum was assessed by anti-Env IgG ELISA, competition-binding and TZM-bl neutralization assays.

**Results:** Analysis of alum-bound iVSV-EnvG-∆G confirmed binding to various bNAbs including PGV04 (CD4 binding site), PGT126 (glycan/V3-loop), PG9 (V1/V2 quaternary epitope), and 2F5 (gp41-MPER), and importantly, the trimer-specific PGT151. At peak, 3x10⁷, 3x10⁸ PFU and gp140 FT control groups exhibited similar anti-Env titers, while the 3x10⁶ group titers were ~1 log lower. In addition, 3x10⁷ and 3x10⁸ PFU-immunized animals exhibited significantly enhanced serum competition for PGV04 and PGT126 binding and neutralization capacity.

**Conclusion:** Animals immunized with IVSV displaying conformationally intact trimer demonstrated an improved quality of Ab response as compared to those immunized with gp140 FT control.

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**P13.06**

**HIV p24 as Scaffold for Presenting Conformational HIV Env Antigens**

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**Background:** Heterologous protein scaffolds engrafted with structurally defined HIV Env epitopes recognized by broadly neutralizing monoclonal antibodies (MAbs) represent a promising strategy to elicit broad neutralizing antibodies. In such regards, a protein scaffold based on the HIV p24 CA protein is a highly attractive approach, providing also Gag epitopes for eliciting HIV non-neutralizing protective antibodies and specific CD4⁺ and CD8⁺ T cell responses.

**Methods:** In the present study, computational techniques were employed to verify the presence of acceptor sites for conformational HIV Env epitopes and, as proof of concept, the analysis of HIV p24 CA-based scaffolds using a complete V3 loop in a MAb-bound conformation is presented. Evaluation of binding of p24:V3 proteins to anti-V3 MAbs was performed by ELISA and SPR analysis.

**Results:** The V3-p24 epitope-scaffold proteins show the formation of capsomers made of hexamers similarly to the p24 wild type protein. Moreover, the conformational V3 loop presented on p24 scaffold is recognized by a panel of anti-V3 MAbs. The results suggest that HIV p24 CA protein has suitable acceptor sites for engrafting foreign epitopes, without disrupting the formation of capsomer hexamer structures, and that the V3 epitope does retain its antibody-bound conformation. ELISA results show that the V3 loop inserted on the p24 scaffold is sufficiently exposed and retained the ability to bind to anti-V3 MAbs. Moreover, SPR analysis indicates that chimeric proteins bound anti V3 MAb in a quite similar fashion providing a KD values ranging from 10 to 13 µM.

**Conclusion:** This strongly support the feasibility of developing a scaffolding strategy based on p24 CA proteins displaying conformational minimal structural, antigenic HIV Env epitopes. Immunization analysis in animal model is currently ongoing and results will possibly presented. (Tagliamonte et al., PLoS One. 2012; 7(8):e43318)
**P13.07**

Development of Novel Simian Adenovirus Based Vaccine Vectors

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**Background:** Human adenovirus serotype 5 is a potent vaccine vector, but its use has been hampered by high seroprevalence amongst people in sub-Saharan Africa. Novel adenoviral vaccine vectors from strains with lower seroprevalence worldwide are being developed that can evade pre-existing immunity. Here we describe the development of a panel of novel old world monkey adenovirus vaccine vectors.

**Methods:** Wild type adenoviruses were isolated from rhesus monkey stool samples tested positive for adenovirus by metagenomic sequencing. Novelty was determined by whole genome sequencing. sAd4287, sAd4310A and sAd4312 were cloned from these new isolates and subjected to immunology and seroprevalence assays. Neutralizing antibodies against sAd287, sAd4310A and sAd4312 were determined using a panel of 106 rhesus macaque sera and 128 human sera from Rwanda and South Africa using a luciferase-based adenovirus neutralization assay. The immunogenicity of a single dose of 10E7, 10E8 or 10E9 virus particles of each sAd-SIV Gag based vector was determined in C57BL/6 mice. SIV-Gag-specific immune responses were assessed by Db/AL11 tetramer binding assays, IFN-γ ELISPOT assays and ICS assays.

**Results:** 85-95% of human sera from sub-Saharan Africa had undetectable neutralizing antibody titers to these novel sAd vectors with 90% of positive tested sera showing titers <200. In comparison, seroprevalence of Ad5 in sub-Saharan Africa is 86.4-89.5% with 61.1-78.7% of this population showing titers >200 and 25.1-46.8% showing titers >1000. Neutralizing antibodies in monkey sera were detected in 5-45% of the population, 90% of which showing titers <200. Gag specific CD8+ T-lymphocyte responses were readily detected with Db/AL11 tetramer as well as IFN-γ ELISPOT assays. SIV Gag specific immune responses elicited by these sAdVs are comparable to those seen with the human Ad26 and Ad28 vectors currently in development at all doses.

**Conclusion:** These data suggest that these novel simian Ad vectors are promising for further studies as candidate vaccine vectors.

**P13.08**

Immunogenicity Potential of MVA Vectors After Removal of the Immunomodulatory A44L and A46R Viral Genes

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**Background:** Modified Vaccinia Ankara (MVA) still retains genes involved in host immune response evasion. We have previously reported the optimization of MVA vaccine potential after the deletion of the C12L gene encoding an IL-18 binding protein. Here we analyze the immunogenicity of MVA vectors harboring the simultaneous deletion of two viral genes: A44L, implicated in synthesis of steroid hormones and A46R, which inhibit transduction signals from TLR receptors (MVAΔA44L-A46R); or with an additional gene deletion: C12L (MVAΔC12L/ΔA44L-A46R).

**Methods:** C57Bl/6 mice were intramuscularly immunized with wild type (MVAwt), or deleted MVAs (dMVAs). T cell responses to VACV (Vaccinia Virus) epitopes were evaluated at acute and memory phases (7 and 45 days post-immunization (dpi) respectively). The proportion of IFN-γ and IL-2 producing cells was measured by ELISPOT. Percentage of cytotoxic CD8-T cells was analyzed by flow citometry through expression of CD44 and CD62L among proliferating (CFSElow) T cell populations. SIV (Vaccinia Virus) epitopes were evaluated at acute and memory phases (7 and 45 days post-immunization (dpi) respectively). The proportion of CD8 and CD4-T cell responses were maintained (p<0,05); and IL-2 anti-VACV CD8- and CD4-T cells (1,5 to two-fold, p<0,01; 2,5 to five-fold, p<0,01 respectively); and IL-2 anti-VACV CD8-T cells (up to five-fold higher; p<0,01). Importantly, increments at 45 dpi in IFN-γ anti-VACV CD8 and CD4-T cell responses were maintained (p<0,05); regarding anti-VACV IL-2 secreting cells, improvements were of two or four-fold against CD4 and CD8 peptides (p<0,05). Proliferating anti-VACV CD8-T cells were incremented from 1% (MVAwt) to 2.8% (MVAΔC12L/ΔA44L-A46R) significantly this vector elicited a higher proportion of specific TCM (45,2%) than TEM (43,5%) compared to MVAwt (TCM:19,5%, TEM:63%). Percentage of specific cytotoxic CD8-T cells that secrete IFN-γ (CD107/IFN-γ), were also incremented by MVAΔC12L/ΔA44L-A46R.

**Conclusion:** Simultaneous deletion of specific viral genes from the MVA genome with inter-related functions as A44R and C12L is an adequate strategy to improve the vaccine potential of this vector.
P13.09

Sequence Evolution in SIVNef Refocuses the CD8 T Cell Response on More Conserved Epitopes

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Background: Vaccination with live attenuated simian immunodeficiency virus (LASIV) induces robust protection against wild-type SIV challenge, including sterile protection, which is rarely observed using other vaccine modalities. Protection induced by LASIV matures with time, significantly increasing at 15-20 weeks after vaccination. Neither the breadth nor the magnitude of the T cell response correlate with this increased protection, here we demonstrate that sequence evolution of LASIV and a concommitant shift in CD8 T cell specificity do.

Methods: Plasma or lymph node CD4 T cell-associated viral RNA from 8 SIVΔnef-vaccinated animals was obtained at time points ranging between 2 and 34 weeks after vaccination. Viral RNA was reverse transcribed, quantitated by qPCR, and sequenced using an Illumina MiSeq next generation sequencing platform. The CD8 T cell response was mapped to the epitope level for 6 animals at weeks 5 and 20 post-vaccination using ELISPOT assays. CD8 T cell responses at the protein level were mapped for 18 animals.

Results: SIVΔnef sequence evolution occurred as early as 3 weeks post-infection and continued into the chronic phase of infection, even in animals with undetectable plasma viral load. Viral mutations were concentrated in mapped and known CD8 T cell epitopes. Epitope escape was followed by a decreased frequency of epitope-specific CD8 T cells relative to the total SIV-specific CD8 T cell response. Epitopes with the lowest Shannon Entropy score did not escape, and their response frequencies were maintained. The Response Conservation Index (RCI), a measure of the extent to which the CD8 T cell response is focused on conserved regions of the virus, increased in all animals between weeks 5 and 20.

Conclusion: Extensive SIVΔnef sequence evolution occurs in vaccinated animals, even at low plasma viral load levels, and drives a concomitant shift in the specificity of the CD8 T cell response, refocusing it on more conserved epitopes.

P13.10

Longitudinal Assessment of Immunogenicity and Pathology of Dual HIV/TB Vaccines Constructed Using Modified Strains of BCG

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Background: An efficacious vaccine is required to combat the dual pandemic of HIV and TB. Our group has recently shown BCGΔpanCD Pasteur to be safer and more immunogenic as an HIV vaccine than wildtype BCG when used in conjunction with a Modified Vaccinia Ankara boost (MVA). Furthermore, TB vaccine studies have shown BCG expressing perfringolysin (pfo) promotes antigen translocation and induces stronger CD8+ T cell responses. In this study, wild type, ΔpanCD, pfo and a combination ΔpanCDpfo strain of BCG from Aeras were constructed to express HIV-1 subtype C Gag. We aimed to longitudinally assess pathology and cellular events leading to the development of HIV-specific immunogenicity.

Methods: rBCG vaccines were given intraperitoneally to BALB/c mice, sacrificed 2, 3, 7, 12 and 28 days post-vaccination. Using flow cytometry and immuno-histochemistry we assessed the roles of dendritic cells, T cells, B cells and macrophages in the development of pathology and immunogenicity. Additional groups of rBCG vaccinated mice were boosted MVA-boost (day 28) and sacrificed (day 40). Using ELISPOT and flow cytometry, we assessed HIV specific immunogenicity at the day 40 time point.

Results: HIV-specific polyfunctional CD4+ and CD8+ T cell responses induced by the modified strains of BCG were of higher magnitude as compared to the wildtype strain following an MVA boost. Increased infiltration of antigen presenting dendritic cells, T cells, B cells and macrophages in the development of pathology and immunogenicity. Additional groups of rBCG vaccinated mice were boosted MVA boost (day 28) and sacrificed (day 40). Using ELISPOT and flow cytometry, we assessed HIV specific immunogenicity at the day 40 time point.

Conclusion: Extensive SIVΔnef sequence evolution occurs in vaccinated animals, even at low plasma viral load levels, and drives a concomitant shift in the specificity of the CD8 T cell response, refocusing it on more conserved epitopes.
P13.11

Elaboration of Imiquimod-Loaded Biodegradable Micelles as HIV-1 Vaccine Adjuvant

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Background: The development of antigen-based vaccines requires the use of particulate adjuvants (emulsions, polymers) to improve antigen immunogenicity. An important challenge to improve efficiency of such vaccines relies on their ability to target and activate dendritic cells (DCs), which play a crucial role in the immune response. This can be achieved by incorporating, in addition to the antigen, DC ligands to the particulate adjuvant. In this context, we have developed polymeric micelles functionalized with HIV-1 p24 at their surface and imiquimod, a TLR7 ligand, in their inner core.

Methods: Micelles of amphiphilic block copolymer poly (D, L-lactide)-b-poly N-vinylpyrrolidone-co-N-acryloxysuccinimide (PLA-b-P(NVP-co-NAS), 19000-22000 g.mol -1 , PDI=1.5) were prepared by nanoprecipitation and characterized in terms of size (DLS) and critical micellar concentration (cmc). Imiquimod was incubated with the aqueous micellar solution for 1 hour and imiquimod loading in the micelles was quantified by fluorimetry. The micelles were further surface functionalized p24 antigen, through amine reaction with the N-succinimidyl esters of the NAS units at micelle surface in PBS pH 7.4 for 20 hours. Protein coupling on the micelles were investigated by SDS-PAGE. The expression of DC maturation/activation markers CD80, CD83, CD86 induced by the micelles was assessed in vitro by flow cytometry.

Results: The micelles initially prepared from the PLA-b-P(NVP-co-NAS) copolymer exhibited a mean diameter of 58 nm and a CMC of 20 mg/mL -1 . Hydrophobic imiquimod was encapsulated at a loading of 1.5% (w/w). The imiquimod-loaded micelles were further highly surface-functionlized with p24 antigen, at a level of 0.12 mg p24 per mg of micelle, with a final size of 110 nm. Imiquimod encapsulated in the micelles was shown to induce up-regulation of the DC maturation markers.

Conclusion: Such novel micelle-based systems could thus be promising for vaccine delivery, particularly considering their ability to potentially reach DCs in the lymph nodes, due to their small size (<120 nm).

P13.12

Immunofocusing to HIV's V2 Loop C β-Strand

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Background: The RV144 trial is the only HIV vaccine clinical trial to show evidence that a vaccine can prevent HIV infection. We aligned the V2 reagents with odds ratios (OR) lower than 0.5 reported in the RV144 immune correlates study, and we determined that at least some protective antibodies (Abs) from the RV144 trial target the peptide segment from positions 165-181 of the V2 loop (V2 165-181), which is centered on the V1V2 domain's C β–strand. Thus, vaccine immunogens that exclusively present V2 165-181 may preferentially elicit Abs that protect against HIV infection.

Methods: We fused a V2 165-181 peptide to a non-HIV scaffold in a manner that recapitulates the conformation of this segment in the native folded V1V2 domain. We tested this designed immunogen by immunization in rabbits using a DNA prime-protein boost approach.

Results: We found that IgG from the immunized rabbits' sera bound a cyclic V2 peptide with high endpoint titers and neutralized Tier 1 HIV viruses from subtypes B, C and AG.

Conclusion: Our data demonstrate that Abs elicited from a synthetic peptide consisting of only the key C β-strand-centered segment of the V1V2 domain can bind to V2 165-181 in its native context within the HIV virus, and in some cases can neutralize the virus. Our results provide proof of concept that a V2 165-181 immunofocused immunogen, which can be further designed to preferentially present the epitopes associated with protection in the RV144 trial, can elicit neutralizing antibodies.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L2, Room 211 – 212. Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L2, Room 211 – 212.

**P13.13**

**Replicating Vesicular Stomatitis Virus Vectors (VSV) Expressing Membrane-Anchored MPER Immunogens**

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**Background:** The membrane-proximal external region (MPER) is an important vaccine target because it is a well-conserved element of Env that is recognized by several broadly neutralizing monoclonal antibodies. Moreover, MPER-specific neutralizing antibodies are generated in some HIV-infected patients emphasizing that the human immune system does recognize MPER structures presented on HIV particles and infected cells. Because the native MPER structure is dependent on its association with the lipid bilayer, we have developed plasmid DNA and live VSV vectors that express the MPER as a membrane-anchored immunogen.

**Methods:** The transmembrane domain and cytoplasmic tail of VSV G were used as a platform (G-stem) for surface expression of an ectodomain composed primarily of MPER sequence. Live VSV and plasmid DNA vectors were constructed encoding G-stemMPER immunogens.

**Results:** Plasmid DNA and VSV vectors were designed to express G-StemMPER immunogens as integral membrane proteins. The VSV vectors coexpress G-stemMPER and native G, thus they are replication-competent. Western blot analysis conducted with transfected or infected cells confirmed expression of G-stemMPER proteins by both vectors, and flow cytometry showed that the MPER epitopes also were present on the cell surface and bound to neutralizing monoclonal antibodies. Consistent with G-stemMPER incorporation into the cell plasma membrane, analysis of purified VSV vectors revealed that the immunogen also was present in virus particles along with native VSV G. A murine study is underway to evaluate the immunogenicity of electroporated DNA prime followed by an intranasal boost with replicating VSV-G-StemMPER vectors and we are currently analyzing immune responses.

**Conclusion:** Plasmid DNA and replicating VSV vectors expressed membrane-anchored G-stem-MPER which were antigenic and have been used to safely vaccinate mice. Data from the ongoing murine immunogenicity study will determine if the G-stemMPER immunogen strategy is an effective approach for inducing antibodies with MPER specificity.

**P13.14**

**Thwarting HIV Evasion of Antibody Avidity with Novel Antibody Architectures**

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**Background:** Despite decades of effort, no current vaccine elicits neutralizing antibodies at concentrations blocking HIV infection. In addition to structural features of HIV’s envelope spike that facilitate antibody evasion, a hypothesis for the ineffective immune response lies in the low density and limited mobility of HIV envelope spikes, which impedes bivalent binding by antibodies, reducing avidity, minimizing the potential for high affinity binding and virus neutralization.

**Methods:** We intend to engineer anti-HIV reagents that bind with high avidity to single spikes, overcoming potential problems with the low density of HIV spikes. Here, we demonstrate a strategy to use dsDNA as a rigid molecular ruler to map epitopes on the HIV envelope protein to gain insight into the relatively unknown spatial environment of the spike trimer. Upon determining the optimal separation distance between epitopes, the dsDNA linker will be replaced with a structured protein linker. This technique should allow for the development of a novel multivalent antibody reagent improving binding and increasing avidity.

**Results:** Optimal HIV binding proteins will be trimerized by attaching a trimerization motif, reducing the concentration required for sterilizing immunity. To date, we have several bispecific DNA reagents that have the ability to neutralize various strains of HIV with greater potency than its individual components.

**Conclusion:** These results demonstrate the promise for discovery of optimal anti-HIV reagents using this technology.
P13.15

Membrane Biophysical and Structural Investigations of HIV-1 Envelope Glycoproteins Epitopes in the Aim of Eliciting Liposomal Vaccine

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Background: A few stable regions have been discovered on the envelope glycoproteins of HIV-1 against which some patients produce neutralizing antibodies. The most promising epitopes are located in the membrane proximal external region and are probably exposed transiently during the fusion of cellular and viral membranes. Whereas the peptides isolated from this region failed to induce neutralizing immunogenic response. Previous studies suggest that the lipid membrane plays a role in antigens structure and in the immunogenic response developed by these patients.

Methods: We investigate these epitopes (one from gp120 and two from gp41) and their insertion into liposomal carriers by structural approaches (Nuclear Magnetic Resonance, Circular Dichroism: CD). This requires the production of the polypeptides by bacterial overexpression and their purification by using biochemical methods, and their reconstitution into liposomal carriers. In addition, the immunogenicity of these epitopes will be investigated by mice immunization and the immune response will be quantified by ELISA.

Results: The conditions for expression and purification of these polypeptides are established. The purified polypeptides solubilised in detergent are reconstituted overnight into liposomal carriers by a dialysis approach. Their secondary structure is investigated by CD. gp120 and gp41a adopt mainly an alpha-helical structure in the detergent. In contact with the lipids, gp41a maintains this helical structure and it is reinforced in liposomes having a similar composition of the viral membrane. In contrast the gp120 construct adopts a predominantly beta-sheet structure.

Conclusion: The comparative analysis of the potential for immunogenicity and structural results obtained will be confronted in order to better understand the fusion of the cellular and viral membranes and thereafter to help in the design of vaccines directed against HIV-1.

P13.16

Construction, Stability and Immunogenicity of Recombinant BCG Expressing HIV-1 Subtype C Gag Under Control of Different Promoters and Signal Peptides

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Background: The HIV epidemic continues to spread globally, thus highlighting the need for the development of an effective HIV vaccine, most especially for developing countries. Mycobacterium bovis bacilli-Calmette-Guerin (BCG) has been widely used as an HIV vaccine vector due to its proven safety record. However, high level expression of viral antigens can often increase the metabolic burden resulting in genetic instability and hence poor immune responses. This study aimed to compare recombinant mycobacteria expressing HIV-1 gag under the control of different promoters and leader sequences. This was done to determine whether the genetic stability of the recombinant mycobacteria could be improved by modification of these vector features.

Methods: The optimal vector backbone was selected by stability assays. A series of shuttle vectors with different promoters (hsp60, psmyc or mtrA) and leader sequences (α-antigen or 19kDa signal peptide) were constructed and their stability was determined by means of relative colony size scoring and growth curves. Lastly the immunogenicity of recombinant BCG expressing HIV-1 Gag alone or Gag fused to either of the leader sequences was evaluated in mice.

Results: It was shown that the addition of various vector features such as 19kDa/α leader sequences and codon optimisation of HIV-1 gag did not have an effect on vector stability as indicated by the similar colony size score. Furthermore, the use of leader peptides resulted in differential expression levels of Gag as shown by p24 assays cultures with pEMαGag2(V3SV5) seemed to express more Gag as compared to pEM19Gag2(V3SV5) and pEMGag2(V3SV5) recombinants (64pg/ml as compared to 39 and 17 respectively).

Conclusion: Despite the low immune responses elicited by the rBCG vaccines, the panBCG[pEMαGag2V3SV5] maybe a promising vaccine as seen by increased immune responses to the V3 CTL epitope as well as Gag p24 levels from M. smegmatis with the same vector.
Development of Broadly Neutralizing Antibody Responses in a Large Sub-Sahara African HIV Primary Infection Cohort


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Methods: The IAVI Protocol C investigates a large longitudinal cohort of primary HIV-1 infection in Eastern and Southern Africa. Plasma neutralizing activity was assessed at Monogram Biosciences on longitudinal samples from 360 donors using a 6 cross-clade pseudo-virus panel predictive of neutralization breadth on larger panels. The specificity of the Env-specific broadly neutralizing antibody response was characterized in 29 Top neutralizers.

Results: At a median time post-infection of 3.7 years, broad neutralizing responses have so far developed in about 15% of individuals, essentially between year 3 and 4. Cross-clade neutralization appears to correlate with low CD4 T cell count and high viral load. Overall, neutralization potency is relatively low as compared to the cross-sectional IAVI Protocol G cohort (median time post-infection > 5 years) (Simek et al. J Virol, 2009). The N332 glycan-dependent epitope appears to be the most commonly targeted (20%) in Top neutralizers. We also found 2 volunteers (7%) with a PG9-like activity and one volunteer with a 10E8-like activity. However, no broad CD4bs neutralizing activity was detected. In contrast, broad CD4bs activity was found in about a quarter of Protocol G Elite neutralizers.

Conclusion: In conclusion, our data suggest that highly potent broadly neutralizing antibody responses, and more particularly responses targeting the CD4bs, may in general require more than 4-5 years of infection to develop. Concurrent longitudinal monoclonal antibody isolation and viral Env sequencing are currently ongoing for Top neutralizers with different broad specificities (PG9-like, N332-Glyc, MPER). Retracing the interplay between B cell and HIV leading to broad neutralizing antibody responses will be crucial for the design of an efficient AIDS vaccine.

Acceptability and Tolerability of Novel Mucosal Secretion Collection Methods in an HIV Vaccine Trial in East Africa

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Background: Mucosal surfaces are important portals of HIV-1 entry and considerable scientific effort has been dedicated to understanding virus-host interactions at these sites. The evaluation of mucosal immune responses presents a significant challenge due to the variability in specimen yields and introduction of unknown dilution factors in certain methods. We evaluated the acceptability of the Instead SoftCup device for collecting genital secretions from women and for collecting semen through masturbation from men in an HIV vaccine trial conducted in East Africa.

Methods: Healthy, low-risk, HIV uninfected, men and women enrolled in a Phase 1 HIV vaccine study (RV 262) conducted in Kenya, Uganda and Tanzania were asked to donate mucosal secretions. Volunteers refusing mucosal collections could still participate in the main trial. Cervico-vaginal (CV) secretions were collected through self-insertion of the SoftCup and semen through masturbation in clinic. Volunteer demographics and reasons for participation or refusal were noted in source documents.

Results: In total, 46 of 63 (73%) male participants agreed to semen collection. Among female participants, 12 of 22 (55%) agreed to provide specimens using the SoftCup. Average yield for semen was 2.12 mL and for CV secretions was 4.7 g, respectively. More men participated in the study than women at all three sites. No social harm or adverse events associated with the mucosal collection was reported. Participants who refused to take part in mucosal collections did so for cultural and religious reasons.

Conclusion: Despite initial concerns regarding the acceptance of the SoftCup device and masturbation, overall acceptability for both methods was high. The clinic staff was able to overcome concerns raised by other clinical staff, volunteers, and regulatory bodies through education and counseling. Our experience shows that SoftCup and masturbation are acceptable and well-tolerated methods for collecting cervico-vaginal secretions and semen to evaluate mucosal responses in low-risk, East African vaccine trial participants.
**P13.19**

**The HIV-1 gp120 C5 Region Shows HLA-like Properties for Immune Activation that May Be Masked by Antibodies**

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**Background:** The 5th constant domain (C5) of HIV-1 gp120 has previously been postulated to contribute to immune hyperactivation and shows serological and sequence similarity to human leukocyte antigens (HLA). Cross clade sequence and structural analysis was used to investigate the similarity of C5 to HLA as well as the potential for C5 antigens (HLA). Cross clade sequence and structural analysis was used and shows serological and sequence similarity to human leukocyte previously been postulated to contribute to immune hyperactivation. Anti-C5 antibodies may mask the C5 domain and prevent pan-DR by representing a 'pseudo HLA' with immune activating properties. By overlapping peptides did not bind HLA class I alleles, nor HLA class II with low-moderate viral load and slow disease progression. C5 conformation. Antibody responses to this C5/gp41 antigen correlated improved serological reactivity to C5, presumably by preserving C5 identified. A peptide antigen combining C5 and this region on gp41. This was used for the design of a peptide structural and serological analysis allowed for the design of a peptide structural and serological analysis was carried out based on curated alignments of HIV env gene sequences (M group) downloaded from the HIV sequence database (www.hiv.lanl.gov/). Sequence, structural and serological analysis for the design of a peptide incorporating both C5 and regions identified on gp41. This was used to determine the prevalence of anti-C5 antibodies in a treatment naive historical longitudinal cohort (n=32) and a cohort of natural viral suppressors (n=58). Overlapping peptides were used to determine the HLA class I and II binding profiles of C5 epitopes using ProImmune REVEAL MHc-binding assay.

**Results:** The C5 region was conserved with limited, largely clade-specific, variation at predominantly two positions reminiscent of the anchor points for epitope binding/presentation on HLA. The C5 region showed partial homology to the third hypervariable domain of HLA-DRβ alleles. A region on gp41 with potential to interact with C5 was identified. A peptide antigen combining C5 and this region on gp41 improved serological reactivity to C5, presumably by preserving C5 conformation. Antibody responses to this C5/gp41 antigen correlated with low-moderate viral load and slow disease progression. C5 overlapping peptides did not bind HLA class I alleles, nor HLA class II (HLA-DR or HLA-DP). C5 epitopes did bind to certain HLA-DQ alleles.

**Conclusion:** C5 is hypothesized to cause immune activation either by presentation as allo HLA-DR epitopes on HLA-DQ molecules or by representing a 'pseudo HLA' with immune activating properties. Anti-C5 antibodies may mask the C5 domain and prevent pan-immune activation.

**P13.20**

**Engineering New E.coli-Mycobacterial Shuttle Vector for a Dual HIV-TB Pediatric Vaccine Vectored by Lysine Auxotroph of BCG**

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**Background:** We previously proposed the use of recombinant BCG prime–recombinant modified vaccinia virus Ankara (MVA) boost pediatric vaccine platform against HIV-1 and Mycobacterium tuberculosis (MtB). Most of the selection methods for recombinant bacterial strains are based on antibiotic resistances. In this study, we have engineered a new mycobacterial vaccine design by using an antibiotic-free plasmid selection system.

**Methods:** We assembled a novel E.coli-mycobacterial shuttle plasmid p2auxo.HIVA, expressing the HIVA immunogen. This shuttle vector employs an antibiotic-resistance-free mechanism for plasmid selection based on glycine complementation in E.coli and lysine complementation in mycobacteria. This shuttle plasmid was transformed into glycine auxotroph of E.coli M15Δgλy strain and further transformed into lysine auxotroph of BCG strain to generate vaccine BCG.HIVA 2auxo. Genetic and phenotypic characterization of BCG.HIVA 2auxo strain was performed. The HIV-1 and MtB-specific mediated immunogenicity was assessed in adult BALB/c mice, in a heterologous prime-boost regimen using MVA.HIVA as a booster. Body mass loss of mice was monitored to assess the biosafety profile.

**Results:** Genetic and phenotypic features of antibiotic marker-less BCG.HIVA 2auxo strain were determined, and we demonstrated that the episomal plasmid p2auxo.HIVA was stable in vivo over a 7 weeks period. The presence of HIVA gene sequence and protein expression was confirmed. The BCG.HIVA 2auxo vaccine in combination with MVA.HIVA induced HIV-1 and MtB-specific interferon γ-producing T cell responses in adult BALB/c mice. Polynuclear HIV-1-specific CD8+ T cells producing IFN-γ, TNF-α and degranulating were induced. No significant difference in body mass was observed between the groups of animals.

**Conclusion:** We demonstrated T cell immunogenicity of a novel and safe BCG-vectored vaccine. In addition, this plasmid selection system based on double auxotrophic complementation might be a new mycobacterial vaccine platform to speed-up the development of recombinant BCG based HIV vaccines for clinical trials, but also for other major pediatric pathogens, to prime protective response soon after birth.
P13.21

Changes in Codon-Pair Bias of Human Immunodeficiency Virus Type 1 Have Profound Effects on Virus Replication in Cell Culture

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Background: Human immunodeficiency virus type 1 (HIV-1) has biased nucleotide compositions different from human genes. This raises the question of how evolution has chosen the nucleotide sequence HIV-1 observed today, or to what extent the actual encoding contributes to virus replication capacity, evolvability and pathogenesis. Here, we applied the previously described synthetic attenuated virus engineering (SAVE) approach to HIV-1.

Methods: Using synonymous codon pairs, we rationally recoded and codon pair-reoptimized and deoptimized different moieties of the HIV-1 gag and protease genes. Then redesigned gag and protease fragments were recombinated with HIV-1 infectious clones that lacked gag or protease. Replication capacity of the resulting viruses was tested in MT-4 cells and peripheral blood mononuclear cells (PBMCs).

Results: Deoptimized viruses had significantly lower viral replication capacity in MT-4 and PBMCs. Varying degrees of ex vivo attenuation were obtained, depending upon both the specific deoptimized region and the number of deoptimized codons. A protease reoptimized virus carrying 38 synonymous mutations was not attenuated and displayed a replication capacity similar to that of the wild-type virus in MT-4 cells. Remarkably, no reversion was observed in the reoptimized virus.

Conclusion: These data demonstrate that SAVE is a useful strategy to phenotypically affect the replicative properties of HIV-1. HIV-1 with different degrees of attenuation can be useful tools for the development of a safe and effective vaccine as well as the development of safer gene-therapy lentiviral vectors.

P13.22

HVTN-Tested Candidate HIV Vaccines Induce Early, Antigen-Specific Plasmablast- and Tfh-like Responses in Peripheral Blood

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Background: Long-term humoral immunity is the primary goal of protective vaccines. To generate this, a vaccine needs to prompt B cell differentiation into plasma cells and into long-lived memory populations. The induction of a functional B cell compartment requires the presence of pathogen-specific T follicular helper cells (Tfh), supporting formation of germinal centers in lymphoid tissue. The kinetics of early B cell responses to vaccination, including plasmablasts as well as peripheral blood CD4+ T cells with Tfh-like characteristics, are not well studied in humans.

Methods: We used PBMC samples from healthy, HIV-negative volunteers vaccinated with either Ad5- (n=10, HVTN 068) or MVA-vectored (n=5, HVTN 205/908) HIV vaccines or an HIV Env protein/MF59 regimen (n=10, HVTN 088), to assess the kinetics of B cell and Tfh-like responses following vaccination.

Results: Irrespective of regimen, early B cell responses (CD19+CD27-CD20-CD38+ plasmablasts) consistently peaked one week after vaccination. Interestingly, no major changes in bulk memory B cell frequencies (CD19+CD27+CD20+IgD+) or naïve B cells (CD19+CD27-CD20+IgD+) were observed for any regimen. Additionally, we identified a peripheral blood T cell subset with Tfh characteristics (CD4+CXCR5+PD1+ICOS+) that also peaked one week following vaccination, suggesting that B cell and Tfh-like responses are synchronized. When we analyzed samples from the Ad5-vectored vaccine regimes, we further found that both, Tfh-like cells and plasmablasts, were antigen-specific.

Conclusion: Our data demonstrate that candidate HIV vaccines induce plasmablast- and Tfh-cell responses in peripheral blood. These cell types could be useful early surrogates for monitoring long-term vaccine-induced functional responses. This work is supported by NIAID UM1 AI068618 and UM1 AI068614.
P13.23

Sequential Immunization Approach for the V3 Epitope Using Constrained Peptides

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Background: The V3 is a well-known target for neutralizing antibodies. However, masking of the V3 loop remains a problem limiting the breadth of V3 directed antibodies. Nevertheless, V3 antibodies with impressive breadth and potency such as KD-247 and F425-B4e8 have been identified. These antibodies can neutralize some resistant primary isolates such as JR-CSF. Interestingly, these antibodies recognize a shorter epitope centered on the GPGR tip. Therefore, our current efforts are aimed at eliciting antibodies with high affinity to a short segment of the V3 tip by active immunization using constrained V3 peptides.

Methods: We have applied a structure based approach for designing constrained V3 peptides. Our most effective immunogen, C4-V3T303C-E322C, was able to neutralize tier1B strains such as ss1196 and 6535. Our present studies combine the application of such C4-V3 peptides used for priming followed by additional boosts with peptides representing shorter constrained V3 segment to focus the immune response to a shorter which may be exposed.

Results: We have examined several immunization strategies using long and short constrained V3 peptides. The neutralization data reveals that sequential immunization using long constrains peptides followed by short constrained peptide immunogens is superior to repeated boosting with long V3 peptides only. Furthermore, boosting with short constrained peptide is more effective than using short linear peptides. Binding data indicated that boosting with short V3 peptide increase the level of antibodies to the V3 tip. Studies to further support and improve our immunization strategy are ongoing.

Conclusion: High level of V3 directed neutralizing antibodies would contribute to any vaccine formulation. We believe that a sequential immunization approach which mimics to some extent natural infection can maximize the effectiveness of the V3 directed antibody response. If proven effective such an approach can be applied to other HIV-1 neutralizing epitopes.

P13.24

The Viral Vector Vaccine VSV-GP Boosts Immune Response upon Repeated Applications

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Background: Vesicular stomatitis virus (VSV) is a potent candidate vaccine vector for various diseases. However, VSV’s inherent neurotoxicity has limited its clinical application. Additionally, VSV induces neutralizing antibodies rapidly and is thus ineffective upon repeated applications. Our group has recently shown that VSV pseudotyped with the glycoprotein (GP) of the lymphocytic choriomeningitis virus, VSV-GP, is not neurotoxic. Here, we evaluated the potential of VSV-GP as a vaccine vector.

Methods: We used Ovalbumin (OVA) as a model antigen and analyzed immunogenicity of GP-pseudotyped and wild-type VSV expressing OVA (VSV-GP-OVA and VSV-OVA) in vitro and in vivo in mouse models.

Results: Both vectors infected murine bone marrow-derived dendritic cells (bmDCs) in vitro. These bmDCs were able to activate OVA specific CD8+ and CD4+ T cells. Mouse experiments revealed that both VSV-OVA and VSV-GP-OVA induced functional OVA-specific CTLs and anti-OVA antibodies upon single immunization. However, boosting with the same vector was only possible for the GP-pseudotype but not for wild-type VSV. The efficacy of repeated immunization with VSV-OVA was most likely limited by the high levels of neutralizing antibodies, which we detected after the first immunization. In contrast, no neutralizing antibodies against VSV-GP were induced even after boosting. CTL responses induced by VSV-GP-OVA were as potent as those induced by an adenoviral state-of-the-art vaccine vector. Additionally, immunization with both vectors completely protected mice from infection with Listeria monocytogenes expressing OVA.

Conclusion: Taken together, VSV-GP is non-neurotoxic, induces potent immune responses, enables boosting and thus is a promising novel vaccine vector.
**P13.25**

**HIV-1 Env-Specific Antibody Responses and Generation of Broadly Neutralizing Antibodies Using Multimeric BAFF and IL-12**

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**Background:** High titers of broadly neutralizing antibodies (bNtAbs) are essential for protection against HIV-1 infection. The hypothesis of this study is that the B cell autonomous function of BAFF increases HIV neutralizing Abs by enhancing the generation of gp120-specific Bmem cells. Env-specific antibody responses are further enhanced by the cytokine IL-12.

**Methods:** BAFF soluble multi-trimers were evaluated for anti-Env antibody responses in DNA and DNA/protein prime/boost mice vaccinations. Antigen plasmid p96ZM651gp140-CD5-opt encoding membrane-bound HIV-1 gp140 protein (AIDS Research Reagent Program) and plasmid encoding soluble 4-trimer pBAFF were tested for immune responses in combination with plasmid expressing murine IL-12p70.

**Results:** Combinations of p96ZM651gp140-CD5-opt with pSPD-BAFF and pIL-12 enhanced humoral immune responses, as determined by significantly higher IgG2a titers when compared to gp140 plasmid plus pL-12 alone (p<0.05). BAFF + IL-12 also increased the number of gp120-specific B cells in the spleen, suggesting enhanced Bmem recall. Soluble multi-trimeric BAFF in combination with IL-12 increased Tier 2 neutralization compared to IL-12 alone. BAFF + IL-12 adjuvants generated neutralizing titers in 13/18 mice relative to only 2/8 in an IL-12 alone control.

**Conclusion:** Vaccination with BAFF soluble multi-trimers together with IL-12 enhanced the induction of humoral immunity against HIV-1 ENV. We propose that the B cell autonomous function of BAFF increases HIV neutralizing Abs by enhancing the generation of ENV-specific Bmem cells and these responses are further enhanced by cytokines such as IL-12.

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**P13.26**

**Deletion of the VACV A46R Gene, Encoding for a TLR Signalling Inhibitor, Enhances the Immunogenicity of the HIV/AIDS Vaccine Candidate NYVAC-C**

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**Background:** Viruses have developed strategies to counteract signalling through Toll-like receptors (TLRs) that are involved in the detection of viruses and induction of proinflammatory cytokines and IFNs. Vaccinia virus (VACV) encodes A46 protein which disrupts TLR signalling by interfering with TLR: adaptor interactions. Since the innate immune response to viruses is critical to induce protective immunity, we studied whether deletion of A46 in a NYVAC vector expressing HIV-1 Env, Gag, Pol and Nef antigens (NYVAC-C) improves immune responses against HIV-1 antigens.

**Methods:** NYVAC-C was used to generate the A46R deletion mutant NYVAC-C-deltaA46R. Cytokines produced by human primary macrophages infected with NYVAC-C or NYVAC-C-deltaA46R were quantified by ELISA. BALB/c mice were subjected to a DNA prime/NYVAC boost immunization protocol. The magnitude, quality and phenotype of the cellular immune response were assayed by polyfunctional flow cytometry (IL-2/IFN-gamma/TNF-alpha/CD107a) on splenocytes from immunized animals collected at days 10 and 53.

**Results:** NYVAC-C and NYVAC-C-deltaA46R replicated similarly in primary chicken embryo fibroblast (CEF) cells. Compared to NYVAC-C, NYVAC-C-deltaA46R markedly increased TNF, IL-6 and IL-8 secretion by primary human macrophages. Analysis of the immune responses elicited in BALB/c mice after DNA prime/NYVAC boost immunization showed that deletion of A46R improved the magnitude of the HIV-1-specific CD4 and CD8 T cell immune responses during adaptive and memory phases and maintained the functional profile observed with the parental NYVAC-C.

**Conclusion:** These findings establish the immunological role of A46 on innate immune responses of macrophages in vitro and antigen-specific T cell responses in vivo and suggest that deletion of viral inhibitors of TLR signalling is a useful approach for the improvement of poxvirus-based vaccine candidates.
**P13.27**

**Broad and Cross-clade CD4+ T Cell Responses Elicited by a DNA Vaccine Encoding Highly Conserved and Promiscuous HIV-1 M-Group Consensus Peptides**

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**Background:** Broad, polyfunctional and cytotoxic CD4+ T cell responses have been associated with control of HIV-1 replication and may represent a beneficial component of an efficacious HIV vaccine. Also, strategies to cope with virus and host genetic diversity are urgently needed.

**Methods:** We used the TEPITOPE algorithm in order to identify conserved, multiple HLA-DR-binding peptides in the HIV-1 M-group consensus sequence. In vitro MHC-peptide binding assay was performed to determine the ability of the selected peptides to bind to different MHC class II molecules. The frequency of peptide recognition was evaluated using PBMC from HIV-1-infected patients. A DNA vaccine encoding the selected peptides was designed and evaluated in BALB/c mice.

**Results:** We identified 27 peptides in the HIV-1 Gag, Pol, Nef, Vif, Vpr, Rev and Vpu sequences. The peptides bound in vitro to an average of 12 out of the 17 tested HLA-DR molecules and also to HLA-DP, -DQ and murine IAb and IAd molecules. Sixteen out of the 27 peptides were recognized by PBMC from HIV-1-infected patients and 72% of such patients recognized at least 1 peptide. Immunization with a DNA vaccine (HIVBr27) encoding the identified peptides elicited IFN-γ secretion against 11 out of the 27 peptides in BALB/c mice; CD4+ and CD8+ T cell proliferation was observed against 8 and 6 peptides, respectively. HIVBr27 immunization elicited cross-clade T cell responses against several HIV-1 peptide variants and polyfunctional CD4+ and CD8+ T cells against the pool of HIVBr27 peptides. In addition, HIVBr27 immunization led to lower viral load in mice challenge with recombinant Vaccinia virus encoding HIV-1 Gag and Pol.

**Conclusion:** We believe that our concept may cope with HIV-1 genetic diversity, provide increased population coverage and elicit protective T cell immunity against HIV-1/AIDS.

**P13.28**

**ABSTRACT WITHDRAWN**
P13.29

Attenuation of Replication-Competent Ad26 Infectivity by Vectorization

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Background: Replication-competent Adenovirus (Ad) vectors may offer important immunological advantages compared with replication-incompetent Ad vectors, but also raise important safety and regulatory issues. In this study, we assessed the effect of vectorization on the infectivity of replication-competent Ad26 (rcAd26). Specifically, we evaluated whether deletion of E3/E4 and insertion of an HIV Env transgene would attenuate replication-competent Ad26 vectors.

Methods: Replication-competent Ad26 (rcAd) vectors were produced by adding the E1 region back into replication-incompetent rAd26. We have produced rAd26 vectors that have either the E3 or E3/E4 regions deleted. The growth of various rAd26 vectors, with and without the Env transgene, were compared to wild-type Ad26 in Per55K (E1-complementing) and A549 (non-complementing) cells at a range of MOI and were scored daily for the presence of CPE.

Results: In the non-complementing A549 cell line, all rAd26 vectors were attenuated as compared to wild-type Ad26. The vectors with both E3 and E4 deleted were attenuated more than those with only E3 deleted. The addition of the HIV Env transgene attenuated the growth of the vector further. The rAd26 vector with E3 and E4 deleted and containing the Env transgene, which is a candidate for clinical studies, exhibited 2 logs less growth than did wild-type Ad26 at 100 MOI. All vectors grew equally well in the E1-complementing cell line Per55K, as expected.

Conclusion: All of the rAd26 vectors were significantly attenuated as compared to wild-type Ad26. The attenuation was most pronounced when both E3 and E4 were deleted and when containing the HIV Env transgene, which reflects the design of our clinical candidate vector. Attenuation as a result of vectorization suggests that replication competent vectors may have the potential to be safely used in humans.

P13.30

Vaccination Directed Against the Human Endogenous Retrovirus-K Envelope Protein Shows Efficiency in a Murine Tumor Model System

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Background: Human endogenous retrovirus (HERV) genomes are chromosomally integrated in all cells of an individual. They are normally transcriptionally silenced and transmitted only vertically. Enhanced expression of HERV-K accompanied by the emergence of anti-HERV-K-directed immune responses has been observed in tumor patients and HIV-infected individuals. As HERV-K is usually not expressed and immunological tolerance development is unlikely, it is an appropriate target for the development of immunotherapies of the HIV infection.

Methods: We generated a recombinant vaccinia virus (MVA-HKenv) expressing the HERV-K envelope glycoprotein (Env), based on the modified vaccinia virus Ankara (MVA), and established an animal model to test its vaccination efficacy. Murine renal carcinoma cells (Renca) were genetically altered to express E. coli beta-galactosidase (RLZ cells) or the HERV-K Env gene (RLZ-HKenv cells). Intravenous injection of RLZ-HKenv cells into syngenic BALB/c mice led to the formation of pulmonary metastases, which were detectable by X-gal staining.

Results: A single vaccination of tumor-bearing mice with MVA-HKenv drastically reduced the number of pulmonary RLZ-HKenv tumor nodules compared to vaccination with wild-type MVA. Prophylactic vaccination of mice with MVA-HKenv precluded the formation of RLZ-HKenv tumor nodules, whereas wild-type MVA-vaccinated animals succumbed to metastasis. Protection from tumor formation correlated with enhanced HERV-K Env-specific killing activity of splenocytes.

Conclusion: These data demonstrate for the first time that HERV-K Env is a useful target for vaccine development and might offer new opportunities as an HIV vaccine.
**P13.31**

A Multivalent Mixture of Clade C HIV-1 Env Trimers Elicits a Greater Magnitude of Neutralizing Antibodies than Individual Trimer Components

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**Background:** Increasing the magnitude and breadth of neutralizing antibody responses to the HIV-1 envelope (Env) protein by vaccination remains an important challenge for HIV-1 vaccine development. We assessed a panel of novel, stable, acute clade C trimeric gp140 Env protein immunogens in vaccination strategies, including a multivalent mixture of trimers.

**Methods:** Three acute clade C HIV-1 Env sequences from HVTN503 (405c, 459c, and 939c) were cloned, stabilized with the T4-fibrin C-terminal trimerization tag, and produced in 293T cells. Characterization of trimers was performed by SDS-PAGE, size-exclusion chromatography (SEC), and surface plasmon resonance (SPR). Guinea pigs were immunized three times with a total of 100 µg of trimer formulated in CpG/Emulsigen adjuvants. Vaccination regimens included all single trimers as well as a mixture of these three trimers. Antibody responses were determined by ELISA and TZM.bl neutralizing antibody assays, and comparisons were determined by Mann-Whitney U tests.

**Results:** Three novel, acute clade C gp140 trimers were confirmed to represent stable and homogeneous populations as assessed by SDS-PAGE and SEC, and contained the epitopes for CD4 and a panel broadly neutralizing antibodies as measured by SPR. A mixture of these trimers combined with our C97ZA012.1 trimer elicited a greater magnitude of neutralizing antibodies against cross-clade viruses, including clade A DJ263.8 (4.6-fold higher titer; p<0.05), and clade B SF162.1S and SS1196.1 (2.8- and 18.0-fold higher titer, respectively; p<0.05), as compared to any individual trimer administered alone. Against clade C viruses (MW965.26, TV1.21, and ZM109F.PB4), the mixture elicited neutralizing antibodies that were comparable to the most immunogenic single trimer component.

**Conclusion:** These data demonstrate that a mixture of acute clade C trimers elicited a greater magnitude of neutralizing antibodies than any individual trimer component against cross-clade viruses. These findings have important implications for the development of multivalent Env immunogen cocktails.

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**P13.32**

Optimized Poly-CTL-Epitope HIV-1 Immunogens for Inducing CD8+ HIV-Specific Immune Responses

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**Background:** CD8+ T cell mediated immunity is crucial to the development of HIV vaccine. Three artificial optimized HIV-1 polyepitope immunogens have been designed on the basic knowledge about antigen processing and presentation to T cells and realize a promising approach for inducing CD8+ HIV-specific immune responses. **Methods:** Using original software TEPredict and PolyCTLDesigner 50 CD8+ and 5 CD4+ T cell epitopes in the structure of HIV-1 antigens have been predicted with the conservatism of 80% among A, B and C HIV-1 isolates. Designed immunogens contain optimized core polyepitope sequence polyE and three flanking “signal” sequences in different combinations, including N-terminal ubiquitin, N-terminal signal peptide and C-terminal tyrosine motif of LAMP-1 protein to improve efficacy of different strategies of polyE processing and presentation on the pathways of MHC-I and MHC-II restriction. For enhancement of CD8+ and CD4+ T cells responses highly conservative HLA-DR binding Thelper peptides and “PADRE” peptide were included in the structure of target immunogens. Genes encoding designed immunogens were cloned in vector plasmid pcDNA3.1 and three DNA vaccine constructs have been obtained: pcDNA3.1_Kozak_polyE (TCI-N), pcDNA3.1_Kozak_ER-signal_polyE (TCI-N1) and pcDNA3.1_Kozak_UB-v76_polyE (TCI-N3). Target genes expression in vitro after transfection of 293T cells with obtained plasmids have been evaluated with mRNA detecting, Western blot analysis and with flow cytometry using antibodies to p24 marker epitope. Immunogenicity of engineered vaccines has been studied on mice model after DNA immunization with created plasmids. Intracellular cytokine staining assay was applied to evaluate HIV-specific CD4+ and CD8+ responses in this study.

**Results:** All plasmid constructs provide intracellular processing and gene expression of engineered artificial immunogens. Following immunization all investigated DNA vaccines elicited HIV-specific cellular immune responses. Immunogens contained ubiquitin and LAMP-1-ER-signal show tendency for increasing CD8+ HIV-specific responses. **Conclusion:** Experiments on animal models demonstrate that optimization of polyepitope HIV-1 immunogen structure influences on quantity and magnitude of CD8+ HIV-specific responses.
P13.33

A gp41 Vaccine with Dual Effect to Both Neutralize HIV and Inhibit CD4 Depletion

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Background: We previously demonstrated that a specific HIV gp41 motif (3S) induces expression of Nkp44L, a ligand of the NK activating receptor (Nkp44L), on CD4 cells. This 3S motif is highly conserved in all HIV-1 clades, and appears critical during HIV infection in inducing both a sharp increase in NK lysis and the CD4 cells decrease. Experiments in macaques showed that both preventive and therapeutic vaccine against the wild-type (WT) 3S motif blocks CD4 depletion, and inhibits cells apoptosis, activation and inflammation, without significant effect on the viral load.

Methods: Mutants were prepared from plasmid pNL4.3 with the QuickChange II XL Site-directed mutagenesis kit, transfected into 293T cells and then tested in purified CD4 cells. Virus production was monitored by p24 antigenometry. Neutralization assays were performed after immuno-purification of antibodies (Abs) on purified CD4 cells and TZB-bl cells. Expression of Nkp44L on CD4 cells and degranulation assay on Nkp44+ NK cells were both performed by flow cytometry. Cross reactivity of 3S Abs was also tested by Elisa.

Results: We show here that a specific substitution in the 3S motif, called W614A, reduces viral infection without affecting gp41 production. Generation of murine Abs against this mutated elicited a capacity to neutralize cross-clade tier 1 and tier2 viruses, impressive in its magnitude, breadth and duration. Importantly, anti-W614A-3S neutralizing Abs (NAbs) were naturally found in sera from some HIV-1 infected patients. Remarkably, these NAbs do not react against the WT 3S peptide, but preserve their capacities to inhibit Nkp44L expression and therefore to protect against CD4 depletion.

Conclusion: These findings suggest that a specific substitution in the 3S-based immunogen might allow the generation of NAbs while protecting CD4 depletion and thus provide a foundation for a rational vaccine design that can neutralize HIV-1 and protect CD4 cells.

P13.34

Safety in a Phase 1 Randomized, Double-Blinded, Placebo-Controlled Trial Evaluating Two Adenovirus HIV Vaccines in Three Different Geographic Regions

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Background: Homologous and heterologous prime-boost regimens were compared using two adenovirus vectors (Ad26.EnvA.01 [Ad26] and Ad35-EnvA [Ad35]) in the USA, East Africa and South Africa. This is the first trial using either vector for an HIV preventive vaccine in Africa. Safety data between these regions were compared.

Methods: The B003/IPCAVD-004/HVTN 091 trial vaccinated 217 healthy HIV-1 negative adults in a randomized, double-blinded, placebo-controlled study in three regions – USA, (Boston, n=51), East Africa (Rwanda, n=45; Kenya, n=40) and South Africa (n=81). Volunteers received Ad26 and/or Ad35 vaccines (5 x 10e10 viral particles) intramuscularly at 0, 3- or 0, 6-month (USA only) intervals. Reactogenicity was collected for 7 days (Days 0 through 7) postvaccination, adverse events (AEs) through one month following second vaccination, serious adverse events (SAEs) throughout the trial and viral cultures for adenovirus post first vaccination (USA only). Local reactogenicity included pain, tenderness, erythema and induration. Systemic reactogenicity included fever, chills, malaise, myalgia, arthralgia, headache, nausea and vomiting.

Results: Safety data are still blinded. Median age was 25.0 (range: 18-50); 49% were female and 78% were black. Reactogenicity was self-limited and did not vary by vaccine regimen or increase with second vaccination. Local reactogenicity was similar across regions and mostly mild (65% overall) in severity. Systemic reactogenicity was also similar across regions with comparable moderate (29.5% overall; range: 28.2%-31.4%) and severe (3.7% overall; range: 1.2%-7.8%) events. Related nonserious AEs (33/452, 7.3%) were mild or moderate and similar (e.g., pruritis, dizziness) between regions. No vaccine-related SAEs were reported. No pregnancies occurred within the disallowed 4-month period post last vaccination. Two volunteers acquired intercurrent HIV infections. No viral shedding was reported.

Conclusion: All regimens were well tolerated. Similar safety profiles were noted in all three regions despite potential differences in Ad26 and Ad35 exposure.
P13.35

Dual Deletion of Type I and Type II Interferon-Binding Proteins in NYVAC-HIV1 Env-Gag-Pol-Nef Poxvirus Vectors Markedly Improves Vector Immunogenicity

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Background: NYVAC, a replication deficient derivative of the Copenhagen vaccinia virus used, is a safe and immunogenic HIV vaccine vector. Deletion of immune evasion genes is an attractive strategy for improving NYVAC immunogenicity. Our aim was to characterize the innate immunological profile of NYVAC expressing the HIV-1 clade C env-gag-pol-nef genes (NYVAC-C) with single or double deletion of genes encoding type I (B19R) or type II (B8R) IFN-binding proteins.

Methods: Human monocytes were infected with NYVAC-C, B19R or ΔB8RB19R. Gene arrays and biological pathways were analyzed using the Illumina BeadChips and Ingenuity Pathway Analysis. Cytokines were quantified by RT-PCR, Western blot and ELISA. Proliferation of allogenic CD4-T cells was measured by H3-incorporation.

Results: Transcriptomic analyses of monocytes infected with NYVAC-C, B19R or ΔB8RB19R revealed a concerted up-regulation of innate immune pathways (IFN-stimulated genes-ISGs) of increasing magnitude with B19R and B8RB19R relative to NYVAC-C. Deletion of B8R or B19R enhanced activation of IRF3, IRF7 and STAT-1 and production of IFNs whose expressions were inhibited by anti-type I IFN antibodies. B8RB19R induced the production of much higher levels of pro-inflammatory cytokines than NYVAC-C or B19R and a strong inflammasome response. Consistent with these findings, B8RB19R-infected monocytes induced stronger type I IFN-dependent and IL-1b-dependent allogenic CD4-T cell responses than NYVAC-C or B19R.

Conclusion: Deletion of type I and II IFN evasion genes in NYVAC markedly enhanced its immunogenic properties via an increased expression of type I IFNs and IL-1b and make it an attractive candidate HIV vaccine vector.

P13.36

Characterization of Anti-SIV Antibody Responses Elicited by a DNA Prime/Trimeric Envelope Protein Boost Vaccine in Rabbits and Non-human Primates

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Background: Recent preclinical studies have demonstrated the use of properly folded trimeric HIV-1 envelope proteins in eliciting broadly neutralizing antibodies. Understanding the type of antibody responses generated by such envelope may be important for the development of an effective HIV-1 vaccine. As a proof of concept, trimeric envelope proteins from SIV may be used in non-human primates to test the efficacy of such vaccine for protection against a pathogenic viral challenge via mucosal route.

Methods: Trimeric gp145 proteins from SIVmac251 and SIVsmE660 isolates expressed in 293H cells were purified and characterized for their oligomeric conformation and high affinity binding to CD4. Rabbits and macaques were electroporated with DNA encoding Env from both isolates and subsequently boosted with homologous proteins either in adjuplex or inulin adjuvant. Sera collected at different times post immunization were examined for antibody affinity and linear epitope specific reactivity. Neutralization assays were performed in TZM-bl cells with SIVmac251 and SIVmacE660 isolates.

Results: Trimeric gp145 immunogen was shown to bind both rhesus and human CD4 with strong affinity comparable to monomeric gp120. Anti-envelope responses to SIVmac251 and SIVsmE660 gp145 were detected in rabbit and macaque sera following DNA immunizations and such responses were markedly enhanced following gp145 protein boost. Reactivity to both variable and constant domains of envelope was noted after protein boost accompanied with significant increase in binding affinity to gp145. At peak response, low to moderate level of neutralizing activity was observed against SIVmac251/M766, SIVsmE660-BR/CG7V and SIVmac251.30 isolates while significantly higher titers were induced against neutralization sensitive isolates SIVmac251.6 and SIVsmE660CG7V.

Conclusion: Trimeric envelope proteins from SIVmac251 and SIVsmE660 isolates elicited broadly reactivity anti-Env antibodies capable of neutralizing both sensitive and partially neutralization resistant SIV isolates. These envelopes together with other antigens that elicit cellular responses may be tested for efficacy against SIV challenge.
Multimeric Scaffolds Displaying the HIV-1 Envelope MPER Induce MPER-Specific Neutralizing Antibodies When Co-immunized with gp160 DNA Vaccines

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Background: Broadly neutralizing antibodies (bNAbs) have been shown to block infection in HIV-1 infection models. These bNAbs target conserved regions of HIV-1 envelope (Env), which allow for their breadth of reactivity. The elicitation of antibodies targeting these conserved regions through vaccination has proven to be difficult. One epitope of particular interest is the membrane proximal external region (MPER) of gp41.

Methods: We developed an MPER-directed vaccine by fusing the MPER epitope to the E2 protein of Geobacillus stearothermophilus. The MPER-E2 fusion protein self-assembles into a particle with icosahedral symmetry, displaying 30 copies of MPER on the surface, refolded with 30 copies of nonrecombinant E2 for stability. These particles were used in conjunction with an HIV-1 SF162 envelope DNA plasmid encoding either full-length gp160 or gp160 lacking hypervariable regions V2, V3, or V1, V2 and V3 to vaccinate rabbits. DNA plus protein vaccinations were given with the MPER-E2 particles delivered by intramuscular injection and the DNA plasmid delivered by Gene Gun inoculation.

Results: All groups developed anti-MPER binding antibodies following the second vaccination, with the gp160 lacking V3 group having significantly higher anti-MPER antibody titers (p<0.0001). Autologous NAbs (ANAbs) were also observed in all groups following the second vaccination with the V3-deleted group again demonstrating significantly higher titers (p<0.01). Low levels of MPER-specific NAbs were observed in all groups following the second vaccination, and increased with the third inoculation. NAbs to Tier 1 and 2 viruses were also observed for all groups with the breadth of the response trending with the MPER-specific NAb titers.

Conclusion: This study provides evidence that an MPER-E2 multimeric particle plus gp160 DNA vaccine can elicit moderate NAbs targeting the highly conserved MPER region, and that MPER-specific responses can be further enhanced by removing the immunodominant variable regions. These findings represent a significant advance in HIV vaccine design.

Development of Germline-Specific HIV-1 Envelope Immunogens Using Directed Molecular Evolution

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Background: Elicitation of broadly neutralizing antibodies against HIV-1 remains a major challenge for vaccine design. VRC01 belongs to a class of potent and broadly neutralizing antibodies targeting the CD4 binding site on gp120 and it has been suggested that the inability to engage germline precursors to these antibodies may be one reason for the failure of current immunogens to elicit VRC01-like responses. A recent study demonstrated that a viral envelope lacking specific N-linked glycosylation sites could activate B cells expressing germline VRC01, indicating that development germline-specific HIV-1 immunogens is possible.

Methods: We used in vitro homologous recombination of genes encoding wild-type Env sequences to generate libraries of gp120 stabilized core variants. We expressed 1,000 individual library clones in HEK-293 cells and screened culture supernatants for binding to VRC01 as well as human and macaque partial germline versions of VRC01. Selected variants from this initial screen were used to create recursive libraries which were screened for further improvements in antibody binding.

Results: Variants with increased affinity to VRC01 and partial germline versions of VRC01 were identified in the recombined libraries. Additionally, some variants showed increased affinity to b12, demonstrating that diverse phenotypic properties can be identified by this approach. Recursive libraries prepared using variants identified in the initial screen contained clones with further improved affinity to partial germline revertants. Importantly, we identified variants that bind to partial germline revertants of the macaque homologue of VRC01, indicating that immunogenicity studies in the macaque model are feasible.

Conclusion: The in vitro recombination method of generating variant proteins is capable of producing libraries of potential immunogens with diverse properties including increased affinity for partial germline antibodies. This directed molecular evolution approach is a valuable tool in the generation of immunogens with desired properties, including the ability to bind to germline precursors to broadly neutralizing antibodies.
P13.39

Modulation of Immune Responses to HIV-1 Env and Gag-Pol-Nef Antigens by Selective Deletions of a Poxvirus Gene

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Background: The poxvirus vector Modified Vaccinia Virus Ankara (MVA) expressing HIV-1 Env, Gag, Pol and Nef antigens from clade B (MVA-B) is currently used as a HIV/AIDS vaccine candidate. A general strategy to try to improve the immunogenicity of poxvirus HIV-1 vaccine candidates is the deletion of known or suggested immunomodulatory vaccinia virus (VACV) genes.

Methods: We have generated and characterized the immunogenicity profile of a new HIV-1 vaccine candidate, which contains a deletion in a VACV gene.

Results: Deletion of this VACV gene from the MVA-B had no effect on virus growth kinetics; therefore this VACV protein is not essential for virus replication. The innate immune signals elicited by the MVA-B deletion mutant in human macrophages and monocyte-derived dendritic cells were characterized. In a DNA prime/MVA boost immunization protocol in mice, flow cytometry analysis revealed that the MVA-B deletion mutant enhanced the magnitude and polyfunctionality of the HIV-1-specific CD4+ and CD8+ T cell adaptive and memory immune responses, with most of the HIV-1 responses mediated by the CD8+ T cell compartment with an effector phenotype.

CD4+ T cell immune responses were preferentially directed against Env in both immunization groups. However, contrary to other MVA-B deletion mutants previously described, where a pattern of GPN-specific CD8+ T cell immune responses were induced, this new MVA-B deletion mutant triggered CD8+ T cell immune responses preferentially directed against Env. Furthermore, MVA-B and the MVA-B deletion mutant are able to induce antibodies against Env.

Conclusion: These findings revealed that this new VACV protein can be considered as an immunomodulator and that deleting this gene in MVA-B confers an immunological benefit by inducing innate immune responses and modulating the magnitude and quality of the T cell adaptive and memory immune responses to HIV-1 antigens. Our observations are relevant for the selective improvement of MVA vectors as HIV-1 vaccines.

P13.40

Modulation of Vaccine Induced Humoral Immune Responses by Heterologous T Cell Help

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Background: The recent RV144 trial has not only renewed the hope in antibody mediated protection, but also the interest in secondary antibody functions. In our mouse studies we have observed a strong bias of HIV-1 Env protein and DNA vaccine regimens towards the less protective mIgG1. Thus, we tried to modulate the humoral immune response towards mIgG2a via heterologous T cell help.

Methods: To determine if BCR-dependent uptake of lentiviral particles enables Env-specific B cells to receive help from Gag-specific CD4+ T cells, we primed animals with Gag and/or Env expressing plasmids by intramuscular electroporation. Successful DNA immunization was verified by intracellular cytokine staining (ICS) and ELISA for cytokine and antibody production. Five and eight weeks after priming animals were boosted with Env and Gag containing virus like particles and antibody responses were followed by ELISA.

Results: DNA priming induced polyfunctional HIV-1 Env- and Gag-specific CD4+ T cells. Compared to Gag, Env-specific restimulation of splenocytes resulted in higher secretion of the prototypic TH 2 cytokines IL-4, 5 and 13. In line with these findings the Env-specific humoral immune response was dominated by mIgG1, while the Gag response was balanced. In the presence of Env-specific T cells the subsequent VLP immunization led to an increase in the humoral immune response against Env with mIgG1 still being the predominant subtype. In contrast, mice primed only with the Gag expression plasmid displayed a balanced mIgG1/mIgG2a ratio for Env-specific antibodies.

Conclusion: These findings revealed that this new VACV protein can be considered as an immunomodulator and that deleting this gene in MVA-B confers an immunological benefit by inducing innate immune responses and modulating the magnitude and quality of the T cell adaptive and memory immune responses to HIV-1 antigens. Our observations are relevant for the selective improvement of MVA vectors as HIV-1 vaccines.
P13.41

Immunogenicity of Novel DosR Candidate Vaccine Antigens of Mtb in ARV-Naïve HIV+ Patients with a Range of CD4 Counts from a High-Burden Country


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Background: Characterizing host immune responses to molecular targets of Mycobacterium tuberculosis (M. tuberculosis) is essential to develop effective immunodiagnostics and better vaccines. M. tuberculosis DosR regulon-encoded antigens are highly immunogenic in M. tuberculosis-infected humans and are associated with latent tuberculosis infection.

Methods: We tested several DosR, resuscitation promoting factors (rpf) and reactivation antigens in HIV positive (CD4 range: 79-641) and negative patients to assess the level of interferon gamma produced in response to stimulation. A 7-day diluted whole-blood assay and IFN-gamma ELISA was used to measure the level of cytokine induced to: Rv0569, Rv0081, Rv1733, Rv1735, Rv1737, Rv2028, Rv2029, Rv3131 (DosR), Rv1131 and Rv1471 (reactivation), Rv0867, Rv1009 and Rv2450 (rpf) and M. tuberculosis classical antigens.

Results: The responses to the antigens were classified as: 55% no responses (<32pg/ml), 15% low responses (32-125pg/ml), 10% intermediate (126-1000pg/ml) and 10% high responses (>1000pg/ml). In the HIV positive patients, TB10.4 was the most immunogenic producing interferon-gamma levels above 32pg/ml in more than 40% of individuals tested. Of the DosR regulon candidates, Rv1737 and Rv2029 produced IFN-gamma in more than 60% of the HIV positive participants. In our study, the latency antigens Rv0569, Rv0867 and Rv1131, but not Rv2031, induced significantly different responses between HIV negative, non-TB diseased and TB diseased participants.

Conclusion: The results indicate that HIV infection affects anti-mycobacterial immune responses, as measured by 7-day whole blood IFN-γ responses, to growth phase specific mycobacterial antigens. These are important findings as the antigens could prove useful as future vaccine constructs or components of diagnostic tests.

P13.42

Immunodominance of HIV-1 Consensus M Gag and Nef Peptides in a Mono- and Multiclade Epidemic: Implications for Vaccine Design and Testing

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Background: Measuring the impact of HIV diversity on immunological responses to new candidate immunogens is an important issue for HIV vaccine design/testing. This study investigated the reactivity and immunodominance patterns of HIV-1 consensus M Gag and Nef in (i) Cameroon, where individuals infected with the predominant CRF02_AG clade were compared with those infected with diverse non-CRF02_AG clades; and (ii) in a multi-clade epidemic, Cameroon, compared with a mono-clade C epidemic, South Africa.

Methods: We analysed CRF02_AG (n=24) and non-CRF02_AG-infected individuals (n=22) from Cameroon and 44 HIV-infected individuals from South Africa for differences in detecting HIV-1 consensus M Gag and Nef T cell responses using the IFN-γ ELISpot assay.

Results: There was no difference in the magnitude and breadth of responses for CRF02_AG and non-CRF02_AG-infected individuals in Gag and Nef. The specificity of peptides targeted, however revealed marked differences. For Gag, CRF02_AG and non-CRF02_AG-infected individuals showed a similar number of targeted peptides (27 and 22 respectively). Less than a third of these (11/38), however, were commonly recognised by both groups, with only one peptide commonly recognised by at least three individuals from both AG and non-AG groups, indicating poor immunodominance. In Nef, this pattern differed, with three times more peptides exclusively targeted in the diverse non-CRF02_AG group compared to the CRF02_AG group (10 vs 3), and more than half of all targeted Nef peptides (14/27) recognised by both groups. In contrast to Gag, four peptides were commonly targeted by at least three individuals in each group. Interestingly, very similar results to the within-Cameroon clade comparison were observed when South Africa, a mono-clade epidemic, and Cameroon, a multi-clade epidemic, were compared.

Conclusion: The central nature of consensus M sequences resulted in their broad recognition, but failed to identify highly immunodominant peptides between homogeneous and diverse epidemics. Further refinement of these immunogens may contribute to the development of a globally relevant vaccine.
Characterization of Antigenicity and Immunogenicity of a Trimeric Envelope Protein from an Indian Clade C HIV-1 Isolate

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Background: An effective HIV-1 vaccine may include properly folded envelope capable of inducing broad neutralizing antibodies. Recent studies have shown that trimeric HIV-1 gp140 fits this profile. However, selection of envelope proteins that can elicit such antibodies is challenging. Many Indian clade C HIV-1 envelopes have open conformation being fairly resistant to neutralization by sCD4 and CD4 binding site antibodies, while being more sensitive to CD4i antibody 17b. Therefore it is of interest to study the immunogenicity of trimeric Env derived from these isolates.

Methods: Trimeric gp145 protein from an Indian isolate 93IN101 was expressed in 293H cells and purified. Antigenicity of trimer was probed by binding of well characterized anti-Env antibodies. Immunogenicity was tested in rabbits as well as macaques. Rabbits were primed with codon optimized DNA vaccine expressing gp145 followed by trimeric gp145 protein boost. Macaques received gp145 protein alone. Immune sera binding affinity to gp145 and reactivity to linear overlapping envelope peptides were measured by ELISA. Neutralization of HIV-1 clade B and C pseudoviruses by immune sera was performed in TZM-bl cells.

Results: Trimeric gp145 showed strong binding to antibodies B6, B12, VRRC01, NIH45-46, 4E10 and PG9. Significant binding was noted with 17b, which was markedly enhanced in the presence of sCD4. In rabbits and rhesus macaques, gp145 elicited strong antibody responses to both variable and conserved domains of gp120. Neutralization of Tier 1 isolates was noted with rabbit sera, and this was enhanced by a subsequent boost with clade B Env. Neutralization by macaque sera extended to some Tier 2 isolates.

Conclusion: Env protein from an Indian isolate with unique antigenic properties is highly immunogenic in rabbits and macaques eliciting broadly reactive antibodies against multiple HIV-1 isolates. This protein, along with other antigens that elicit cellular responses, will be tested against appropriate SHIV challenge in future efficacy studies.

HIV-Recombinant Murine Leukemia Virus-like Particle Based Vaccines Induce Mucosal and Systemic Immune Responses in Mice

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Background: We developed DNA-vaccines expressing recombinant retrovirus-based VLPs (plasmoVLPs) pseudotyped with envelope glycoproteins expressed in their wild-type conformation. This strategy combines the efficiency of VLP-based vaccines with the simplicity and versatility of DNA-based vaccines. We previously demonstrated that plasmoVLPs induce significantly better epitope-specific CTL and envelope-specific neutralizing responses than standard plasmids expressing non-particulate antigens.

Methods: We designed HIV-specific retrovirus-derived VLP-based vaccines and investigated their immunogenicity after mucosal administration. BALB/c mice were immunized intranasally with PEI-formulated plasmoVLPs and boosted (or not) with VLPs or proteins by intravaginal or intrarectal routes. Immune responses were evaluated by ELISA in sera and lavages, and by T-, B cell ELISpot and ICS in spleen, lung, vagina and gut mucosal tissue.

Results: Our results show that the delivery vehicle and route of immunization are critical factors governing the vaccine efficacy. Remarkably, we demonstrated that potent HIV-specific mucosal and systemic immune responses are induced after intranasal immunization with plasmoVLPs in contrast to standard DNA vaccines. Using a prime-boost strategy, our results show strengthening T and B responses in both systemic and mucosal compartments. Remarkably, high numbers of HIV-specific IgA-secreting cells were observed in mucosa after intravaginal or intrarectal VLP administration, demonstrating the importance of local antigen administration to improve mucosal immunity. Importance of antigen form delivery was investigated by comparing VLP to protein boost. We observed that VLPs lead to similar systemic responses than protein boost but only VLPs induce high level of T cell multifunctionality at both systemic and mucosal level. Finally, we demonstrated that addition of genetic adjuvants encoding CCL28 or TSLP to our VLP-based vaccines have no effect on antibody responses but enhance significantly systemic T cell response.

Conclusion: Taken together, our VLP-based vaccines (plasmoVLPs and VLPs) represent efficient vaccine candidates that can be used to induce HIV-specific systemic and local immunity. Supported by CUT'HIVAC (EU FP7 #241904) and ANRS.
**P13.45**

**Somatic Hypermutation in a Broad and Potent Neutralizing Antibody Response to HIV as Probed by Deep Sequencing**

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**Background:** Broadly neutralizing HIV antibodies (bNAb) can protect against mucosal challenge in macaque models suggesting that elicitation of bNAb should be a high priority for candidate HIV vaccines. To date, however, no immunogen has reliably elicited significant levels of HIV bNab. Part of this failure might be due to the unusually high levels of somatic hypermutation (SHM) levels described for bNAb and the inability of immunization approaches to elicit such levels. The level of SHM that is absolutely required for both breadth and potency neutralization remains to be determined.

**Methods:** We used 454 sequencing and designed a novel phylogenetic method to construct lineage evolution of the bNAb PGT121-123. We characterized lineage intermediates for the development of neutralization breadth and potency and evaluated changes in how lineage variants recognize HIV Env.

**Results:** We found a positive correlation between the level of SHM and the development of neutralization breadth and potency. We also identified putative antibody intermediates that are only 6-10% mutated from germline in VH, which is approximately half the mutation level of PGT121-123, but are still capable of neutralizing 40-80% of viruses in a large panel with a potent median IC50 that is only 14 to 1.5-fold higher than the affinity-matured antibodies.

**Conclusion:** These lower mutation levels are close to the SHM levels that are commonly elicited through human vaccination and the antibody potency and breadth of neutralization at the levels of SHM described in this work have not been discovered previously. The results imply that elicitation of a combination of antibody specificities of lower SHM than the most mutated mature bNAb might represent a viable approach to HIV vaccine design. Patterns of neutralization of putative bNAb intermediates further suggest hypotheses as to how SHM may lead to potent and broad HIV neutralization and provide important clues for immunogen design.

**P13.46**

**Immunogenicity of Oligomeric gp41 Immunogens Exposing the MPER of HIV-1 Envelope Glycoprotein**

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**Background:** To overcome HIV diversity, vaccines for HIV/AIDS may need to elicit neutralizing antibodies to several conserved sites on the envelope glycoprotein (Env). Here we describe our efforts to focus antibody responses to the poorly immunogenic MPER by designing stable, oligomeric gp41 immunogens that have features of the fusion-intermediate conformation of Env. Our prior studies of conformational changes in Env suggested that immunogens based on fusion intermediate-type conformations may expose the MPER better than the native pre-fusion conformation or the final post-fusion conformation of Env in which gp41 forms a stable six-helix bundle (6HB) structure.

**Methods:** We created a panel of gp41 immunogens that lack gp120 to eliminate its contribution to epitope masking, but retain trimeric structure in part due to the inclusion of a partial six-helix bundle structure upstream of the MPER. Several iterations of this basic gp41 scaffold were analyzed in single antigen and prime-boost protocols involving gp41 and gp160 DNA primes and gp41 and gp140 protein boosts.

**Results:** We found that our gp41 protein immunogens elicited moderately strong MPER binding antibodies in a variety of assays, though significant neutralization to this determinant was not seen. We further describe how the various modifications of our immunogen or immunization protocol, such as inclusion of a V3 immunodominant epitope in the protein antigen or use of gp160 or gp41 DNA priming affected MPER-directed responses.

**Conclusion:** These findings inform the design of next-generation MPER immunogens and immunization protocols.
**P13.47**

**Early Life Immunity and Ability to Respond to Vaccines Hindered by Myeloid Derived Suppressor Cells: Implications for Infant HIV Vaccinology**

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**Background:** Despite great progress in reducing HIV mother-to-child transmission (MTCT), there are still serious challenges due to ART adherence. Elimination of new childhood HIV infections will likely require an effective infant HIV vaccine. However, development of such a vaccine may necessitate different approaches from the development of an adult HIV vaccine. Young infants respond poorly to infections and vaccines but the basis of reduced immunity is ill defined. Historically the infant’s immune system has been regarded as immature. Here, we describe, for the first time, myeloid-derived suppressor cells (MDSC) in the infant’s immune system.

**Methods:** The prevalence of MDSC during early life was analyzed by flow cytometry in blood mononuclear cells isolated from healthy cord blood (CB), infants and adults. Phenotypically, MDSC were identified as HLA-DRneg, CD14neg, CD33pos, CD11bpos, and CD15 pos. To determine the effect of MDSC on T cell proliferation, T cells and MDSC were depleted/purified from CBMC using CD3 or CD15 magnetic beads respectively. CFSE-labeled CBMC or CB-derived CD3pos T cells were stimulated with anti-CD3/anti-CD28 beads with or without MDSC.

**Results:** We identified a prominent population of MDSC in CB at 6-24 months and 0.8% in adults). The amount of suppression imposed by MDSC after CD3 stimulation of CBMC directly correlated with the frequency of MDSC. Addition of MDSC resulted in significantly reduced proliferative capacity of neonatal T cells (p<0.001, Wilcoxon-Matched-Pair-Signed-Rank-test).

**Conclusion:** In order to achieve effective infant immune responses to HIV vaccination or to control of HIV infection, MDSC populations will need to be manipulated in vivo. This project is funded by NIH (R01AI100018, K08HD069201) and UW CFAR (P30 AI027757).

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**P13.48**

**HIV Envelope Immunogens Derived from Critical Timepoints in the Development of Breadth Rapidly Elicit Heterologous Neutralizing Antibodies in Rabbits**

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**Background:** A major goal of vaccine efforts is the design of Envelope (Env)-based immunogens effective at eliciting broadly neutralizing antibodies (NAbs). During infection, HIV-1 evolves rapidly within the host, resulting in the development of a viral quasispecies population. We hypothesize that B cells become programmed to develop broad NAbs by exposure to Envs presented by viral quasispecies variants. We propose that similar programming could be achieved by a vaccine concept exposing the host to selected Env quasispecies variants isolated from individuals who rapidly developed broad NAbs.

**Methods:** Full-length functional env genes were cloned longitudinally from VC10014, VC20013 and CAP257, three clade B and C subjects, who developed neutralization breadth within two years of infection. Vaccine candidates from each subject were rationally selected using a combination of in silico phylogeny analyses and in vitro neutralization criteria. Rabbits were co-immunized with gp160-DNA and gp140-trimeric Envelopes from each subject to assess whether broad NAbs could be induced by Env immunogens derived from specific timepoints.

**Results:** NAbs were detected after only two DNA-protein co-immunizations and increased after subsequent co-immunizations. Importantly, Env immunogens derived from timepoints preceding or contemporaneous with the development of neutralization breadth elicited significantly higher NAbs than a single early immunogen or a longitudinal collection of Envelopes. Depending upon the Env source, neutralization breadth was elicited against clade A and B viruses and the epitope targeting observed in the broad neutralizer was replicated.

**Conclusion:** Taken together, these vaccine experiments show that HIV-1 Envelopes derived from timepoints preceding and contemporaneous with the appearance of neutralization breadth are a promising source of immunogens to elicit heterologous neutralizing antibodies via DNA-protein co-immunizations. This study exploring the use of multiple native and related Envs as immunogens emphasizes the value of understanding the development of breadth in subjects with broad NAbs.
**P13.49**

Deletion of C7L and K1L Genes Leads to Significantly Decreased Virulence of Recombinant Vaccinia Virus TianTan

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**Background:** The vaccinia virus TianTan (VTT) has been modified as an HIV vaccine vector in China and has shown excellent performance in immunogenicity and safety. However, its adverse effects in immunosuppressed individuals warrant the search for a safer vector in the following clinical trials.

**Methods:** In this study, we deleted the C7L and K1L genes of VTT and constructed six recombinant vaccinia strains VTTΔC7L, VTTΔK1L, VTTΔC7LK1L, VTKgpeΔC7L, VTKgpeΔK1L and VTTΔC7LK1L-gag. The pathogenicity and immunogenicity of these recombinants were evaluated in mouse and rabbit models.

**Results:** Comparing to parental VTT, VTTΔC7L and VTTΔK1L showed significantly decreased replication capability in CEF, Vero, BHK-21 and HeLa cell lines. In particular, replication of VTTΔC7LK1L decreased more than 10-fold in all four cell lines. The virulence of all these mutants were decreased in BALB/c mouse and rabbit models; VTTΔC7LK1L once again showed the greatest attenuation, having resulted in no evident damage in mice and erythema of only 0.4 cm diameter in rabbits, compared to 1.48 cm for VTT. VTKgpeΔC7L, VTKgpeΔK1L and VTTΔC7LK1L-gag were immunized twice, and elicited as strong cellular and humoral responses against HIV genes as did VTKgpe, while humoral immune response against the vaccinia itself was reduced by 4-8-fold.

**Conclusion:** These data show that deletion of C7L and K1L genes leads to significantly decreased virulence without compromising animal host immunogenicity, and may thus be key to creating a more safe and effective HIV vaccine vector.

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**P13.50**

Env-Specific Immunogenicity of Asian Mosaic HIV-1 Subtype AE/B gag/pol/env Combination and HIV-1 env Alone DNA Vaccine in BALB/c Mice

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**Background:** To overcome the genetic diversity of HIV-1 among Asian countries, mosaic design of HIV-1 DNA vaccines encompassing gag, pol and env regions derived from HIV-1 CRF01_AE and Asian subtype B were constructed.

**Methods:** Full-length gag, pol and env sequences derived from 156, 101 and 113 CRF01_AE and 72, 30 and 59 Asian subtype B, respectively, were computerized to generate 3 representative each of Asian mosaic HIV-1 gag/pol/env DNA. The mosaic were humanized and cloned into pCMV expression vector. The immunogenicity of the combination of gag/pol/env and env alone was tested in BALB/c mice by electroporation with 100 µg total DNA at the 1st dose and 20µg DNA at the 2nd and 3rd doses and an additional group of 12 and 5 million (4 million each of gag,pol,env in gag/pol/env and in env alone groups) pfu AE-vaccinia boosted. Thus, total env dose was 3-fold less when compared env alone to the env/gag/pol combination. Cellular immune responses were measured from splenocytes by IFN-gamma ELISpot assay by both HIV-1 AE and B pooled truncated peptides.

**Results:** The median ELISpot responses to env AE and B peptides of the Asian mosaic HIV-1 env alone group were 1088 and 680, while the responses of the gag/pol/env combination were 81 and 18 SFU/million splenocytes, respectively. Boosting with recombinant env-vaccinia alone (5x10⁶ pfu) or recombinant gag/pol/env (12x10⁶ pfu) to the above respective groups significantly enhanced (p = 0.002) the responses to 2012 and 1550 and 1833 and 1285 SFU/million splenocytes against AE and B pooled peptides, respectively.

**Conclusion:** Our results suggest that the Asian mosaic env HIV-1 DNA vaccine was more immunogenic when immunized alone at high dose, but it was much less immunogenic when 3-fold lower dose was used in the gag/pol/env combination. The DNA priming effect of both groups was however similarly effective when boosted with recombinant AE-vaccinia.
**P13.51**

**Expression of HIV Envelope Glycoprotein at the Yeast Cell Surface for Vaccine Development**

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**Background:** We are developing a system in which random mutagenesis and screening approaches are being used to identify forms of the HIV envelope glycoprotein (Env) that can act as effective immunogens for the elicitation of broadly neutralizing antibodies. In particular, we are trying to identify variants of Env with enhanced affinity for germine precursors to neutralizing antibodies, since poor affinity of most forms of Env for such precursors appears to be a major limitation on the ability to develop protective immune responses.

**Methods:** A synthetic gene encoding the sequence of YU2 Env using optimal codons for yeast expression has been used for cloning of constructs encoding gp120, gp140, and additional truncated forms of gp140 fused to sequences from the yeast Aga2p and Sag1p proteins in order to anchor the expressed proteins to the outer surface of the yeast cell wall. Expression of constructs containing a variety of signal sequences, flexible linker sequences, trimerization sequences, introduced protease cleavage sites, and normal or mutated furin cleavage sites has been evaluated by immunoblotting and flow cytometry using anti-Env and anti-epitope tag antibodies.

**Results:** We detect expression and cell surface display of gp120 and some longer Env fragments. The expressed proteins are glycosylated and undergo at least partial cleavage at the furin site apparently catalyzed by the endogenous yeast Kex2p protease. Reactivity of different forms of expressed Env with a battery of anti-HIV antibodies is currently being evaluated.

**Conclusion:** Expression of variants of Env displayed at the yeast cell surface system appears to be a useful system for identifying improved immunogens for HIV vaccine development. Libraries of variant forms of Env created by large-scale targeted random mutagenesis are being screened to identify variants exhibiting enhanced stability, preservation of oligomeric state, and affinity for germ-line precursors to broadly neutralizing antibodies.

**P13.52**

**Immunogenicity and Safety of Vaccinia Virus LC16m8Δ Expressing SIV Gag Under a Strong or Moderate Promoter in BCG Prime-Vaccinia Virus Boost Protocol**

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**Background:** Previously, we developed the very strong vaccinia virus (VV) pSFJ1-10 promoter that consists of ten repeats of the mutated early region of the p7.5 promoter and the ATI late promoter. But, too much expression of foreign protein decreased the propagation of VV. Balance between expression of the foreign antigen and viral propagation in vivo might be crucial for optimal immunogenicity. Thus, we compared the effect of the pSFJ1-10 and moderately strong p7.5 promoters on the immunogenicity and pathogenicity of the replication-competent VV LC16m8 (m8) harboring the SIV gag gene. We adopted vaccination regimen consisting of a recombinant BCG-SIVGag prime followed by a recombinant VV boost. This regimen may be expected to maintain effector memory T cells for long time because of persistent infection of BCG-SIVGag, and to elicit potent immunities because of replication competency of m8 recombinants.

**Methods:** C57BL/6 mice were injected with rBCG-SIVGag followed with rVV. Mice splenocytes were analyzed by intracellular staining assay. For safety evaluation, suckling CD1 mice were intracerebrally inoculated with various amounts of rVVs.

**Results:** m8/pSFJ/SIVGag synthesized more Gag protein than m8/p7.5/SIVGag but replicated less efficiently in vitro. In addition, m8/pSFJ/SIVGag was less pathogenic and elicited Gag-specific IFN-γ+, CD107a+, CD8+ cells more efficiently than m8/p7.5/SIVGag. Vaccination by this regimen elicited long-lasting Gag-specific CD8+ T cells, the majority of which showed a CCR7- phenotype. Tetramer staining analyses revealed maintenance of Gag specific tetramer+, CD62L-, CD8+ T cells, phenotype characteristic of effector memory T cells. Although both recombinants were less pathogenic than parental m8 that harbors intact HA gene, an insertion site for the gag gene, Gag expression increased the neurotoxicity of the empty HA-m8VNC110, indicating the necessity of safety testing for each recombinant VV.

**Conclusion:** We propose that this recombinant BCG prime-m8Δ/pSFJ/ HIVGag boost regimen would be a promising vaccination procedure for preventing HIV infection.
P13.53

Dissection of Antibody-Induced Structural Changes in a Soluble HIV-1 Trimer by Hydrogen/Deuterium-Exchange Mass Spectrometry

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Background: The HIV Envelope glycoprotein (Env) is the sole target for neutralizing antibodies on the virus surface. Recent efforts have led to the discovery of several broadly neutralizing antibodies (bNAbs), capable in some cases of neutralizing >90% of circulating isolates. High-resolution structures exist for many antibody Fabs complexed to fragments of Env, and electron microscopy has imaged large-scale changes in the trimer. The underlying, detailed structural changes resulting from bNAb binding to the native trimer, however, have yet to be determined.

Methods: Hydrogen/deuterium-exchange mass spectrometry (HDX-MS) reports on the protection of backbone amides resulting primarily from their involvement in secondary structure hydrogen-bonding. This analysis provides a detailed map of local structural dynamics as well as changes in structural order resulting from ligand binding. Here, we investigate the extent of structural reorganization in trimeric SOSIP gp140s when complexed with 17b, b12, VRC01, and PGT123 Fabs.

Results: 91% sequence coverage of Clade A KNH1144.664 SOSIP trimer enabled changes throughout most of the glycoprotein to be monitored. Antibody 17b, which binds a CD4-induced epitope, stabilizes portions of gp120 while loosening trimeric interactions involving the loops V1/V2 and V3. CD4 binding site-targeted antibody b12 stabilizes the binding site, but also produces a moderate disruption of trimeric interactions involving the variable loops. In contrast, another CD4 binding site-directed antibody, VRC01, stabilizes the CD4 binding loop without altering V1/V2 structure relative to the unliganded trimer. Variable loop and glycan-targeted PGT123 appears to stabilize both the loops and the CD4 binding site.

Conclusion: HDX-MS provides a robust method for dissecting conformational changes induced by antibody binding, including detection of distal allosteric effects. This study indicates that multiple mechanisms of neutralization may exist. Some bNAbs lock the trimers in the “closed” conformation, while others disrupt trimer interactions inducing it to adopt an “open” form.

P13.54

A Pure Native Env Trimer Vaccine Elicits Potent Tier 2 Neutralization to a Quaternary, Glycan-Sensitive Epitope

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Background: As the exclusive target of nAbs, the native Env trimer is a logical basis for a nAb vaccine. Given its immunorecessive nature, purity may be essential for its success as a nAb immunogen. In other words, it must be free of contamination by more immunogenic forms of Env.

Methods: Four groups of rabbits were immunized with: 1) “trimer VLPs”, expressing pure native JR-FL Env trimer, cleared of non-functional Env by a protease digest, 2) undigested control VLPs, 3) a JR-FL gp140F trimer DNA prime-protein boost, and 4) JR-FL gp120 prime-boost. Immune sera were characterized for binding, neutralization and specificity.

Results: All four immunogens were effective in eliciting anti-Env antibodies, as judged by gp120 ELISA, although “trimer VLP”-immune sera bound only weakly. One serum each from groups 1 and 3 exhibited remarkable IC50s >1:1,000 against the JR-FL index virus in the TZM-bl assay. Two group 4 sera exhibited titers of ~1:100. Mapping revealed that both potent sera targeted sites unusually lacking glycans. Specifically, the group 1 serum neutralization was regulated by a glycan at residue 197 in the V1V2 loop stem and the group 3 serum was regulated by a glycan at residue 230 in the C2 of gp120. Interestingly, the N197-regulated activity recognized a quaternary epitope. The two neutralizing sera of group 4 mapped to the C3-V4 region.

Conclusion: It is perhaps no coincidence that the two most potent sera targeted sites that unusually lack a glycan. Similar glycan-deprived sites have also accounted for autologous neutralization in natural infection. We are now working on strategies to elicit this activity more consistently, and to broaden its neutralization coverage.
**P13.55**

**HIV-1 VLP Production Platform Based on Transient Transfection of HEK 293 Suspension Cultures in Bioreactors**

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**Background:** Robust and scalable Gag VLP production processes are required to generate VLPs for preclinical and eventually clinical testing. Transient gene expression offers a convenient route to accelerate the identification of candidate VLP vaccines as it allows for the production of a large number of product variants with relative ease. Considerable progress has been made in the past several years towards establishing large-scale transient transfection protocols. The human embryonic kidney 293 (HEK 293) is the preferred host system due to the many industrially relevant features this cell line offers including high transfectability, ability to grow in suspension, ability to grow to high cellular densities and adaptation to serum-free culture conditions.

**Methods:** HEK 293 mammalian cell cultures were grown in suspension and serum-free conditions in disposable shake flasks and WAVE bioreactors. VLPs were generated by transient transfection using a Gag-GFP plasmid construct and polyethilenimine as transfection reagent. The GFP tagged VLPs were quantified using a spectrofluorometer.

**Results:** An optimized platform for the generation of Gag VLPs in mammalian suspension cultures is described in this work. To facilitate process optimization, a fast and reliable quantitation technique based on fluorescence was developed and validated. An in house culture medium supplemented with non-animal derived components was defined and the different transient transfection parameters were fine-tuned to allow maximum cell growth and VLP production. The great majority of Gag-GFP recovered from cell culture supernatants was shown to be correctly assembled into VLPs of the expected size and morphology. The scalability of this strategy was demonstrated using a 1L WAVE bioreactor.

**Conclusion:** The quantity and quality of Gag VLPs generated using the described platform is suitable for pre-clinical studies in mice. By using a GMP-compliant suspension cell line, disposable bioreactors and a culture medium devoid of animal-derived components, a fast translation into clinical trials is envisioned.

**P13.56**

**A Common Transitional Structure Revealed by a New Broadly Neutralizing Antibody as a Novel HIV-1 Vaccine Candidate**


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**Background:** Large numbers of broadly neutralizing antibodies (bnAbs) have been cloned in recent years. However, they typically show unusually high levels of somatic hypermutation and autoreactivity that might be major roadblocks to using them as templates for rational HIV-1 vaccine design. Novel bnAbs with less somatic hypermutation and no autoreactivity, therefore, should be explored as templates for rational HIV-1 vaccine design.

**Methods:** By functional screening, we have identified a novel bnAb, N60-B1.1 that is encoded by VH4-39 with 13.3% mutation at the nucleotide level. It uses a germline-like VK3-15*01 light chain. Further, it is not autoreactive. N60-B1.1 recognizes a transitional co-receptor associated region of Env involving epitopes in V1/V2 and V3 region. The neutralization pattern of N60-B1.1 is complementary to other bnAbs in that it potently neutralizes viruses that are often resistant to other bnAbs. N60-B1.1 also shows moderate ADCC function in a bound-virion assay.

**Results:** The binding of N60-B1.1 to gp120 was competed by V1V2 mAbs, V3 mAbs, traditional CD4i mAbs and non-broadly neutralizing CD4bs mAbs. In contrast, broadly neutralizing CD4bs mAbs, b12 and VRC01, as well as non-neutralizing ADCC Abs against conserved N terminal region of Env substantially enhance N60-B1.1 binding to gp120. Importantly, fusion proteins of gp120 with single-chain fragment variable regions (ScFv) of these enhancing mAbs substantially expose epitopes of N60-B1.1 and other epitopes recognized by the bnAbs, PG9 and PGT128.

**Conclusion:** Therefore, N60-B1.1 stands as a new, unique bnAb against HIV-1. Our results suggest that N60-B1.1 locks gp120 in an inactive conformation similar to those reported for the broadly neutralizing mAbs b12 and VRC01. The ScFv-gp120 fusions with improved and stabilized exposure of the N60-B1.1 epitope as well as other epitopes recognized by the bnAbs, PG9, PGT128 and VRC01, represent a common transitional structure that is a new candidate for evaluation in preclinical studies as a HIV-1 vaccine.
P13.57

VAC-3S, an Immunoprotective HIV Vaccine Directed to the 3S Motif of gp41, in Patients Receiving ART: Safety, Dose and Immunization Schedule Assessment

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Background: We have developed a novel immunotherapeutic vaccine (VAC-3S), comprised of 3S peptide, a highly conserved HIV gp41 motif coupled to commercially used carrier and adjuvant. The 3S motif leads, by NKp44L expression on CD4, to apoptosis of uninfected CD4. In vitro, anti-3S antibodies (Ab) protect CD4 from apoptosis. In cohort studies, anti-3S Ab correlate with lack of CD4 decrease and/or disease progression. VAC-3S primate proof-of-concept showed effect on immune and inflammatory biomarkers. Dose ranging studies, GLP toxicity, local tolerance studies were performed in rats and rat/mice, respectively.

Methods: First-Time-In-Human randomized, double-blind, placebo-controlled study conducted in multiple sections. (A) Dose escalation of 0.1, 1, 10 µg microg given IM, 4weeks in virologically ART controlled patients (pts) to assess safety. (B) a booster injected in 1 & 10 µg microg groups 6 month post-initial vaccination.

Results: First 2 study sections reported: 25 HIV pts (23 m) completed vaccination in 3 groups of 8. Median (range) age 47 yrs (32-54), CD4 710 c/mm3 (311-1187), CD4 nadir 336 c/mm3 (127-739), ART initiated for HIV and many infectious diseases because of its safety, lack of negative anti-vector serology, stability, and ease of manufacture. However, the immunogenicity of DNA vaccines has traditionally been low compared with viral vectors and recombinant protein. Recent work has established that the immunogenicity of DNA vaccines has been significantly enhanced by delivery with in vivo electroporation. Further improvements in delivery design allow electroporation to be performed in the dermis, which likely will impact tolerability and with improved delivery, further enhancing immunogenicity.

Methods: Guinea Pigs and non-human primates received intradermal DNA vaccination followed by in vivo electroporation at 0.2 or 0.1A. Results: Here we report that the current of intradermal vaccination impacts antigen expression, inflammation, and the induction of both humoral and cellular immunity to HIV antigens as well as reporter genes delivered into both guinea pigs and non-human primates. In contrast to prior studies with a variety of diverse delivery devices, we observed that by using a new focused EP array and much lower (0.1A) current we saw a reduction in inflammation which significantly improved antigen expression resulting in a much longer immune priming in vivo. In contrast, using higher current expression, in vivo priming was reduced. The improved antigen expression resulted in higher cellular immune responses with strong breadth and more consistent antibody responses.

Conclusion: This study supports the further development of electroporation technology in order to balance enhanced plasmid transfection with a loss of expression due to tissue inflammation and necrosis. We have also studied multiple delivery arrays and conditions for improving DNA transfer into the dermis. These all produce significant antibody responses in multiple animal species. These results have major importance for next generation DNA/EP approaches with improved delivery, improved immune potency and also improved immunogenicity.
**P13.59**

**Prime-Boost Vaccine Strategies Using Different Subtype Antigens Can Induce Broader Neutralized Antibodies**

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**Background:** Vaccine development to induce broadly neutralizing antibodies against HIV-1 is a global health priority. We proposed using different vaccine immunogens in series to trigger the appropriate maturation pathway of neutralized antibodies.

**Methods:** Guinea pigs were divided into 3 groups; Guinea pigs in group 1 and 2 were primed with DNA vaccines expressing HIV-1 clade A/E gag and env three times at two weeks intervals. Then they were boosted with rMVA carrying clade B/C gag and env twice at weeks 8 and 12. Additionally, at week 16, guinea pigs in group 2 were boosted once with rAd5 vaccine containing clade B gag and gp160. As negative control, guinea pigs in group 3 were inoculated PBS.

**Results:** Binding antibody responses from immunized groups were significantly higher than the PBS-injected group. By using TZM-bl cell neutralizing antibody assay, we measured post-immunization sera from each guinea pigs of three groups using HIV-1 clade B/C pseudovirus-pool containing 2 sensitive strains (tier 1 viruses, SC19-15 and BJ22-5) and 4 epidemic strains (tier 2 viruses, SC17-32, SC21-28, CX78-8, and XJ16-6). The endpoint titers were determined by ID50 value 2-fold higher than that from pre-immunization sera. Guinea pigs of both group 1 and 2 could induce moderate neutralizing antibodies compared with negative controls group. And sera form guinea pigs boosted with rAd5 showed better neutralization ability, in both intensity and width than those only boosted with rMVA.

**Conclusion:** By using this heterologous prime-boost immunization strategy, immunization with our DNA, rMVA and rAd5 vaccines could elicit high-titer binding antibodies, as well as broad neutralizing antibodies. Although the strength of induced neutralizing antibodies were moderate, these neutralizing antibodies could neutralize both tier 1 and 2 viruses which is important for vaccine design. Further exploration and refinement of this strategy may contribute to the development of an effective vaccine in China.

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**P13.60**

**Screening of HIV Envelope Spike Variants for Binding to Broadly Neutralizing Antibodies by Yeast and Mammalian Cell-Surface Display**

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**Background:** HIV-1 broadly neutralizing antibodies (bnAbs) are challenging to elicit in a vaccine setting. Reasons could be the very low to absent affinity of bnAb germline precursors to HIV-1 spike-derived immunogens or a non-adequate structural representation of the native spike. Protein display technologies are powerful tools to evolve proteins towards desired properties. We here investigated yeast and mammalian cell surface display to evolve an HIV-1 spike protein towards improved binding to bnAbs.

**Methods:** JR-FL gp140 was displayed on the surface of S. cerevisiae or HEK293F cells. Displayed spike proteins were characterized for binding to bnAbs using flow cytometry. HEK293F cell-displayed test libraries were subjected to multiple rounds of fluorescence-activated cell sorting (FACS) for binding to quaternary structure-specific bnAb pg16. Between sort cycles, episomal vector DNA encoding for gp140 was recovered, amplified in E. coli and transfected. Sorted spike populations were analyzed for enrichment of variants with improved binding to PG16.

**Results:** Yeast-displayed gp140 showed a restricted binding profile to CD4bs-, V1/2/3 loop- and quaternary structure-specific bnAb pep16. Between sort cycles, episomal vector DNA encoding for gp140 was recovered, amplified in E. coli and transfected. Sorted spike populations were analyzed for enrichment of variants with improved binding to PG16.

**Conclusion:** By using this heterologous prime-boost immunization strategy, immunization with our DNA, rMVA and rAd5 vaccines could elicit high-titer binding antibodies, as well as broad neutralizing antibodies. Although the strength of induced neutralizing antibodies were moderate, these neutralizing antibodies could neutralize both tier 1 and 2 viruses which is important for vaccine design. Further exploration and refinement of this strategy may contribute to the development of an effective vaccine in China.
P13.61

Developing an Anti-HIV Vaccine Using Nucleoside-Modified mRNA Encoding Envelope

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Background: Despite enormous progress in understanding the molecular mechanisms of HIV-1 infection, effective vaccine development has been protracted. Recent advancements in mRNA-based vaccination, however, provides a promising new modality to generate potent vaccines, which is further enhanced by using nucleoside-modified mRNA.

Methods: To create a vaccine with maximal potency, in vitro transcribed mRNAs were optimized for higher levels and extended translation with optimized UTRs, modified nucleosides, 5’ cap, 3’ poly(A)-tail and were HPLC-purified. In the present approach, sequences of HIV-1 ENV derived from different viral isolates (iR3A and 89.6) were used. The mice were immunized with naked mRNAs administered intradermally. Flow cytometry was used to evaluate T cell responses after a single or multiple administrations. To increase the robustness of the immune response, we co-injected mRNAs encoding adjuvant molecules, including CD40 ligand and constitutively active TLR3 and TLR4.

Results: Significant antigen-specific CD4+ and CD8+ T cell responses could be measured following one or more rounds of mRNA injections.

Conclusion: The promising preliminary results show that the use of mRNA might open up new possibilities in the field of HIV-1 vaccination. It is important to identify the most immunogenic HIV-1 ENV isolate and determine the optimal parameters and conditions, in order to elicit robust anti-HIV-1 B and T cell immune responses.

P13.62

Attempt at HIV Elimination by Enfuvirtide-IgG

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Background: There is a need for new anti-HIV agents or approaches, with the ultimate challenge of eradicating latent HIV-1 reservoirs in infected patients.

Methods: The proprietary Dock-and-Lock (DNL) platform technology was used to label human monoclonal antibody of anti-HIV or other specificities with four copies of enfuvirtide (T20), a peptide that inhibits HIV fusion to cell membranes. Labelling provides target specificity, prolonged serum half-life, higher binding avidity, and enhanced potency of anti-HIV activity. This was determined by virus neutralization, cell-to-cell virus inhibition assays and inhibition of virus-infected cells in an experimental in vivo murine HIV-1 model using HIV-1/MuLV. The potential of IgG-(T20-4) to purge latently infected cells was investigated by measuring the efficacy of compounds to inhibit the spread of HIV-1 following activation with suberoylanilide hydroxamic acid (SAHA).

Results: The variant of hIg-(T20-4), inhibited and neutralized in vitro HIV subtype B strains of primary and cell-adapted origins to 20-100 fold that of either antibody or T-20 alone. The potential of IgG-(T20-4) to purge latently infected cells was investigated by measuring the efficacy of compounds to inhibit the spread of HIV-1 following activation with suberoylanilide hydroxamic acid (SAHA).

Results: The variant of hlg-(T20-4), inhibited and neutralized in vitro HIV subtype B strains of primary and cell-adapted origins to 20-100 fold that of either antibody or T-20 alone. The potential of IgG-(T20-4) to purge latently infected cells was investigated by measuring the efficacy of compounds to inhibit the spread of HIV-1 following activation with 100 nM SAHA was measured. The substantially activated HIV replication was suppressed by each of the T20 labelled agents to 0-3%. Enhanced potency was observed for a doxorubicin DNL conjugated anti-HIV IgG to neutralize free virus, inhibit intercellular spread of viral infection, as well as to eliminate HIV-1/MuLV infection in vivo.

Conclusion: The combination of HIV targeting molecules by the DNL method resulted in compounds with highly enhanced anti-HIV potency. HIV virions, HIV infected cells and infected non-producing cells adjacent to HIV-infected cells could be targeted, leading to new possibilities to decrease cells in the viral reservoir.
P13.63

Study 1: Optimizing DNA Vaccines that Target the 2F5 Epitope of the Membrane Proximal External Region of HIV-1 gp41

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Background: HIV vaccines targeting the membrane proximal external region of HIV-1 gp41 (MPER), the site of three broadly neutralizing (bNt) monoclonal antibodies (Abs), we hypothesize must mimic the neutralization-competent structure (NCS) of the MPER, as defined by tight binding by broadly Nt Abs and weak binding by their non-Nt mutant counterparts. We developed two DNA vaccines that express the NCS of the MPER in the context of the cell membrane, and a liposome-based vaccine bearing peptides covering the MPER and portions of the gp41-TMD.

Methods: Immunization 1: Rabbits were immunized via gene gun delivery with DNA vaccines encoding the MPER fused to the transmembrane domain (TMD) of the platelet-derived growth factor receptor (PGDFR-TMD), or to the gp41-TMD + 27 residues of the cytoplasmic tail (TM1). Immunization 2: Rabbits were immunized with DNA vaccines encoding PGDFR-TMD and boosted intra-muscularly with liposomes bearing peptides expressing the 2F5 epitope (POPG: Npre-TM). Immune sera were analysed by ELISA and neutralization assays.

Results: Serum Abs elicited by the MPER-PGDGR vaccine mapped to the 2F5 epitope and had equivocal neutralizing activity. While the MPER-TM1 vaccine exposes both the 2F5 and 4E10 epitopes well, it elicited Abs that mostly cross-react with the TM1 cytoplasmic tail. N-terminal addition of flagellin to DNA vaccines did not alter 2F5 and 4E10 antigenicity, and MPER reactivity was boosted by MPER-TM1 DNA vaccines encoding it. POPG-N-pre-TM liposomal vaccines were found to elicit 2F5-like Abs. As such, MPER-PGDFR and POPG-N-pre-TM vaccines are currently being tested for its ability to elicit high-titer 2F5-like Nt Abs in a DNA-prime liposome-boost immunization study.

Conclusion: We identified DNA- and protein-based vaccines that expose the 2F5 epitope in the context of lipids, and will report on the results of this novel immunization strategy. Our studies suggest DNA vaccines must be re-engineered to fully expose the 4E10 epitope via alternative TMDs.

P13.64

Study 2: Engineering DNA Vaccines Expressing the Membrane Proximal External Region of HIV-1 gp41 to Optimize Exposure of Neutralizing Epitopes

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Background: We hypothesize that to elicit HIV-neutralizing antibodies (Abs), vaccines targeting the membrane proximal external region of HIV-1 gp41 (MPER) must mimic its neutralization-competent structure (NCS), defined as being bound tightly by broadly neutralizing Abs 2F5 and 4E10, and weakly by their non-Nt mutant counterparts. Our first-generation vaccine, MPER-TM1, fully exposed the 2F5 and 4E10 epitopes, but failed to elicit neutralizing Abs against them, due to immunodominance of elements within the gp41 cytoplasmic domain. Here, we engineered ecto- and transmembrane domains (TMDs) surrounding the MPER to enhance exposure of its neutralizing epitopes.

Methods: Our first-generation DNA vaccine tethered the MPER via the gp41-TMD. Hypothesizing that alternative TMDs can optimize exposure of neutralizing epitopes of the MPER, we fused the MPER to the membrane soluble region of GCN4 (MS1), a trimeric TMD. DNA vaccines, MS1.1-MS1.7, were constructed with each encoding the MPER followed by MS1 shifted C-terminally by a single residue. These constructs and the MPER-TM1 were “capped” with a trimeric coil-VaLd(C) domain inserted N-terminal to the MPER. DNA vaccines were expressed in cells and analyzed for exposure of the 2F5 and 4E10 epitopes using a cell-lysate ELISA.

Results: Capped and uncapped MPER-MS1.1-1.7 were expressed in the context of the plasma membrane and were bound by MAbs 2F5 and 4E10, though 4E10 binding was not to levels observed for the MPER-TM1 vaccine. Optimal MPER reactivity was observed for the MS1-TM shifted C-terminally by a single residue (MPER-TMMS1.1) or by four residues (MPER-TMMS1.4). Capping increased 2F5 and 4E10 binding for the MS1.1 construct (CC-MPER-TMMS1.1) and decreased it for CC-MPER-TM1; capping had no effect for other MS1 constructs (MS1.2 – MS1.7).

Conclusion: Our results suggest the MS1 TMD, and possibly capping, can support optimal 2F5 and 4E10 epitope exposure. Immunogenicity studies of these MPER-MS1 DNA vaccines are planned.
**P13.65**

**Lentiviral-Based Anti-HIV Therapeutic Vaccine: Design, Preclinical Studies and Phase I/II Clinical Trial Preliminary Results**

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**Background:** Theravectys, a spin-off the Pasteur Institute, develops a new generation of prophylactic and therapeutic vaccines using optimized lentiviral vectors. It’s most advanced product, a therapeutic anti-HIV vaccine treatment, has entered clinical Phase I/II end of 2012. This vaccination should allow seropositive patients to gain an immunological status identical to the so-called “Functional Cured” patients who develop an efficient immunological response capable of controlling the infection without therapy.

**Methods:** Vaccine candidates are integrative and self-inactivated live-recombinant lentiviral vectors. They encode an HIV antigen, under the regulation of a patented promoter that is preferentially induced in APC (generating the specific immune response), and showing a basal level expression in all cells (allowing their elimination by the settled immune response). Furthermore, Theravectys developed a vaccination regimen based on iterative immunizations with lentivectors encoding the same HIV transgene, relying on different VSV-G serotypes for pseudotyping without generating cross-neutralizing antibodies. These vaccine candidates are classified as “Live recombinant vectored vaccines” (EMA, 2011).

**Results:** Preclinical studies demonstrated i) the generation of a strong, specific and very long lasting T cell immune response (up to 2 years in murine animal models), ii) the restricted diffusion of the vaccine candidates after injection and iii) their fast disappearance within few weeks, correlated with an absence of macroscopic and microscopic toxicity.

**Conclusion:** These data allowed the settlement of an anti-HIV therapeutic Phase I/II clinical trial that has received the authorizations of the French and Belgium regulatory agencies in 2012. This trial is held in France and Belgium and is actually enrolling 36 HIV-1 infected patients. Theravectys’ anti-HIV vaccine treatment is assessed at three doses and safety, tolerability and immunogenicity compared to a placebo group. Furthermore, vaccine efficiency will be evaluated by the interruption of the HAART treatment in all patients, including placebo. Results are expected by 2014 with intermediary analysis in September 2013.

**P13.66**

**HIV CD4 Based Vaccine Increases CD8 T Cell Responses and Confers Protection Against Challenge with Recombinant Vaccinia Encoding HIV Gag-Pol Proteins**

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**Background:** Generation of competent and long-lived memory CD8+ T cells is highly dependent on CD4+ T cell help. A vaccine able to provide cognate help may efficiently prime CD8+ T cell responses establishing an appropriate immune control of HIV. Furthermore CD4 T cells responses also have been correlated with HIV infection control. Our group has identified a set of 27 conserved and promiscuous CD4 epitopes from 8 HIV-1 proteins. HIVBr27 encoding such epitopes was highly immunogenic in BALB/c mice inducing strong and broad CD4+ responses.

**Methods:** Here we tested whether pre-immunization with HIVBr27 could provide cognate help and improve CD4 and CD8 T cell responses against other HIV proteins (Gag, Pol and Vif) correlated with HIV protection/disease control. To answer that we used ELISPOT assays as well as CFSE based proliferation assays against 15 mer overlapping peptides from Gag, Pol and Vif.

**Results:** The pre-immunization with the CD4 based vaccine increased and broadened the CD8 T cell response against Gag, Pol and Vif. Higher proliferation and cytokine secretion were observed in pre-immunized animals. In relation to the CD8 T cells, we observed an overall increase in 57% and 40% of the breadth and magnitude of the responses, respectively, when comparing the CD4 based vaccine pre-immunized group to the control. Into the CD4 T cell compartment we noticed an increase of 3 and 2 times in the breadth and magnitude of responses, respectively, comparing CD4 versus control primed group. We also observed 1 log reduction on the PFUs after recombinant Gag-Pol vaccinia virus challenge as well as a 32 fold increase in the anti-p24 antibody levels in the HIVBr27 pre-immunized group when compared to the control one.

**Conclusion:** These results highlight importance of a CD4 inducer based component as primer for global responses, impacting in both cellular and humoral immune response.
High Affinity Recognition of Synthetic Glycopeptides by HIV-1 gp120 V1V2 Loop Broadly Neutralizing Antibodies and Their Unmutated Common Ancestors

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Background: Current HIV-1 vaccines elicit dominant strain-specific neutralizing antibodies. Broadly neutralizing antibodies (BnAbs) are not dominant and arise in ~20% of HIV-1-infected individuals after several years of infection. One strategy for induction of subdominant antibody responses is to produce homogeneous immunogens that selectively express BnAb epitopes but do not express dominant strain-specific epitopes. An HIV-1 vaccine should target the unmutated common ancestor (UCA) B cell receptors of naïve B cells, but to date, no HIV-1 envelope constructs have been found that bind to the UCA of V1V2 BnAb PG9.

Methods: We have designed and chemically synthesized homogeneously glycosylated peptides reflective of the HIV-1 envelope V1V2 region with Man3GlcNAc2 or Man5GlcNAc2 glycans at N156 and N160 (JACS, in press, 2013). We tested the reactivity of these synthetic glycopeptides with mature antibodies and their unmutated common ancestors (UCAs) of both broadly neutralizing antibodies (BnAbs) PG9 and CH01 and strain-specific V2 neutralizing antibody CH58.

Results: The Man5GlcNAc2 V1V2 glycopeptide bound avidly to V1V2 BnAbs PG9 and CH01 with Kds of 29 nM and 46 nM respectively, and bound as well to their UCAs at 98 nM and 118 nM, respectively. Whereas PG9 and CH01 BnAbs bound similarly well to the Man3GlcNAc2 V1V2 glycopeptide, their UCAs bound with an order of magnitude weaker affinity to Man3GlcNAc2 V1V2 glycopeptide, indicating a requirement for Man5GlcNAc2 glycans for naïve B cell UCA binding. In contrast, the V2 strain-specific neutralizing antibody CH58 bound minimally with unmeasurable affinity to either V1V2 glycopeptide.

Conclusion: The selective high-affinity binding of these homogeneous V1V2 glycopeptides to BnAbs and their UCAs, but not to strain-specific neutralizing antibody CH58, makes these synthetic glycopeptide constructs promising immunogens for targeting subdominant HIV-1 envelope V1V2 neutralizing antibody producing B cells.

Novel R5 Tropic Founder Env SHIV for Nonhuman Primate Immunoprophylactic Studies

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Background: Optimal use of nonhuman primate (NHP) models to evaluate in vivo antibody-mediated protection from HIV infection requires appropriate challenge viruses. Challenges with chimeric simian-human immunodeficiency viruses (SHIV) bearing HIV-1 Envs are particularly attractive because they allow for direct testing of HIV-1 Env-directed approaches, obviating the need for multi-step evaluations. New CCR5-tropic SHIVs that authentically represent transmitted HIV-1 Envs are needed to enhance the relevance of NHP studies of antibody-based vaccine modalities.

Methods: A novel, mucosally transmissible CCR5-tropic SHIV bearing an unadapted founder HIV-1 envelope (Clade B) capable of high level replication in rhesus macaques was identified via in vivo competition after infecting naïve rhesus macaques with pools containing multiple unique SHIVs (16-21 SHIVs per pool) bearing distinct CCR5-tropic founder HIV-1 Envs. To assess the utility of this SHIV for studies designed to evaluate neutralizing antibody-based prevention strategies, six rhesus macaques were passively immunized with the broadly neutralizing anti-HIV-1 gp120 antibody PGT-121, 24 h prior to intrarectal SHIV challenge; six control animals received an antibody of irrelevant specificity (DEN03).

Results: All six PGT-121-infused animals were protected from infection. In contrast, all six control animals became infected, with peak plasma viral loads between 10^6-10^7 viral RNA copies/ml. At the time of challenge, all six PGT-121 infused animals had serum PGT-121 concentrations >5,000-fold higher than the in vitro IC_{50} of PGT-121 for the challenge virus and serum IC_{50} neutralization titers >3,000.

Conclusion: Through in vivo selection, we have identified a novel CCR5-tropic SHIV bearing a transmitted/founder HIV-1 envelope that is mucosally transmissible and replicates to high titers in rhesus macaques and is suitable for in vivo NHP studies to evaluate antibody-based protection strategies.
P13.69 LB

Multiple Pathways of HIV-1 Autologous Neutralizing Antibodies Cooperate to Drive CD4 Binding Site Broadly Neutralizing Antibody Responses

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Background: Defining the virus pathways that drive HIV-1 broadly neutralizing antibodies (BnAbs) is critical for HIV-1 vaccine design.

Methods: We have previously reported the evolution and structure of a CD4 binding site (CD4bs) broadly neutralizing antibody (BnAb) CH103 clonal lineage from an African donor followed from time of infection. In this report we probed the initial events driving the CD4bs BnAb CH103 lineage in which contact residues of the BnAb included the CD4bs, the V5 loop and loop D in envelope glycoprotein gp120.

Results: As the CH103 BnAbs matured, they potently neutralized the transmitted/founder (T/F) virus. Whereas a mutation in the CD4bs and insertions in the V5 loop 30 weeks after transmission led to virus escape from the CH103 lineage, mutations in the loop D did not lead to virus escape; rather loop D mutant viruses were 8-fold more sensitive to the T/F virus to neutralization by the CH103 BnAb, implying the existence of non-CH103 lineage Nabs targeting the gp120 loop D. Demonstration of such a lineage required the isolation and measured fitness of the corresponding mutants for the original virus escape from the CH103 lineage, mutations in the loop D did not lead to virus escape; rather loop D mutant viruses were 8-fold more sensitive to the T/F virus to neutralization by the CH103 BnAb, implying the existence of non-CH103 lineage Nabs targeting the gp120 loop D. Demonstration of such a lineage required the isolation and measured fitness of the corresponding mutants for the original virus escape from the CH103 lineage.

Conclusion: The dominant initial antibody response was an autologous NAb response different from the CH103 lineage that targeted the gp120 loop D, and in turn, induced escape mutants with greater affinity for the CH103 CD4bs neutralizing antibody lineage. Including both the T/F Env and loop D mutant Env in HIV-1 immunogen design will be a critical component of experimental immunogens designed to recapitulate this sequence of antibody maturation events.

P13.70 LB

Validation of the Predicted Fitness Landscape in HIV-1 Gag: A Rational Approach to HIV Immunogen Design

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Background: HIV-1 immune evasion by sequence variation is a major hindrance to vaccine design. To address this challenge, our group has developed a computational model, rooted in physics, that predicts HIV-1 fitness landscapes and may thereby inform design of vaccine immunogens that maximise targeting of sites harmful to HIV-1 when mutated simultaneously, to block viable HIV-1 escape. Here, we aimed to address previous computational model limitations and also directly tested model predictions in vitro fitness measurements of HIV-1 strains containing multiple Gag mutations. We further assessed whether refined models improved predictive power for fitness measurements.

Methods: To address under-sampling, a regularisation term was introduced into our published model. Further, an extended model was developed to introduce residue-specific resolution (while our previous models used a binary approximation – consensus versus non-consensus). Gag mutation combinations (17 pairs, 1 triple and 25 single mutations within these) predicted to be either harmful to HIV-1 viability or fitness-neutral were introduced into HIV-1 NL4-3 by site-directed mutagenesis. Replication capacities of mutants were assayed in an HIV-1-inducible green fluorescent protein reporter cell line.

Results: A strong correlation was observed between the predicted and measured fitness of the corresponding mutants for the original model (R=-0.74, p=3.6 x 10-6), and this was further strengthened in the two new models (R=-0.83, p=3.7 x 10-12 and R=-0.73, p=9.7 x 10-9, respectively).

Conclusion: Our results support the ability of computational models to robustly predict the relative fitness of mutant HIV-1 Gag viral strains that are phylogenetically not too distant, and the potential value of these to address the problem of HIV-1 immune evasion.
P13.71 LB

Human HIV-1 Vaccine Induced Antibody Durability and Env IgG3 Responses


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Background: Effective HIV-1 vaccine design requires induction of long-lived protective antibody responses. Substantial differences in the IgG subclass profile among vaccine strategies have been previously demonstrated and in RV144, Env IgG3 was correlated with decreased risk of infection. Notably, several bNAbs are of IgG3 origin, suggesting that induction of this antibody subclass may be an important goal for HIV-1 vaccine design.

Methods: Vaccine-induced Env IgG subclass responses were evaluated from 4 human HIV-1 vaccine clinical trials with distinct immunogen and adjuvant strategies. Antibody kinetics and/or durability of Env IgG3 were measured in 40 RV144 (ALVAC prime, Clade B/E gp120/alum) vaccinees at 3.5 yrs post vaccination, in 120 VAX003 (Clade B/E gp120/alum) vaccinees between protein boosts, in 30 GSK PRO HIV-002 (Clade B gp120, AS01B) vaccinees out to 18 months post vaccination, and in 34 HVTN 088 (Clade C gp140/M59) vaccinees boosted 6-17 yrs after prior vaccination.

Results: GSK PRO HIV-002 (Clade B gp120/AS01B) elicited more durable vaccine induced IgG responses compared to RV144 (Clade B/E gp120/alum) vaccinees with a significantly slower decline. Additionally, HVTN 088 (Clade C gp140/M59) IgG antibody levels were detectable 6-17 years after prior vaccination in 64-79% of vaccinees tested. However, in each study we found that Env IgG3 responses declined more rapidly compared to overall IgG responses (29% Env IgG and 0% IgG3 response rates 3.5 yrs post-vaccination in RV144).

Conclusion: The more rapid decline in vaccine induced IgG3 responses compared to overall IgG responses indicates that the character of the vaccine induced antibody responses is time-dependent. The changing levels among antibody subclasses after vaccination can potentially influence the functional properties of the vaccine induced humoral responses. Evaluating differences in vaccine induced antibody durability is important for immunogen and adjuvant designs that aim to strategically target the durability of HIV-1 Env antibodies with optimal antiviral properties.

P13.72 LB

Single Glycan Binding by a Broadly Neutralizing Antibody to HIV-1 Env

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Background: The HIV-1 envelope glycoprotein is a primary target of the neutralizing antibody response. However, sequence variable loops on gp120 mediate antibody escape, and extensive N-linked glycosylation shields much of the gp120 protein surface from immune recognition. Recently, a number of highly potent broadly neutralizing antibodies (bNAbs) have been discovered that bind to a glycosylated region around the base of the V3 loop. Although these bNAbs penetrate the glycan shield to make interaction with the underlying protein, most of their interactions are with the conserved N-linked glycans. While antibody-protein interactions are well characterized, antibody-glycan interactions are relatively poorly understood. Therefore, further structural studies of glycan-dependent bNAbs in complex with their glycosylated antigens are needed, especially if glycosylated immunogens are to be designed to elicit glycan-dependent broadly neutralizing responses.

Methods: Towards this end, we have combined various biophysical techniques including x-ray crystallography, electron microscopy, glycan arrays, isothermal titration calorimetry, neutralization assays, and deep sequencing, to characterize how a novel glycan-dependent bNAb, PGT 124, interacts with gp120.

Results: The crystal structure shows that PGT124 primarily interacts with a high mannose glycan linked to Asn332 of gp120, accounting for over 50% of the contacts in the antibody-antigen interaction. Indeed, removal of the N332 glycan by alanine mutagenesis completely abrogated neutralization on a cross-clade 6 virus panel, while removal of other glycan sites in the high-mannose patch demonstrated no effect. Although protein-glycan interactions typically achieve mM to uM Kd in the absence of multimerization, surprisingly, the PGT 124-gp120 interaction, apparently mediated primarily by the glycan at 332 with limited protein involvement, achieves a Kd of 20 nM.

Conclusion: Thus, these findings provide a framework for engineering high affinity monovalent antibody-glycan interactions, and thereby contribute to HIV-1 vaccine design.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L2, Room 211 – 212.

Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L2, Room 211 – 212.

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**P13.73 LB**

**HIV-1 Envelope Glycoprotein Signatures that Correlate with the Development of Cross-Reactive Neutralizing Activity**

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**Background:** Current HIV-1 envelope glycoprotein vaccines are unable to induce cross-reactive neutralizing antibodies. However, such antibodies are elicited in 10-30% of HIV-1 infected individuals, but it is unknown why these antibodies are induced in some individuals and not in others. We hypothesized that the envelope glycoproteins (Env) of early HIV-1 variants in individuals who develop cross-reactive neutralizing activity (CrNA) may have unique characteristics that support the induction of CrNA.

**Methods:** We retrospectively generated and analyzed env sequences of early HIV-1 clonal variants from 31 individuals with diverse levels of CrNA. The Sequence Harmony (SH) algorithm was used to analyze amino acid differences between the env sequences of the individuals who developed CrNA and the individuals who did not develop CrNA. SH is an entropy-based method that measures the overlap in distribution of amino acid types between two groups of sequences.

**Results:** Our analyses revealed a number of Env signatures that coincided with CrNA development. These included a statistically shorter V1 and a lower probability of glycosylation as implied by a high ratio of NXS versus NXT glycosylation motifs. Furthermore, lower probability of glycosylation at position 332, which is involved in the epitopes of many broadly reactive neutralizing antibodies, was associated with the induction of CrNA. Finally, Sequence Harmony identified a number of amino acid changes associated with the development of CrNA. These residues mapped to various Env subdomains, but in particular to the V1 and V4 as well as the underlying α2 helix of the C3.

**Conclusion:** These findings imply that the development of CrNA might depend on specific characteristics of early Env. Env signatures that correlate with the induction of CrNA might be relevant for the design of effective HIV-1 vaccines.

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**P13.74 LB**

**Using an Autologous HIV Vaccine/activator (Based on the Full Length Genome and Intrapatient Virus Population) to Induce Latent HIV and Boost Immunity**

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**Background:** HIV persists in cellular reservoirs as latent proviral integrants even with fully suppressive HAART. Prior to treatment, HIV-1 primarily infects HIV-specific CD4+ T cells that then transitions to the latently infected memory T cells. Thus, we propose that the most effective and specific activator of latently infected T cells is the patient’s HIV-1 quasispecies prior to treatment. This same autologous activator can act as an immunogen.

**Methods:** The entire HIV-1 genome and population was amplified from multiple plasma samples prior to HAART. The HIV-1 genome was RT-PCR amplified in overlapping halves or thirds, which is then recombined into a yeast-based vector via homologous recombination/gap repair. The vector lacks the 5’LTR and through PCR, we destroyed the RNA packaging sites, integrase active site, and PPT. Defective proviral plasmids are transfected into 293T cells to produce a replication-incompetent vector based on the intrapatient HIV-1 population prior to treatment.

**Results:** Autologous HIV-1 vector, a NL4-3-based vector, and a cocktail of flu/tetanus/CMV antigens were loaded onto the patient DCs and then co-cultured with T cells, both obtained and isolate from samples: 3 yrs on stable HAART. In five different patients, the autologous HIV vector, presented by DCs, induced at least 30-fold higher HIV-1 production from the T cells than did the NL4-3 vector and 100-fold higher than the Flu/TT/CMV cocktail. In contrast, gamma interferon ELISPOTS on the DC-antigen-T cell cocultivations revealed >10-times more sfu’s with the Flu/TT/CMV antigen cocktail than with the autologous vector.

**Conclusion:** These findings suggest that the intrapatient HIV-1 population in a safe, dead vector may be the most effective, specific stimulus to drive HIV-1 out of the latent T cells. Activation of the HIV-specific T cells (lacking latent HIV-1) may also provide help for both the humoral and CTL responses, boosted by this autologous HIV-1 immunogen.
P13.75 LB

Filling in the Immunological Space: Low Seroprevalent Ad HIV Vaccines +/- Adjuvanted Protein or Electroporated DNA Elicit Different Immune Profiles


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Background: Since correlates of immune protection for HIV are still poorly defined, it is difficult to prioritize vaccines for further development. Assigning distinct immunological profiles to HIV vaccine candidates may be helpful. IAVI and partners have assessed the clinical safety and immunogenicity profiles of Ad35 vectored HIV vaccines alone or combined with HIV vaccines based on Ad26, adjuvanted protein or electroporated DNA.

Methods: Four randomized, placebo-controlled, phase I trials of 4-5 arms each enrolled 498 healthy HIV-uninfected participants at low risk for HIV in the US and Africa. Trial 1: dose escalation of a replication-defective Ad35 vector containing HIV-1 subtype A epitopes encoding gag, RT, integrase, nef (GRIN) and envelope (Env). Trial 2: adjuvanted HIV-1 gag-pol-Nef Fusion Protein (F4/AS01) co-administered or sequentially administered with Ad35-GRIN. Trial 3: heterologous prime-boost with Ad35-Env and homologous sequences of Ad35-HIVEnv and Ad26-HIVEnvA01. Trial 4: multi-antigenic HIV plasmid DNA (HIVMAG) vaccine encoding clade B gag-pol, env, nef-tat-vif, alone or co-administered with pDNA IL12 given IM/EP using TriGrid™ Delivery System, and Ad35GRIN-Env. Central laboratories with qualified assays assessed immunogenicity across the trials.

Results: The IFN-gamma ELISPOT response rates, magnitude and breadth in the best arms from the trials were similar 2-4 weeks post-boost (for Env and for Gag-RTNef). However, the immune profiles differed. CD8 T cell responses predominated after Ad35 and Ad35-Ad26; co-administration of F4/AS01 + Ad35-GRIN induced balanced, persistent CD4 and CD8 T cell responses; and DNA EP +/- IL-12 + Ad35GRIN-Env elicited CD4 and CD8 T cell responses after prime and boost respectively. Polypeanutin T cell responses were detected across all arms, to different degrees. Antibody responses to adjuvanted protein were strongest and durable compared with other regimens. Adenovirus neutralization titers remained low in all groups.

Conclusion: Distinct immunological profiles were revealed in the 4 trials. Further characterization of the responses will enhance understanding of the profiles.

P13.76 LB

Antigen Design to Maximize Anti-HIV CD4+ T Cell Responses: Provision of Cognate Help, Increased Coverage and Coping with HIV Genetic Variability

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Background: Purposely eliciting CD4+ T cell responses has been successfully explored in the HIV vaccine field, despite increasing evidence for the importance of the CD4+ T cell response in protection against HIV. In the RV144 trial, Env-specific CD4+ but not CD8+ T cell responses were demonstrated in a fraction of vaccines. Direct effectors CD4+ T cells have been associated with better outcomes in SIV and HIV infection, and Rhesus macaques protected by SIV vaccines displayed robust CD4+ T cell responses.

Methods: We used rational vaccine design to develop a DNA vaccine encoding 18 HIV-1 B subtype (HIVBr18) or M-type (HIVBr27) conserved, multiple HLA-DR-binding CD4 epitopes, known to be recognized by multiple HIV-1-infected patients.

Results: Both vaccines elicited broad, polyfunctional, and long-lived CD4+ T cell responses in BALB/c and several HLA class II transgenic mice. In addition, HIVBr27 elicited extensive cross-clade immunity. Immunization with HIVBr27 increased CD8 T cell responses against subsequent whole HIV protein immunization, and reduced viral titers after challenge with a recombinant vaccinia virus encoding HIV Gag-Pol proteins. Immunization with HIVBr18 prior to recombinant gp140 HIV envelope protein drastically increased the IgG2a/IgG1 ratio of elicited anti-gp140 antibodies under multiple adjuvants.

Conclusion: By virtue of inducing broad responses against multiple conserved CD4+ T cell epitopes that can be recognized across widely diverse, common HLA class II alleles, this vaccine concept may induce T cell responses against multiple peptides in large proportion of the genetically heterogeneous population. By increasing the chance of matching the responses with multiple epitopes in the infecting HIV isolate, the vaccine concept may also cope with HIV genetic variability. The vaccine concept may be a candidate for standalone use or in association with conventional immunogenics, to increase the amplitude, coverage and effectiveness of the induced response.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L2, Room 211 – 212.

Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L2, Room 211 – 212.

**Topic 13: Vaccine Concepts and Design**

**P13.77 LB**

**Evaluation of Dendritic Cell Targeted Consensus B and MOSAIC HIV Gag Protein Vaccines In Vitro in PBMC of Treatment Naïve HIV-1 Infected People**

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**Background:** Our consortium has developed a first generation dendritic cell (DC) targeted consensus B HIV gag protein vaccine for proof of concept studies that selected delivery of proteins to DC will allow proteins to be more immunogenic. This should provide a cheaper and effective way to immunize people repeatedly with no negative impact of pre-existing immunity. This vaccine is already in an ongoing phase 1 clinical trial in New York however it is not known whether this consensus B based vaccine would work in sub Saharan African where unrelated strains of HIV-1 are predominant.

**Methods:** To assess the consequence of targeting a consensus B and MOSAIC gag protein vaccines to DC from people infected with unrelated strains of HIV-1 in Africa we added the protein vaccines to their blood cells in vitro and measured proliferation and IFNγ production by bulk PBMCs as well as a coculture between monocyte derived DC and T cells of treatment naïve HIV-1 infected people.

**Results:** Dendritic cell targeted Consensus B and MOSAIC HIV gag Protein vaccines recalled pre-existing T cell responses in blood cells of treatment naïve people infected with unrelated strains of HIV-1. DC targeted MOSAIC gag vaccine was more efficient than the consensus B vaccine and stimulated significant proliferation of HIV gag specific cells (P<0.001).

**Conclusion:** Thus dendritic cell targeted consensus B and MOSAIC HIV gag protein vaccines could recall pre-existing CD4 and CD8 T cell responses in vitro in PBMC of treatment naïve people infected with unrelated strains of HIV-1 in Africa. DC targeted MOSAIC gag protein vaccine should be further evaluated for possible incorporation in future vaccines especially for therapeutic vaccination in Sub Saharan Africa.

**P13.78 LB**

**Novel Epitopes of Nef, Suitable for an HIV Vaccine in Africa**

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**Background:** Understanding of host responses to HIV antigens is of importance for continued rational development of HIV vaccines. Immunogenic epitopes of HIV proteins have predominantly been identified for HLA alleles commonly expressed in Caucasian populations, but the need for HIV vaccines is particularly urgent in many African countries.

**Methods:** By combining analysis tools of the Immune Epitope Database Analysis Resource (IEDB), the HIV/AIDS database of Los Alamos and proteasomal cleavage evaluation, we predicted high affinity novel peptides for five alleles common in African populations. The de novo predicted HLA epitopes were validated by biologically identified HLA epitopes for A*02:01.

**Results:** We identified previously not described epitopes for the common African HLA alleles HLA-A*02:01, A*30:01, A*30:02, B*58:01 and C*07:01. Among the ten best for these alleles, all had low percentile ranks below 10, indicating high affinities related to induction of cellular immunity. Locations for mutations to preserve immunogenicity of the Nef protein, modified not to confer HLA- or CD4+ down-regulating activities were identified.

**Conclusion:** Our study of the HIV-1 Nef aa sequence has brought to light several candidate epitopes, which indicates that incorporation of the Nef protein or gene in an HIV vaccine should contribute effectively to stronger and broader cellular immunogenicity of an HIV-1 vaccine. This appears to be true particularly for African populations.
**P13.79 LB**

**Env-Specific Tfh Responses and Neutralizing Antibodies Develop Concomitantly Following Co-Immunization of Rhesus Macaques with HIV Env DNA and Protein**

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**Background:** RV144 vaccinees harbored Env-specific IgG and HIV-1 specific CD4⁺ T cells. Thus, the modest success of RV144 may be due to induction of both HIV-1 specific B cell and Tfh responses. We designed DNA and protein immunogens, using Env sequences from two HIV-infected subjects who developed broadly neutralizing antibodies (bNAb) and co-immunized macaques (DNA+protein) to explore the ontogeny of bNAb development. This strategy rapidly elicits strong NAb responses, and our goal here was to measure Tfh responses in lymph nodes from these immunized macaques.

**Methods:** Twelve macaques, divided evenly into two groups, were co-immunized with gp160 Env DNA and gp140 protein at weeks 0, 4, 12 and 20. Serum antibodies were monitored for Env binding, neutralization, and avidity. Lymphocytes collected from inguinal lymph nodes at weeks 21 and 22 were incubated with soluble gp140 Env DNA and Protein

**Results:** DNA+protein-immunized macaques developed neutralization breadth against HIV-1 Tier 1 and Tier 2 viruses. Using previously described rhesus macaque CD4⁺ Tfh cell phenotypes, we examined Tfh responses in inguinal lymph nodes two weeks after the fourth immunization. All animals developed measurable Env-specific Tfh responses specific for HIV Env-SF162 and for cognate EnvS derived from broad neutralizers.

**Conclusion:** This is the first reported identification of Env-specific Tfh responses in vaccinated rhesus macaques. The significance of memory T cell and protective B cell responses induced by vaccination is central to HIV vaccine success. Non-human primates are valuable models to monitor vaccine induction of cellular immunity by measuring HIV Env-specific Tfh responses in lymph nodes to investigate their kinetics and frequency in response to vaccination and to correlate their appearance and strength with the emergence of binding and neutralizing antibodies. NIH/NIAID P01AI078064 N.L.H.

**P13.80 LB**

**Redesign of Replicating LucR HIV-1 Reporter Viruses with Innovative IRES Elements for Expanded Immune Monitoring Applications**

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**Background:** We previously reported on replication competent HIV-1 reporter viruses expressing Renilla luciferase (Env-IMC-LucR; Edmonds, 2010) which have enabled a novel T-cell based assay with heightened sensitivity for neutralizing antibody in vaccinee sera for testing of samples from RV144 and Vax003 trials (Montefiori, 2012). Furthermore, these reporter viruses are enabling novel CD8 T cell virus inhibition assays (LucR-IMC-VIA; Naarding, in press), ADCC and other approaches. Env-IMC-LucR technology (in which a self-cleaving T2A peptide enables Nef expression in frame with, and release from, LucR) has found wide application. However, the molecular approach may not be optimal for certain approaches to Correlates of Protection discovery which aim at utilizing "whole-genome" Transmitted/Founder reporter IMC, or those which require unaltered Nef.

**Methods:** In Env-IMC-LucR viruses, LucR is stable over multiple replication cycles, and read-out is highly sensitive and robust. The small T2A peptide is advantageous in that regard and allows nef expression at normal levels, but it adds an N-terminal residue to Nef which may affect some Nef function. Thus, we have explored if novel IRES elements of small size or with attenuating features can outperform "classic", and potentially destabilizing, EMCV IRES in HIV-1 proviruses. We designed 8 novel IRES containing HIV-1 reporter viruses with IRES from insect viruses, HCV, and modified EMCV IRES.

**Results:** Compared to Env-IMC-LucR, all IRES containing proviruses were similarly infectious and expressed LucR. Nef was not detected from insect IRES elements. Expression of Nef from HCV and some “attenuated” EMCV IRES elements was similar to that of parental virus. Constructs with physiologic Nef levels were tested for replication kinetics and genetic stability of LucR, and behaved encouragingly similar to Env-IMC-LucR.

**Conclusion:** Redesigned reporter viruses with modified IRES elements promise to be useful virologic tools for augmenting HIV vaccine immune monitoring efforts, including neutralization, ADCC, and CD8 VIA approaches, and novel assays.
Odd-numbered posters will be presented in Poster Session 01 on Tuesday, 8 October from 17:30 – 19:00 in L2, Room 211 – 212.

Even-numbered posters will be presented in Poster Session 02 on Wednesday, 9 October from 17:30 – 19:00 in L2, Room 211 – 212.

**P13.81 LB**

**Exogenous Expression of HIV-1 Envelope Glycoprotein Trimer in Suspension Culture-Adapted HEK293 and CHO-K1 Cells**

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**Background:** HIV-1 Env is the only virus-specific protein presented on the virion surface and thus, it’s the principal target of neutralizing antibody. Until recently, gp120 and gp41 structures did not include several functionally important regions. This knowledge gap limits immunogen design vaccine research. Using Env trimer purified from 293F cells, Mao et al, 2012, recently described an ~11-Å cryo-EM structure of an unliganded, fully glycosylated Env trimer in its pre-fusion state, elucidating new structural information on gp120/gp41 subunit interactions in context of the Env trimer. These findings demonstrated utility of single-particle cryo-EM for structural analysis of the trimeric HIV-1 Env glycoprotein complex.

**Methods:** To augment structure studies of the Env trimer, TRE-env-puromycin-T2A-EGFP expression cassettes were stably integrated into DNA of CHO and HEK293 cells via lentiviral vectors. Env expression and conformation were assessed by analyzing binding of monoclonal antibodies. Env was purified from the plasma membrane using detergents, density-gradient ultracentrifugation and affinity chromatography.

**Results:** Stable high-producer clonal cell lines were obtained that over-expressed variants of the JRFL env, including cleavage deficient and cleavage competent full-length and truncated variants. Exogenous Env exhibited native conformation based on recognition by MAb that bind diverse epitopes in gp120 and gp41, including conformation-dependent neutralizing antibodies. Microgel filtration chromatography of Env solubilized and purified from the cell plasma membrane suggested the presence of intact trimeric glycoprotein. The suspension cells were readily scalable to 10 liter batch cultures, comprising 50-70 billion cells with peak Env expression observed 15-24 hrs after doxycycline induction. Analysis of microsomal membranes indicated 0.1-0.5 pg of Env production per cell.

**Conclusion:** Our findings suggest that the robust nature of the expression system offers new opportunities for characterizing biophysical and biochemical properties of native HIV-1 Env.
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Certificate of Attendance
AIDS Vaccine 2013 Conference

This document is to certify that

attended the AIDS Vaccine 2013 Conference
held 7–10 October at the International Convention Center (CCIB),
Barcelona, Spain.

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