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Sanofi Pasteur
May 8-11, 2013 – Utrecht, the Netherlands

Wednesday, May 8, 2013
5:00 pm  Registration
7:00 pm  Reception & Welcome Dinner

Thursday, May 9, 2013
8:00 am  Breakfast

SESSION 1: Transmission and prevention
Ron Swanstrom & Tuenis Geijtenbeek
9:00  Opening Remarks
9:15  TRANSMISSION AND EARLY EVOLUTION OF HIV-1, Ron Swanstrom (#108)
9:30  INNATE SIGNALING BY C-TYPE LECTINS IN HIV-1 INFECTION, Teunis Geijtenbeek (#112)
9:45  A MOLECULAR DYNAMICS APPROACH TO INVESTIGATE THE INFLUENCE OF N-LINKED GLYCOSYLATION ON HIV-1 CORECEPTOR TROPISM, Natasha Wood (#61)
10:00 DIMINISHED TRANSMISSION OF DRUG RESISTANT HIV-1 VARIANTS WITH REDUCED REPLICATIVE CAPACITY IN A HUMAN TRANSMISSION MODEL, Marieke Pingen (#36)
10:15 CHARACTERIZATION OF HIV-1 GENOMES FROM 24 ACUTELY INFECTED SUBJECTS, Morgane Rolland (#99)
10:30  Coffee Break

SESSION 2: HIV Therapy & Resistance evolution
John Coffin & Monique Nijhuis
11:00 Persistent Elevation in HIV viremia during cART with Identical WT Sequences Implies Expansion of a Clonal Source, John Coffin (#114)
11:15 HIV-1 SEQUENCE EVOLUTION DURING PERSISTENT LOW-LEVEL VIREMIA, Saran Vardhanabhuti (#81)
11:30 A COMPREHENSIVE, ACCURATE, FAST, CROSS-PLATFORM AND USER-FRIENDLY PIPELINE FOR THE ANALYSIS OF HIV-1 NEXT GENERATION SEQUENCING DATA, N. Lance Hepler (#97)

11:45 FROM IN VITRO TO IN VIVO QUANTIFICATION OF ANTIRETROVIRAL DRUGS EFFECTS BASED ON DYNAMICAL MODELS OF HIV, Mélanie Prague (#16)

12:00 PRE-EXISTENCE AND EMERGENCE OF VIRAL DRUG RESISTANCE, Helen Alexander (#34)

12:15 A DECADE OF HIV-1 DRUG RESISTANCE IN THE UNITED STATES: TRENDS AND CHARACTERISTICS IN A LARGE PROTEASE/REVERSE-TRANSCRIPTASE AND CO-RECEPTOR TROPISM DATABASE FROM 2003 TO 2012, Mojgan Haddad (#3)

12:30 Lunch

SESSION 3: Latency, reservoirs & cure

Steven Wolinsky & Ben Berkhout

2:00 LYMPHATIC TISSUES SHOW PERSISTENT REPLICATION, EVOLUTION AND DISPERAL OF HIV-1 DESPITE ANTIRETROVIRAL THERAPY, Trevor Bedford (#106)

2:15 Ben Berkhout

2:30 PREDICTING OUTCOMES OF TREATMENTS TO ERADICATE THE HIV LATENT RESERVOIR, Daniel Rosenbloom (#23)

2:45 QUANTIFYING THE TURNOVER OF TRANSCRIPTIONAL SUBCLASSES OF HIV-1-INFECTED CELLS, Christian Althaus (#72)

3:00 HIV-1 ACTIVITY AND COMPARTMENTALIZATION AT THE ANAL MUCOSA IN MSM WITH INTRAEPITHELIAL NEOPLESIA, Georgios Pollakis (#67)

3:15 CO-RECEPTOR AND PROLIFERATION MARKER EXPRESSION AND CHARACTERIZATION OF THE VIRAL RESERVOIR IN CD4+ T-CELLS AFTER LONG-TERM THERAPY, Jori Symons (#29)

3:30 Coffee Break

SESSION 4: Immunity I

Jim Mullins & Debbie van Baarle

4:00 MANIPULATION OF IMMUNDOMINANCE WITH A CONSERVED ELEMENT-BASED HIV-1 VACCINE, Jim Mullins (#109)

4:15 AIDS-PROTECTIVE HLA-B*27/B*57 AND CHIMPANZEE MHC CLASS I MOLECULES TARGET ANALOGOUS CONSERVED AREAS OF HIV-1/SIVCPZ, Natasja de Groot (#8)

4:30 CO-EXPRESSION OF HLA-B27, BUT NOT B57, RESULTS IN ENHANCED RESPONSIVENESS OF HIV-SPECIFIC CTL RESTRICTED THROUGH OTHER HLA-ALLELES, Ingrid Schellens (#63)
4:45  
**CTL ESCAPE DYNAMICS AND THE EFFECTS OF SHORT PULSE ART IN PATIENTS WITH ACUTE HIV-1B INFECTION**, Hannah Roberts (#56)

5:00  
**PROPERTIES OF MHC CLASS I PRESENTED PEPTIDES THAT ENHANCE IMMUNOGENICITY**, Jorg Calis (#54)

5:15  
**DO PROTECTIVE HLA-B ALLELES CONFER PARTIAL TOLERANCE AGAINST HIV?**, Roland Regoes (#41)

5:30 – 7:00  Poster Session

7:00  
Dinner

8:30  
Key Note Lecture: Asier Sáez-Cirión

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**Friday, May 10, 2013**

8:00 am  
Breakfast

**SESSION 5: Immunity II**

*Mary Carrington & Ronald Bontrop*

9:00  
**THE INFLUENCE OF HLA EXPRESSION ON HIV DISEASE**, Mary Carrington (#111)

9:30  
**APOBEC3G-DRIVEN SUPPRESSION OF PRODUCTIVE HIV-1 INFECTION**, Narendra Dixit (#12)

9:45  
**THE PARADOX OF HIV-1 ADAPTATION TO NK-CELL-MEDIATED IMMUNE PRESSURE**, Marjet Elemans (#33)

10:00  
**CHEMOKINE CO-RECEPTOR TROPISM, IMMUNE ACTIVATION AND IN VIVO PROLIFERATION OF CD4+ T-CELLS IN HIV-1 INFECTION**, Derek Macallan (#44)

10:15  
**T-CELL TURNOVER IN HIV-1 INFECTED PATIENTS ON ANTIRETROVIRAL THERAPY: DIFFERENCE BETWEEN IMMUNOLOGICAL RESPONDERS AND NON-RESPONDERS**, Julia Drylewicz (#21)

10:30  
Coffee Break

**SESSION 6: Progression within host**

*Alan Perelson & Jose Borghans*

11:00  
**MODELING HCV INFECTION AND TREATMENT**, Alan Perelson (#110)

11:15  
**EVOLUTIONARY DYNAMICS OF IMMUNE ESCAPE MUTATIONS IN HIV-1 HAPLOTYPES**, Aridaman Pandit (#14)

11:30  
**JOINT ASSOCIATION ANALYSIS OF GENOME-WIDE HUMAN AND HIV-1 VARIATION**, Istvan Bartha (#35)

11:45  
**ESTIMATING THE CONTRIBUTION OF GUT TO VIRAL LOAD IN EARLY SIV INFECTION FROM THE DYNAMICS OF ESCAPE**, Janka Petravic (#50)
12:00  PREDICTING THE IMPACT OF CD8+ T CELL DYSFUNCTIONALITY ON HIV DISEASE PROGRESSION, Frederik Graw (#27)

12:15  Lunch

SESSION 7: Phylogeny & Epidemiology I
Thomas Leitner & Can Kesmir

2:00  PRIME: PROPERTY-INFORMED MODELS OF EVOLUTION, Konrad Scheffler (#98)

2:15  NON-B HIV TRANSMISSION NETWORKS IN THE UK, Manon Ragonnet-Cronin (#25)

2:30  MEASURING EMERGENCE AND TRANSMISSION OF HIV DRUG RESISTANCE USING PHYLOGENETICS, George Shirreff (#59)

2:45  ANALYSIS OF PCR FOUNDER EFFECT AND SEQUENCING ERRORS IN ILLUMINA SEQUENCING, Wei Shao (#94)

3:00  SIMULTANEOUSLY ESTIMATING VIRAL EVOLUTIONARY HISTORY AND PHYLOGENETIC TRAIT SIGNAL, Philippe Lemey (#17)

3:15  IDENTIFYING HIV-1 TRANSMISSION RISK FACTORS AND THE SOURCE OF TRANSMISSION FROM MOLECULAR DATA, Erik Volz (#43)

3:30  Coffee Break

SESSION 8: Phylogeny & Epidemiology II
Jan Albert & Simon Travers

4:00  CHALLENGES WITH USING PRIMER ID’S TO IMPROVE ACCURACY OF NEXT GENERATION SEQUENCING, Jan Albert (#65)

4:15  THE APPLICATION OF SEQ2RES TO FACILITATE LARGE-SCALE, COST-EFFECTIVE HIV DRUG RESISTANCE GENOTYPING USING 454 PYROSEQUENCING, Simon Travers (#75)

4:30  INTEGRATING GENEALOGICAL AND DYNAMICAL MODELLING TO INFERENCE ESCAPE AND REVERSION RATES IN HIV EPITOPEG, Duncan Palmer (#24)

4:45  HIV COMPETITION DYNAMICS OVER SEXUAL NETWORKS, Viktor Müller (#42)

5:00  EXTRACTING EPIDEMIC DYNAMICS FROM VARYING HIV-1 EVOLUTIONARY RATES, Ethan Romero-Severson (#83)

5:15– 7:00  Poster Session

7:00  Dinner

9:00  City Tour
Saturday, May 11, 2013

8:00 am Breakfast

SESSION 9: Vaccines
Hanneke Schuitemaker & Doug Richman

9:00 TOWARDS A GLOVAL HIV VACCINE, Hanneke Schuitemaker

9:30 CO-EVOLUTION OF A BROADLY NEUTRALIZING HIV-1 ANTIBODY AND FOUNDER VIRUS FROM TIME OF INFECTION, Peter Hraber (#45)

9:45 MONITORING OF SIV MUTATIONS IN PROTEASE CLEAVAGE SITE REGIONS AFTER HIGH DOSE OF SIV MAC239 CHALLENGE IN CYNOMOLGUS MACAQUES, Ben Liang (#9)

10:00 THE INFLUENCE OF ANTIBODY TITRATION CURVE SLOPES ON THE EVOLUTION OF ANTIBODY RESISTANCE IN HIV-1 INFECTION, Carsten Magnus (#53)

10:15 THE EVOLUTION OF BROADLY NEUTRALIZING ANTIBODIES: WHAT CAN DEEP SEQUENCING TELL US?, Sergei Kosakovsky Pond (#86)

10:30 Coffee Break

SESSION 10: Within vs Between Host Evolution
Andrew Leigh Brown & Rob de Boer

11:00 HIV-1 VIRULENCE EVOLUTION IN AN HLA-POLYMORPHIC HOST POPULATION, Christiaan van Dorp (#104)

11:15 THE PARADOX OF VIRAL HERITABILITY AND STABILITY OF SET POINT VIRUS LOAD IN HIV, Sebastian Bonhoeffer (#32)

11:30 IS HIV SHORT-SIGHTED?, Katrina Lythgoe (#30)

11:45 WITHIN-HOST AND BETWEEN-HOST EVOLUTIONARY RATES ACROSS THE HIV-1 GENOME, Samuel Alizon (#31)

12:00 VIRAL GENOTYPE IN SUBTYPE C SIGNIFICANTLY INFLUENCES PLASMA VIRAL LOAD, Emma Hodcroft (#55)

12:15 Final Adjournment
# Table of Contents

<table>
<thead>
<tr>
<th>#</th>
<th>Abstract Title and Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>POTENTIALS OF FAS RECEPTORS AND LIGANDS IN THE MONITORING OF HIV-1 DISEASE IN CHILDREN IN YAOUNDÉ, CAMEROON. George Ikomey, M.-C. Okomo Assoumou, Julius Atashili, Martha Mesembe, Emilia Lyonga, Agnes Eyoh</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>MODELING AND SIMULATING THE DYNAMICS OF TWO GROUP PATIENTS’ ANTI-HIV INFECTION THERAPY Qilin Sun, Lequan Min, Xiao Chen, Ying Liu</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>A DECADE OF HIV-1 DRUG RESISTANCE IN THE UNITED STATES: TRENDS AND CHARACTERISTICS IN A LARGE PROTEASE/REVERSE-TRANSCRIPTASE AND CO-RECEPTOR TROPISM DATABASE FROM 2003 TO 2012 Agnes Paquet, Jeannette Whitcomb, Laura Napolitano, Christos Petropoulos, Mojgan Haddad</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>ROLE OF HUMAN MANNOSE RECEPTOR IN SEXUAL TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS IN SERODISCORDANT COUPLES Shivaji K, Atmaram H</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Withdrawn</td>
<td></td>
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<tr>
<td>6</td>
<td>Withdrawn</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>Withdrawn</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>MONITORING OF SIV MUTATIONS IN PROTEASE CLEAVAGE SITE REGIONS AFTER HIGH DOSE OF SIV MAC239 CHALLENGE IN CYNOMOLGUS MACAQUES ben liang, david la, rupert capina, xinyong yuan, shaun tyler, blake ball, paul sandstrom, gary van domselaar, ma Luo, francis plummer</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>DELETERIOUS SYNONYMOUS MUTATIONS IN ENV HITCHHIKE TO HIGH FREQUENCY Fabio Zanini, Richard Neher</td>
<td>7</td>
</tr>
<tr>
<td>11</td>
<td>BEHAVIORAL INTERVENTION FOR REDUCTION OF HIV/STD TRANSMISSION AMONGST SEX WORKERS IN UGANDA Philip Batwala, Esther Musoke, Muwanga Benson</td>
<td>8</td>
</tr>
<tr>
<td>12</td>
<td>APOBEC3G-DRIVEN SUPPRESSION OF PRODUCTIVE HIV-1 INFECTION Pulari Thangavelu, Vipul Gupta, Narendra Dixit</td>
<td>8</td>
</tr>
<tr>
<td>13</td>
<td>GAG-SPECIFICITY BUT NOT HLA-RESTRICTION OF CTL RESPONSES IMPACTS THE LIFESPAN OF HIV-INFECTED CELLS Hilde Spits, Ingrid Schellens, Sanne Spijkers, Tania Mudrikova, Nening Nanlohy, Anne Wensing, Debbie van Baarle, José Borghans</td>
<td>9</td>
</tr>
<tr>
<td>#</td>
<td>Abstract Title and Authors</td>
<td>Page</td>
</tr>
<tr>
<td>----</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>14</td>
<td>EVOLUTIONARY DYNAMICS OF IMMUNE ESCAPE MUTATIONS IN HIV-1 HAPLOTYPES</td>
<td>9</td>
</tr>
<tr>
<td>15</td>
<td>MODELING THE COURSE OF THE HCV EPIDEMIC AMONG HIV-POSITIVE MSM IN THE NETHERLANDS</td>
<td>10</td>
</tr>
<tr>
<td>16</td>
<td>FROM IN VITRO TO IN VIVO QUANTIFICATION OF ANTIRETROVIRAL DRUGS EFFECTS BASED ON DYNAMICAL MODELS OF HIV.</td>
<td>11</td>
</tr>
<tr>
<td>17</td>
<td>SIMULTANEOUSLY ESTIMATING VIRAL EVOLUTIONARY HISTORY AND PHYLOGENETIC TRAIT SIGNAL</td>
<td>11</td>
</tr>
<tr>
<td>18</td>
<td>NETWORK BASED MODELING OF INHIBITION AND RESISTANCE MECHANISMS IN HIV-1 REVERSE TRANSCRIPTASE</td>
<td>12</td>
</tr>
<tr>
<td>19</td>
<td>RESERVOIRS AS A POSSIBLE MECHANISM FOR THE CD4+ T CELL DEPLETION IN HIV INFECTION</td>
<td>12</td>
</tr>
<tr>
<td>20</td>
<td>MODELING THE EFFECT OF INTERLEUKIN 7 ON CD4+ T CELLS</td>
<td>13</td>
</tr>
<tr>
<td>21</td>
<td>T-CELL TURNOVER IN HIV-1 INFECTED PATIENTS ON ANTIRETROVIRAL THERAPY: DIFFERENCE BETWEEN IMMUNOLOGICAL RESPONDERS AND NON-RESPONDERS</td>
<td>14</td>
</tr>
<tr>
<td>22</td>
<td>IMPROVED DETECTION OF HIV-1 CORECEPTOR USAGE FROM SEQUENCE VARIATION AND V3 STRUCTURAL INFORMATION</td>
<td>15</td>
</tr>
<tr>
<td>23</td>
<td>PREDICTING OUTCOMES OF TREATMENTS TO ERADICATE THE HIV LATENT RESERVOIR</td>
<td>16</td>
</tr>
<tr>
<td>24</td>
<td>INTEGRATING GENEALOGICAL AND DYNAMICAL MODELLING TO INFERENCE AND REVERSION RATES IN HIV EPI TOPES</td>
<td>17</td>
</tr>
<tr>
<td>25</td>
<td>NON-B HIV TRANSMISSION NETWORKS IN THE UK</td>
<td>17</td>
</tr>
<tr>
<td>26</td>
<td>GENERALIZED DYNAMICS OF DRUG RESISTANCE IN SUSCEPTIBLE-INFECTED-MULTIPLE TREATMENT MODELS</td>
<td>18</td>
</tr>
<tr>
<td>#</td>
<td>Abstract Title and Authors</td>
<td>Page</td>
</tr>
<tr>
<td>----</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>27</td>
<td>PREDICTING THE IMPACT OF CD8+ T CELL DYSFUNCTIONALITY ON HIV DISEASE PROGRESSION</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Frederik Graw, Roland R. Regoes</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>LACK OF X4-TROPIC HIV PREVENTS VIRAL REBOUND POST CCR5-?32 STEM CELL TRANSPLANTATION IN THE “BERLIN PATIENT”</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Jori Symons, Steven Deeks, Gero Hütter, Annemarie Wensing, Petra van Ham, Linos Vandekerckhove</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>CO-RECEPTOR AND PROLIFERATION MARKER EXPRESSION AND CHARACTERIZATION OF THE VIRAL RESERVOIR IN CD4+ T-CELLS AFTER LONG-TERM THERAPY</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Jori Symons, Rachel McGovern, Annemarie Wensing, Sigrid Otto, Theresa Mo, Dorien de Jong, Kiki Tesselaar, Andy Hoepleman, Richard Harrigan, Monique Nijhuis</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>IS HIV SHORT-SIGHTED?</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Katrina Lythgoe, Lorenzo Pellis, Christophe Fraser</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>WITHIN-HOST AND BETWEEN-HOST EVOLUTIONARY RATES ACROSS THE HIV-1 GENOME</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Samuel Alizon, Christophe Fraser</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>THE PARADOX OF VIRAL HERITABILITY AND STABILITY OF SET POINT VIRUS LOAD IN HIV</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Anna Hool, Gabriel Leventhal, Sebastian Bonhoeffer</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>THE PARADOX OF HIV-1 ADAPTATION TO NK-CELL-MEDIATED IMMUNE PRESSURE</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Marjet Elemans, Becca Asquith</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>PRE-EXISTENCE AND EMERGENCE OF VIRAL DRUG RESISTANCE</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Helen Alexander, Sebastian Bonhoeffer</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>JOINT ASSOCIATION ANALYSIS OF GENOME-WIDE HUMAN AND HIV-1 VARIATION</td>
<td>24</td>
</tr>
<tr>
<td>36</td>
<td>DIMINISHED TRANSMISSION OF DRUG RESISTANT HIV-1 VARIANTS WITH REDUCED REPLICATIVE CAPACITY IN A HUMAN TRANSMISSION MODEL</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Marieke Pingen, Ramin Sarrami Forooshani, Annemarie M.J. Wensing, Petra M. van Ham, Charles A.B. Boucher, Teunis B.H. Geijtenbeek, Monique Nijhuis</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>IDENTIFICATION OF SOURCES OF HIV-1 TRANSMITTED DRUG RESISTANCE USING PHYLOGENETICS</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Sara Drescher, Viktor von Wyl, Sabine Yerly, Jürg Böni, Cyril Shah, Vincent Aubert, Thomas Klimkait, Patrick Taffé, Bruno Ledergerber, Huldrych Günthard, Roger Kouyos</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>TRANSMISSION DYNAMICS OF THE WORLDWIDE HIV-1 SUBTYPE B EPIDEMIC REGARDED FROM A CARIBBEAN ISLAND</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Daniela Bezemer, Hillegonda Hermanides, Nuno Rodrigues Faria, Ard van Sighem, Christophe Fraser, Frank de Wolf, Ashley Duits</td>
<td></td>
</tr>
<tr>
<td>#</td>
<td>Abstract Title and Authors</td>
<td>Page</td>
</tr>
<tr>
<td>----</td>
<td>------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>39</td>
<td>HIV SUPERINFECTION DOES NOT CONTRIBUTE TO TRANSMITTED DRUG RESISTANCE</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Istvan Bartha, Matthias Assel, Peter Sloot, Maurizio Zazzi, Carlo Torti, Eugen Schüller,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Andrea de Luca, Anders Sönnerborg, Ana B Abecasis, Anne-Mieke Vandamme, Roger Paredes,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>David van Vijver, Viktor Müller</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>HIV-1 SUBTYPE B TRANSMISSION NETWORKS OF HETEROSEXUALLY INFECTED PATIENTS CO-INFECTED WITH</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>HCV</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Daniela Bezemer, Colette Smit, Ard van Sighem, Frank de Wolf</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>DO PROTECTIVE HLA-B ALLELES CONFER PARTIAL TOLERANCE AGAINST HIV?</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Roland Regoes, Jacques Fellay, Amalio Telenti, Swiss HIV Cohort Study</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>HIV COMPETITION DYNAMICS OVER SEXUAL NETWORKS</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Bence Ferdinandy, Enys Mones, Tamás Vicsek, Viktor Müller</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>IDENTIFYING HIV-1 TRANSMISSION RISK FACTORS AND THE SOURCE OF TRANSMISSION FROM MOLECULAR</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>DATA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Erik Volz, Simon Frost</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>CHEMOKINE CO-RECEPTOR TROPISM, IMMUNE ACTIVATION AND IN VIVO PROLIFERATION OF CD4+ T-CELLS</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>IN HIV-1 INFECTION</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yan Zhang, Catherine de Lara, Andrew Worth, Andrea Hegedus, Peter Beverley, Derek</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Macallan</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>CO-EVOLUTION OF A BROADLY NEUTRALIZING HIV-1 ANTIBODY AND FOUNDER VIRUS FROM TIME OF</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>INFECTION</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peter Hraber, Hua-Xin Liao, Rebecca Lynch, Tongqing Zhou, Feng Gao, S Munir Alam,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Scott Boyd, Andrew Fire, Krishna Roskin, Chaim Schramm, Zhenhai Zhang, Jiang Zhu,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lawrence Shapiro, James Mullikin, Sandrasegaram Gnanakaran, Kevin Wiehe, Garnett Kelsoe,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Guang Yang, Shi-Mao Xia, David Montefiori, Robert Parks, Krissey Lloyd, Richard Scearce,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kelly Soderberg, Myron Cohen, Gift Kaminga, Mark Louder, Lillian Tran, Yue Chen, Fangping</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cai, Sheri Chen, Stephanie Moquin, Xiulian Du, Gordon Joyce, Sanjay Srivatsan, Baoshan</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zhang, Anqi Zheng, George Shaw, Beatrice Hahn, Thomas Kepler, Peter Kwong, John Mascola,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barton Haynes, Bette Korber</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>GENETIC COMPONENTS OF ENV SENSITIVITY TO CROSS-REACTIVE ANTIBODY NEUTRALIZATION</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Peter Hraber, Alan Lapedes, Elena Giorgi, Tanmoy Bhattacharya, Hongmei Gao, Kelli</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Greene, Allan DeCamp, Raphael Gottardo, Steve Self, Michael Seaman, Robert Bailer, John</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mascola, David Montefiori, Bette Korber</td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>HIV-1 EVOLUTION IN CAMEROON: 1995-2012</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Bimela Jude Saber, Atohgo Tiedeu Barbara, Judith Ndongo Torimiro</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>A MULTIPLE-ALIGNMENT BASED PRIMER DESIGN ALGORITHM FOR GENETICALLY HIGHLY VARIABLE DNA</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>TARGETS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Johanna Brodin, Mohan Krishnamoorthy, Gayathri Athreya, Will Fischer, Peter Hraber,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cheryl Gleasner, Cliff Han, Lance Greene, Bette Korber, Thomas Leitner</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>COMPLEX PATTERNS OF HIV ESCAPE FROM THE CTL RESPONSE</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>RESULTING FROM THE INTERPLAY OF MUTATION COST AND PARTIAL RECOGNITION LOSS.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rebecca Batorsky, Rinat Sergeev, Igor Rouzine</td>
<td></td>
</tr>
<tr>
<td>#</td>
<td>Abstract Title and Authors</td>
<td>Page</td>
</tr>
<tr>
<td>-----</td>
<td>-------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>50</td>
<td>ESTIMATING THE CONTRIBUTION OF GUT TO VIRAL LOAD IN EARLY SIV INFECTION FROM THE DYNAMICS OF ESCAPE</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Janka Petravic, Thomas H. Vanderford, Guido Silvestri, Miles Davenport</td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>UP TO 20% OF SUBSTITUTION MUTATIONS DURING REVERSE TRANSCRIPTION OCCUR IN ASSOCIATION WITH RECOMBINATION.</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Timothy Schlub, Redmond Smyth, Andrew Grimm, Abha Chopra, Simon Mallal, Vanessa Venturi, Johnson Mak, Miles Davenport</td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>PHYLODYNAMIC EVIDENCE FOR THE IMPACT OF HIGHWAY CORRIDORS ON HIV-1 SPREAD IN EAST AFRICA</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Nuno R Faria, Kim C Sigaloff, David AMC van de Vijver, Andrew J Tatem, Andrea Pineda, Carolle L Wallis, Marc A Suchard, Tobias F Rinke de Wit, Raph L Hamers, Philippe Lemey, Nicaise Ndemb</td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>THE INFLUENCE OF ANTIBODY TITRATION CURVE SLOPES ON THE EVOLUTION OF ANTIBODY RESISTANCE IN HIV-1 INFECTION</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Carsten Magnus, Rusert Peter, Huldrych F Günthard, Roland R Regoes, Alexandra Trkola</td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>PROPERTIES OF MHC CLASS I PRESENTED PEPTIDES THAT ENHANCE IMMUNOGENICITY</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Jorg Calis, Matt Maybeno, Jason Greenbaum, Daniela Weiskopf, Aruna de Silva, Alessandro Sette, Can Kesmir, Bjorn Peters</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>VIRAL GENOTYPE IN SUBTYPE C SIGNIFICANTLY INFLUENCES PLASMA VIRAL LOAD</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Emma Hodcroft, Esther Fearnhill, Andrew Philips, David Dunn, Deenan Pillay, Siobhan O’Shea, William Tong, Jarrod Hadfield, Andrew Leigh Brown</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>CTL ESCAPE DYNAMICS AND THE EFFECTS OF SHORT PULSE ART IN PATIENTS WITH ACUTE HIV-1B INFECTION</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Hannah Roberts, Jacob Hurst, John Frater, Rodney Phillips, Angela McLean</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>VIRAL ESCAPE FROM CD8+ T CELLS: EVIDENCE FOR A LYTIC EFFECTOR MECHANISM?</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>K Seich al Basatena, K Chatzimichalis, M Elemans, F Graw, SDW Frost, RR Regoes, B Asquith</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>IMPACT OF THE GENETIC BACKGROUND AND POPULATION SIZE ON THE EVOLUTION OF RALTEGRAVIR RESISTANCE</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Axel Fun, Thomas Leitner, Linos Vandekerckhove, Martin Daumer, Alexander Thielen, Bernd Buchholz, Jacobien Maarschalk-Ellerbroek, Clemens Richter, Pauline Schipper, Annemarie Wensing, Monique Nijhuis</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>MEASURING EMERGENCE AND TRANSMISSION OF HIV DRUG RESISTANCE USING PHYLOGENETICS</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>George Shirreff, Tanja Stadler, Roger Kouyos, Wan-Lin Yang, Sabine Yerly, Jürg Böni, Thomas Klimkait, Vincent Aubert, Huldrych Günthard, Sebastian Bonhoeffer</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>ANALYSIS OF MUTATIONS RESPONSIBLE FOR THE “IN VITRO” FITNESS ALTERATIONS IN HIV-1 CLONES AFTER DIFFERENT SERIAL PASSAGES.</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Cecilio Lopez-Galindez</td>
<td></td>
</tr>
</tbody>
</table>
Abstract Title and Authors

61  A MOLECULAR DYNAMICS APPROACH TO INVESTIGATE THE INFLUENCE OF N-LINKED GLYCOSYLATION ON HIV-1 CORECEPTOR TROPISM
Natasha Wood, Elisa Fadda, Robert Woods, Simon Travers 49

62  UNIQUE BC RECOMBINANT FORMS OF HIV-1 IDENTIFIED IN CAPE TOWN, SOUTH AFRICA
Graeme Jacobs, Eduan Wilkinson, Shahieda Isaacs, Georgina Spies, Soraya Seedat, Susan Engelbrecht 50

63  CO-EXPRESSIO/N OF HLA-B27, BUT NOT B57, RESULTS IN ENHANCED RESPONSIVENESS OF HIV-SPECIFIC CTL RESTRICTED THROUGH OTHER HLA-ALLELES
Ingrid Schellens, Hilde Spits, Marjon Navis, Margreet Westerlaken, Nening Nanlohy, Neeltje Kootstra, Frank Miedema, Hanneke Schuitemaker, Jose Borghans, Debbie van Baarle 51

64  MOLECULAR EPIDEMIOLOGY OF FELINE IMMUNODEFICIENCY VIRUS (FIV) IN THE KRUGER NATIONAL PARK (KNP), SOUTH AFRICA
Tanya Kerr, Sonja Matthee, Danny Govender, Conrad Matthee, Wolfgang Preiser, Susan Engelbrecht 52

65  CHALLENGES WITH USING PRIMER ID’S TO IMPROVE ACCURACY OF NEXT GENERATION SEQUENCING
Johanna Brodin, Charlotte Hedskog, Alexander Heddini, Mattias Mild, Jan Albert 52

66  RAMICS - RAPID AMPLICON MAPPING IN CODON SPACE FOR ACCURATE HIV-1 DRUG RESISTANCE GENOTYPING
Imogen Wright, Simon Travers 53

67  HIV-1 ACTIVITY AND COMPARTMENTALIZATION AT THE ANAL MUCOSA IN MSM WITH INTRAEPITHELIAL NEOPLESIA
G Pollakis, O Richel, DJ Vies, WA Paxton, HJC de Vries, JM Prins 54

68  HIV DRUG RESISTANCE GENOTYPING OF PROTEASE, REVERSE TRANSCRIPTASE AND INTEGRASE GENES USING ILLUMINA MISEQ
Dawn Dudley, Ryan Westergaard, Adam Bailey, Jacquie Astemborski, Shruti Mehta, Gregory Kirk, David O’Connor 55

69  USING AN EPIDEMIOLOGICAL MODEL FOR PHYLOGENETIC INFERENCE REVEALS DENSITY-DEPENDENCE IN HIV TRANSMISSION
Gabriel Leventhal, Huldrych Günthard, Sebastian Bonhoeffer, Tanja Stadler 56

70  RECONCILING HOST AND PATHOGEN TREES FOR A LARGE HIV TRANSMISSION CHAIN
Bram Vrancken, Andrew Rambaut, Alexei Drummond, Guy Baele, Marc Suchard, Eric Van Wijngaerden, Anne-Mieke Vandamme, Kristel Van Laethem, Philippe Lemey 57

71  Withdrawn

72  QUANTIFYING THE TURNOVER OF TRANSCRIPTIONAL SUBCLASSES OF HIV-1-INFECTED CELLS
Christian Althaus, Beda Joos, Alan Perelson, Huldrych Günthard 58

73  T-CELL REPERTOIRE DYNAMICS DURING CHRONIC HIV-1 INFECTION
Dan Koning 58
<table>
<thead>
<tr>
<th>#</th>
<th>Abstract Title and Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>74</td>
<td>NATURAL EXTINCTION OF HIV-2 IN RURAL GUINEA-BISSAU</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Helen Fryer, Carla van Tienen, Maarten Schim van der Loeff, Matthew Cotten, Peter Aaby,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zacarias da Silva, Hilton Whittle, Sarah Rowland-Jones, Thushan de Silva</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>THE APPLICATION OF SEQ2RES TO FACILITATE LARGE-SCALE, COST-EFFECTIVE HIV DRUG RESISTANCE</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>GENOTYPING USING 454 PYROSEQUENCING.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ram Shrestha, Imogen Wright, Natasha Wood, Hanah Ajoge, Irene Ketseoglou, Maria Papathanasopolous, Ian Sanne, Robin Wood, James McIntyre, Wendy Stevens, Simon Travers</td>
<td></td>
</tr>
<tr>
<td>81</td>
<td>HIV-1 SEQUENCE EVOLUTION DURING PERSISTENT LOW-LEVEL VIREMIA</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Saran Vardhanabhuti, Babafemi Taiwo, Daniel Kuritzkes, Joseph Eron, Ronald Bosch</td>
<td></td>
</tr>
<tr>
<td>82</td>
<td>INTERPLAY BETWEEN LONG-LIVED ANTIBODY RESPONSES AND SHORT-LIVED CD8+ T CELL RESPONSES CAN</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>EXPLAIN THE MAINTENANCE AND LOSS OF HIV-1 CONTROL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paul Wikramaratna, Paul Klenerman, Oliver Pybus, Sunetra Gupta</td>
<td></td>
</tr>
<tr>
<td>83</td>
<td>EXTRACTING EPIDEMIC DYNAMICS FROM VARYING HIV-1 EVOLUTIONARY RATES</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Ethan Romero-Severson, Irina Maljcovic Berry, Nicholas Hengartner, Namrata Patel, Thomas</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leitner</td>
<td></td>
</tr>
<tr>
<td>84</td>
<td>GLOBAL HAPLOTYPE RECONSTRUCTION OF HIV-1 GENOMES</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Francesca Di Giannonardo, Melanie Rey, Armin Töpfer, Sandhya Prabhakaran, Yannick Duport,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rita Lecca, Martin Däumer, Huldrych F Günthard, Niko Beerenwinkel, Volker Roth, Karin J</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Metzner</td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>ORIGIN AND PHYLOGENETIC RELATIONSHIPS OF AN HIV-1 SUBTYPE F1 TRANSMISSION CLUSTER RAPIDLY</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>EXPANDING IN MEN WHO HAVE SEX WITH MEN IN SPAIN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Elena Delgado, Yolanda Vega, Marina Cabello, Francisco Domínguez, Aurora Fernández-García,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>María Ángeles Castro, Ana Mariño, Vanessa Montero, Antonio Ocampo, María José López-Álvarez,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Raúl Rodríguez, Matilde Trigo, Lucía Pérez-Álvarez, Michael Thomson</td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>THE EVOLUTION OF BROADLY NEUTRALIZING ANTIBODIES: WHAT CAN DEEP SEQUENCING TELL US?</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Sergei Kosakovsky Pond, N Lance Hepler, Douglas Richman, Devin Sok, Dennis Burton, Pascal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Poignard, Davey Smith</td>
<td></td>
</tr>
<tr>
<td>87</td>
<td>ANALYSIS OF THE HETEROLOGOUS NEUTRALIZATION CAPACITY OF PLASMA FROM PATIENTS SUPERINFECTED</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>WITH CONCORDANT SUBTYPE STRAINS OF HIV-1.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Luzia Mayr, Johnson Ngai, Bladine Asaah, Aubin Nanfack, #Phillipe Nyambi</td>
<td></td>
</tr>
<tr>
<td>88</td>
<td>OVERESTIMATED HETEROSEXUAL TRANSMISSION OF HIV-1 SUBTYPE B IN THE UK</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Stephane Hue, Samantha Lycett, David Dunn, Esther Fearmhill, David Dolling, Valerie Delpech,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alison Brown, Deenan Pillay, Andrew Leigh-Brown</td>
<td></td>
</tr>
<tr>
<td>89</td>
<td>Withdrawn</td>
<td></td>
</tr>
</tbody>
</table>


# Abstract Title and Authors

<table>
<thead>
<tr>
<th>#</th>
<th>Abstract Title</th>
<th>Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>DISCORDANT DRUG PENETRATION PROMOTES THE EVOLUTION OF MULTI-DRUG RESISTANCE</td>
<td>Stefany Moreno-Gamez, Alison Hill, Daniel Rosenbloom, Martin Nowak, Pleuni Pennings</td>
<td>68</td>
</tr>
<tr>
<td>91</td>
<td>SELECTIVE INTERFERENCE AND CTL ESCAPE IN HIV</td>
<td>Roland Regoes, Victor Garcia</td>
<td>68</td>
</tr>
<tr>
<td>93</td>
<td>Withdrawed</td>
<td></td>
<td>68</td>
</tr>
<tr>
<td>94</td>
<td>ANALYSIS OF PCR FOUNDER EFFECT AND SEQUENCING ERRORS IN ILLUMINA SEQUENCING</td>
<td>Wei Shao, Valerie F. Boltz, Mary F. Kearney, Frank Maldarelli</td>
<td>70</td>
</tr>
<tr>
<td>95</td>
<td>DETAILED PHYLOGENETIC AND PHYLOGEOGRAPHIC ANALYSIS OF HIV-1 IN THE SCANDINAVIAN REGION</td>
<td>Joakim Esbjörnsson, Mattias Mild, Anne Audelin, Helena Skar, Louise Bruun Jörgensen, Kirsi Liitsola, Per Björkman, Göran Bratt, Magnus Gisslén, Anders Sönnerborg, Claus Nielsen, SPREAD programme, Patrik Medstrand, Jan Albert</td>
<td>71</td>
</tr>
<tr>
<td>96</td>
<td>Withdrawed</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>97</td>
<td>A COMPREHENSIVE, ACCURATE, FAST, CROSS-PLATFORM AND USER-FRIENDLY PIPELINE FOR THE ANALYSIS OF HIV-1 NEXT GENERATION SEQUENCING DATA</td>
<td>N Lance Hepler, Ellen Paxinos, Michael Brown, Jason Chin, David Alexander, Patrick Marks, Yan Guo, Douglas Richman, Davey Smith, Sergei Kosakovsky Pond</td>
<td>72</td>
</tr>
<tr>
<td>98</td>
<td>PRIME: PROPERTY-INFORMED MODELS OF EVOLUTION</td>
<td>Konrad Scheffler, Ben Murrell, Davey Smith, Sergei Kosakovsky Pond</td>
<td>73</td>
</tr>
<tr>
<td>100</td>
<td>SPATIAL DISTRIBUTION AND PHYLOGEOGRAPHY OF THE HIV TRANSMISSION NETWORK IN SAN DIEGO, CALIFORNIA</td>
<td>Sanjay Mehta, Antoine Challion, Hayden Lowenstein, Susan Little, Joel Wertheim, Doug Richman, Sergei Pond, Davey Smith</td>
<td>75</td>
</tr>
<tr>
<td>101</td>
<td>Withdrawed</td>
<td></td>
<td>75</td>
</tr>
<tr>
<td>102</td>
<td>HIV-1 CRF02_AG INTRA-PATIENT EVOLUTION IN RELATION TO DISEASE PROGRESSION RATE</td>
<td>Joakim Esbjörnsson, Angelica Palm, Verónica Rodríguez Fernández, Fredrik Månsson, Hans Norrgren, Philippe Lemey, Patik Medstrand</td>
<td>76</td>
</tr>
<tr>
<td>#</td>
<td>Abstract Title and Authors</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------</td>
<td></td>
</tr>
</tbody>
</table>
| 103 | **TIMING OF MOTHER-TO-CHILD TRANSMISSION OF THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 BASED ON VIRAL MOLECULAR EVOLUTION**  
Antoine Chaillon, Tanawan Samleerat, Faustine Zoveda, Sebastien Ballesteros, Nicole Ngo-Giang-Huong, Gonzague Jourdain, Pranee Leechanachai, Marc Lallemant, Francis Barin, Frantz Depaulis | 77   |
| 104 | **HIV-1 VIRULENCE EVOLUTION IN AN HLA-POLYMORPHIC HOST POPULATION.**  
Christiaan van Dorp, Rob de Boer, Michiel van Boven                                                                                                              | 78   |
| 105 | **CXCR4- USING HIV-1 SUBTYPE C STRAINS IDENTIFIED BY SINGLE GENOME SEQUENCING FROM PATIENTS FAILING ANTIRETROVIRAL TREATMENT IN BOTSWANA**  
Lotta Pramanik Sollerkvist, Simani Gaseitsiwe, Madisa Mine, Max Essex, Anneka Ehrnst                                                                               | 79   |
| 106 | **LYMPHATIC TISSUES SHOW PERSISTENT REPLICATION, EVOLUTION AND DISPERAL OF HIV-1 DESPITE ANTIRETROVIRAL THERAPY**  
Trevor Bedford, Andrew Rambaut, Eun-Young Kim, Ramon Lorenzo-Redondo, John Archer, Ashley Haase, Tim Schacker, Steven Wolinsky | 80   |
| 107 | **THE COLOMBIAN EPIDEMIC IS DOMINATED BY HIV-1 SUBTYPE B OVER TIME: A MOLECULAR EPIDEMIOLOGY AND PHYLODYNAMICS STUDY**  
Andrea-Clemencia Pineda-Peña, Nuno Rodrigues Faria, Francisco-Javier Diaz, Patricia Olaya, Casper Møller Frederiksen, Li Guangdi, Arley Gomez-Lopez, Philippe Lemey, Anne-Mieke Vandamme | 81   |
| 108 | **TRANSMISSION AND EARLY EVOLUTION OF HIV-1**  
Ronald Swanstrom                                                                                                                                                | 81   |
| 109 | **MANIPULATION OF IMMUNDOMINANCE WITH A CONSERVED ELEMENT-BASED HIV-1 VACCINE**  
Viraj Kulkarni¹, Christian Brander², Geoffrey Stone³, Sylvie Le Gall⁴, Morgane Rolland⁵, Siriphan Manocheewa⁶, Niranjan Y. Sardesaï⁶, Barbara K. Felber¹, George N. Pavlakis⁷, James I. Mullins⁸   | 82   |
| 110 | **MODELING HCV INFECTION AND TREATMENT**  
Alan S. Perelson, Ph.D.                                                                                                                                               | 82   |
| 111 | **IMMUNOGENETIC FACTORS THAT IMPACT THE COURSE OF HIV INFECTION**  
M. Carrington, 1                                                                                                                                                  | 82   |
| 112 | **INNATE SIGNALING BY C-TYPE LECTINS IN HIV-1 INFECTION**  
Teunis B.H. Geijtenbeek                                                                                                                                              | 83   |
| 113 | **COMPARATIVE STUDY OF THE MOLECULAR EPIDEMIOLOGY OF HIV IN TWO DIFFERENT PERIODS AMONG DIFFERENT SPANISH REGIONS REVEALED THE PRESENCE OF LOCAL SUBEPIDEMICS**  
González-Alba JM¹, García R², Ortiz de Lejarazu R³, García-Bermejo I⁴, Cardeñoso L⁵, Pumarola T⁶, Alonso R⁷, Martín D⁸, Delgado R⁹, García-Bujalance S¹⁰, Páez-Peña M¹¹, Suárez A¹², Viciana I¹³, Gómez C¹⁴, Aguilera A¹⁵, Fernández-Pereira L¹⁶, Martín P¹⁷, Porter M¹⁸, González-Palacios M¹⁹, Galán JC¹ | 83   |
<table>
<thead>
<tr>
<th>#</th>
<th>Abstract Title and Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>114</td>
<td>PERSISTENT ELEVATION IN HIV VIREMIA DURING CART WITH IDENTICAL WT SEQUENCES IMPLIES EXPANSION OF A CLONAL SOURCE</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>John Coffin</td>
<td></td>
</tr>
<tr>
<td>115</td>
<td>INFERRING HIV ESCAPE RATES FROM MULTI-LOCUS GENOTYPE DATA</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Taylor A. Kessinger¹, Alan S. Perelson² and Richard A. Neher¹</td>
<td></td>
</tr>
</tbody>
</table>
ABSTRACT Background: Monitoring of HIV-infected children prior to and after the initiation of antiretroviral therapy is important for adequate management. The difficulties in systematically implementing monitoring through HIV-viral load in resource-limited settings prompts the search for cheaper and accurate alternate approaches. One potential alternative being explored is the plasma levels of Fas, a mediator of cell apoptosis involved in HIV pathogenesis. Objective: To assess the correlation between plasma levels of soluble forms of Fas receptor and Fas ligands with standard indicators of HIV disease progression (HIV viral load, CD4 absolute counts and CD4%) in children. Methods: In a cross-sectional study, 22 consecutive HIV-1 positive children attending the Pediatric unit of Chantal Biya Foundation Hospital were enrolled. Ten ml of whole blood was collected from each participant. CD4 cell absolute counts and CD4% counts were assayed from whole blood using an Automated Facscount machine from (Becton Dickenson, Belgium) and a cell Dyne 3200, (Abbott, France) respectively. Plasma levels of soluble Fas receptors and Fas ligands were determined using two different quantitative sandwich ELISA kits (Quantikine®, R&D Systems, UK). Plasma HIV-1 RNA levels were measured using a commercial quantitative reverse transcriptase polymerase chain reaction assay (Amplicor HIV Monitor version 1.5, Roche Molecular Diagnostic, Germany). Results: Participants were aged between 9 months and 7 years. Fas ligand levels ranged from 36-299 (median=126) pg/ml while soluble Fas receptor levels ranged from 987-3217 (median=1571.5) pg/ml. The correlation coefficients (p-values) between Fas ligand levels and each of HIV-1 viral load, CD4 absolute counts and CD4% were respectively 0.56 (0.01), -0.29 (0.18), 0.30 (0.18). On the other hand the correlation coefficients (p-values) between soluble Fas receptor levels and each of HIV-1 viral load, CD4 absolute counts and CD4% were respectively 0.12 (0.60), -0.30 (0.18), -0.29 (0.19). Conclusion: The significant correlation between plasma levels of HIV-1 viral load and Fas ligands suggests that the latter could potentially be an alternative approach to monitor HIV-1 disease progression children. However these findings will need to be confirmed in a larger, diverse study population of children in prospective studies.

Supported by a grant from amfAR to attend this meeting.
MODELING AND SIMULATING THE DYNAMICS OF TWO GROUP PATIENTS’ ANTI-HIV INFECTION THERAPY

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Objectives and Background: Study the dynamics of two group patients’ 12 weeks anti-HIV infection therapy. Group I has 15 patients and Group II 13 patients. During the treatment, the two group patients took the same dose of protease inhibitors: ritonavir and saquinavir. Additionally Group I took strvudine, and Group II took strvudine and dideoxyinosine as the nucleoside reverse transcriptase inhibitors, respectively.

Methods: Based on Nowark et al.s’ basic virus infection model, an amended anti-HIV infection therapy model with a saturated infection rate was introduced to describe the dynamics of anti-HIV infection therapy. The model has infection-free state (Q1) and endemic infection state (Q2). LaSalle’s invariance principle was used to study the conditions of the global attraction of Q1 and Q2, which will imply that treatment makes patients’ virus infection free and endemic infection. Matlab platform was used to simulate the dynamics of anti-infection therapy model.

Results: The model has a basic infection reproductive number R. It is proved that if R<1 then Q1 is globally attractive. Otherwise Q2 is globally attractive. The simulations have shown that the first 4 and 8 weeks’ treatment made the two group patients’ R reduced but still slightly more than one, respectively. After the two periods, the drug resistance appeared which made patients’ R increased. The results interpret why patients’ CD4+ T cells mean level raised and HIV RNA mean level declined rapidly in the first two periods, but contrary in the following weeks.

Conclusion: The analysis of the model suggests that a patient with R>1 may be endemic infection even if infected with only one HIV. The simulations show that HIV in vivo persistently existed, because the two group patients’ R was more than one.

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A DECADE OF HIV-1 DRUG RESISTANCE IN THE UNITED STATES: TRENDS AND CHARACTERISTICS IN A LARGE PROTEASE/REVERSE-TRANSCRIPTASE AND CO-RECEPTOR TROPISM DATABASE FROM 2003 TO 2012

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Background:
Drug resistance testing and co-receptor tropism determination are key components of the management of antiretroviral therapy for individuals infected with HIV-1. The purpose of this study was to examine phenotypic drug resistance patterns in protease- (PI), nucleoside-reverse-transcriptase- (NRTI), and non-nucleoside-reverse-transcriptase-inhibitors (NNRTI) over time, as well as prevalence of co-receptor usage by surveying Monogram’s commercial patient testing database.

Methods:
We examined samples submitted for routine phenotypic and genotypic patient testing that show phenotypic resistance to at least one drug within PIs, NRTIs, and NNRTIs as measured by fold-change in IC50 (FC) > lower cutoff (CO). A total of 78,867 resistant samples collected from 2003 through 2012 were grouped into specimens that had FC > CO for minimum of 1 drug in each drug-class. We studied the temporal trends of % phenotypic 1-, 2-, and 3-class resistance. Furthermore, we examined the prevalence of CCR5 (R5) and CXCR4 (X4) usage among 8,114 samples that had phenotypic PI, NRTI, and NNRTI resistance information as well as co-receptor tropism as determined by Monogram’s Trofile assay.

Results:
Among samples that show any phenotypic drug resistance, percentage of samples with 1-class resistance increased from 30.7% in 2003 to 54.6% in 2012, while 2-class resistance remained relatively stable (40% to 35.3%), and 3-class resistance sharply declined from 29.3% to 10% over the same time period. Prevalence of X4 using viruses among samples with matched PR/RT genotype and phenotype increased significantly: 36.4%, 41.3%, 46.8%, and 50.2% for 0-, 1-, 2-, and 3-class resistance, respectively (Jonckheere-Terpstra p=0.02).

Conclusion:
A strong trend of decreasing prevalence of 3-class resistance, and increased prevalence of 1-class resistance was identified within samples collected between 2003 and 2012. CXCR4-mediated entry was more prevalent among patient viruses with multiple drug class resistance. This increased prevalence may be due to the more advanced disease stage of treatment experienced patients. These trends have important implications for antiretroviral drug selection, clinical trial design as well as future drug discovery and development.

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ROLE OF HUMAN MANNOSE RECEPTOR IN SEXUAL TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS IN SERODISCORDANT COUPLES

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Introduction: HIV binds specifically to human Mannose Receptor (hMR) on vaginal epithelial cells which are devoid of conventional CD4 receptor. HIV binding to hMR on vaginal epithelial cells induces the production of Matrix Metalloproteinase 9 (MMP9) leading to degradation of extracellular matrix which may increase the risk of sexual transmission of HIV.

Methods: Localization of hMR on vaginal epithelial cells of the seronegative females from general population (n=52) and seronegative females from Serodiscordant couples was studied using FITC labelled antibodies to hMR (FITC AbhMR). PCR amplification of DNA from PBMCs of the serodiscordant females for CCR5 gene flanking for CCR5-?32 region. Translated amino acid sequence of C2-V3 region of env gene of HIV PBMCs and sperm of the infected male partners of the Serodiscordant couples was determined.

Results: Presence of hMR on 0-11 % of the vaginal epithelial cells of seronegative females (n=39) from serodiscordant couples and 90-95% that of control group of females (n=52). Nine of these serodiscordant females did not show CCR5-?32 deletion. Translated amino acid sequence of C2-V3 region of env gene of HIV1C in PBMCs (n=9) and sperm (n=5) of the male partners showed the presence of distinct variants and the variation in PBMCs and sperm of serodiscordant males was almost similar to that of infected males from concordant couples.

Conclusion: Presence of hMR in lower number of vaginal epithelial cells of Serodiscordant females prevented binding and HIV entry into these cells. The study suggests the association of hMR in sexual transmission of HIV.

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DELIVERY OF CARE TO HIV INFECTED PATIENTS.

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Withdrawn
UNIFORMED MEN: A VULNERABLE AND A RISK GROUP TO HIV/AIDS

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Withdrawn

MALE CIRCUMCISION PRACTICE: ASSOCIATION WITH HIV/AIDS PREVENTION

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Withdrawn
AIDS-PROTECTIVE HLA-B*27/B*57 AND CHIMPANZEE MHC CLASS I MOLECULES TARGET ANALOGOUS CONSERVED AREAS OF HIV-1/SIVCPZ

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Human immunodeficiency virus type-1 (HIV-1) causes, without treatment, in most infected human individuals acquired immunodeficiency syndrome (AIDS). However, a minority of infected individuals is long-term non-progressor, and this is strongly associated with the presence of the MHC class I molecules HLA-B*2705 and -B*5701. Chimpanzees (Patr), humans’ closest living relative, are also susceptible to HIV-1 infection, but most infected animals do not contract AIDS. As compared to humans, we showed that chimpanzees experienced an ancient selective sweep affecting the MHC class I repertoire, and we hypothesized that this was caused by HIV-1/SIVcpz or a closely related ancestral retrovirus. To examine this hypothesis and whether the observed repertoire skewing resulted in the preferential selection of Patr molecules that are similar to AIDS-resistant molecules in human long-term non-progressors, we have determined the peptide binding properties of various Patr class I molecules. Subsequently peptide-binding studies were performed in which we show that 94% of the studied subjects possess at least one Patr class I molecule that targets similar areas of HIV-1/SIVcpz, as does HLA-B*27/B*57. Many chimpanzees express several molecules that can bind multiple peptides originating from various conserved areas, which suggest that chimpanzees may have developed a “double-lock” strategy. Accordingly, chimpanzees of an experimentally infected HIV-1 cohort display broadly reactive CTL responses. Hence, the chimpanzee MHC class I repertoire skewing favored the survival of animals that possess Patr molecules targeting similar conserved areas of HIV-1/SIVcpz as does HLA-B*27/B*57-positive human long-term non-progressors. The functional characteristics of the contemporary chimpanzee MHC class I repertoire suggest that the sweep was caused by a lentiviral pandemic. As a consequence most chimpanzees seem to be able to cope with retroviral infections such as HIV-1/SIVcpz, like AIDS-resistant HLA-B*27/B*57-positive humans. This information may shed light on the consequences of the contemporary HIV-1 pandemic in humans.
Protease cleavage sites (PCSs) of HIV-1 are highly conserved among major subtypes of HIV-1. Direct immune responses against these PCSs destroy the virus before its permanent establishment in the host. Thus, a vaccine targeting HIV-1 PCSs could force the virus to accumulate mutations eliminating the normal function of the HIV protease thus eliminating viable virions.

We conducted a pilot study to monitor dynamics of SIV mutations in/or around 12 PCSs, mediating cleavage of Gag, Gag-Pol and Nef precursor polyproteins, after immunization of 39 cynomolgus macaques by the newly designed recombinant *Vesicular Stomatitis Virus* (VSV)-peptides combined with a nano-delivery system over 20 weeks. Viral mutations were identified and monitored by analyzing 454 pyrosequencing data, covering 12 PCS regions, generated from SIVmac239. Results showed that there were extensive mutations in the PCSs and the flanking regions. There was not an obvious trend that diversity of PCS and flanking regions increased over time. However, it was found that diversity of PCS regions was higher in the vaccine group than in the control group. Furthermore, the extensive mutations at/or around PCS sequences, especially frame shift mutations, were shown to be correlated with lower viral load ($P < 0.0001$). In conclusion, this study is capable of precisely interpreting the correlation between the mutations in PCS or flanking regions and host immune responses and thus helps examine the effectiveness of generating immune responses to PCS regions in the protection against HIV infection using Cynomolgus macaque model.

Deleterious synonymous changes hitchhike to high frequency

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The variable loops V1-V5 in env are under strong positive selection imposed by the host immune system, and escape mutations frequently appear. We track the allele frequency of synonymous changes in published longitudinal, intrapatient data sets and observe that many synonymous mutations in the flanking C1-C5 regions also reach high frequencies. They rarely fix, however, and usually are lost within two years, a clear signature of deleterious fitness effects. We suggest that deleterious synonymous changes hitchhike on the background of an escape mutant, decouple via recombination, and are eventually lost via purifying selection. Both data and comparisons to computer models indicate a deleterious effect size of approximately 0.001. Exploiting published data on SHAPE assays (Watts et al. Nature, 2009), we show that synonymous changes that disrupt base pairs in RNA secondary structures are lost more often than other synonymous changes, hinting at a fitness effect of RNA stems. Finally, we observe a reduced fixation of nonsynonymous changes as well. Comparison to computational models points at two possibly concomitant explanations. On the one hand, escape mutations might be only transiently beneficial, until recognition by the host; on the other, several escape mutants at the same epitope could arise in different parts of the viral population and compete with each other until only one is fixed.
11  BEHAVIORAL INTERVENTION FOR REDUCTION OF HIV/STD TRANSMISSION AMONGST SEX WORKERS IN UGANDA

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Background: The objectives of this intervention research were to increase knowledge and awareness among sex workers and their clients regarding STD’s including HIV/AIDS, (1) to study the clinical -social aspects among STD patients. Methods: Referred cases 100 between July 2011 and March 2012) intervention activities were conducted through health care education, intensive counseling, awareness and condom use. Patients attending STD clinic were screened for anti-HIV antibodies. 96 male STDs in the age range of 17-50 years (27.35, 7.69) and 32 female sex workers in 15-45 years (26.94, 6.82) were included in the study. Information regarding their STD/AIDS perceptions, condom use, and partner relationship was obtained on a specialty prepared pro-forma, Although, very few sex workers used F.P. method, 10% males reported a condom usage at the some time, despite reporting risky behavior with multiple sex partners. Results: In the beginning of the study very few sex workers (60%) were aware of AIDS disease. through, some of them (10%) were aware on the usefulness of condoms but practically none of them were using the same. However, after providing health care education and intervention counseling to the sex workers, 32.03% of the sex workers ensured that all their clients used condoms but regularly. STD was detected in 36.4% male and 37.5% female sex workers through case histories and clinical examinations including laboratory investigations. Conclusions: The prevalence of both HIV1 and HIV2 infection amongst the sex workers in Uganda indicates that there is a need for an intensive health case education, counseling and awareness programmes to affect behavior change and STD control amongst the high risk groups

12  APOBEC3G-DRIVEN SUPPRESSION OF PRODUCTIVE HIV-1 INFECTION

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The interaction between the host factor APOBEC3G (A3G) and the HIV-1 protein Vif is an attractive target of intervention. A3G incorporated into virions induces significant GG to AG hypermutations in the viral genome and curtails infection. Vif targets A3G for proteasomal degradation and restricts its incorporation into virions. Several drug molecules targeting the A3G-Vif axis are under development. The extent to which the A3G-Vif interaction must be suppressed in order to tilt the balance in favour of A3G remains unknown. We performed stochastic simulations of the within-host dynamics and evolution of HIV-1 to estimate the fraction of virions that must incorporate A3G to render productive infection unsustainable. We let A3G induce GG to AG hypermutations, which could alter the quasispecies structure as well as result in premature stop codons inhibiting viral production. Using a full length HIV-1 genome, the twin-gradient hypermutation pattern observed, and other parameters representative of HIV-1 in vivo, we found that a sharp transition occurred from sustained infection to complete suppression of infection when the fraction of virions that incorporated A3G crossed ~0.8. We advanced the basic model of HIV dynamics to explicitly incorporate A3G activity and found that the latter estimate was consistent with the extinction threshold predicted by the model crossing which rendered \( R_0 < 1 \). Intriguingly, the estimate lies at the upper end of the range 0.3-0.8 of A3G units/virion observed experimentally with fully functional Vif, suggesting that Vif activity is evolutionarily optimized to suppresses A3G incorporation just enough to avert crossing this threshold. We found further that the quasispecies remained localized in sequence space upon crossing this threshold indicating that suboptimal targeting of the A3G-Vif axis may be less fraught with the risk of increasing viral adaptability than perceived. The threshold identified presents a quantitative guideline for intervention strategies targeting the A3G-Vif axis.
GAG-SPECIFICITY BUT NOT HLA-RESTRICTION OF CTL RESPONSES IMPACTS THE LIFESPAN OF HIV-INFECTED CELLS

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It is widely believed that cytotoxic T lymphocytes (CTL) play an important role in the control of HIV infection, amongst others by shortening the lifespan of productively infected (PI) cells. Recent studies have shocked the field, however, by showing that complete depletion of the CD8+ T-cell pool of SIV-infected rhesus macaques does not influence the lifespan of their productively SIV-infected cells. We hypothesize that a protective CTL response which successfully reduces the lifespan of PI cells is only present in a minority of HIV-infected individuals, and may therefore easily be overlooked. Indeed, protective HLA-alleles and highly functional CTL responses against HIV-Gag, which correlate with slow disease progression, rarely occur. Likewise, macaque studies are typically limited to macaques that do progress to AIDS, which could explain why CTL depletion had no effect.

We determined the lifespans of PI cells in 31 patients, of which 45% possessed one of the rare protective HLA-alleles HLA-B57 or B27, during untreated HIV-1 infection. This was estimated by measuring their viral load declines immediately after start of HAART. The breadth of the CTL response against HIV-Gag was determined by a matrix-elispot with an overlapping 15-mer Gag-peptide pool. The average lifespan of the PI cells varied considerably between individuals, from 0.77 to 2.71 days. Strikingly, the lifespan of PI cells did not correlate with the presence or absence of a protective HLA allele. In contrast, a broad CTL response against HIV-Gag correlated with a shortened lifespan of PI cells (p=0.03). Taken together, these data suggest that protective HLA alleles exert their effect via another route. Quite possibly they control HIV-1 by targeting infected cells before they start producing virus. However, shortening the lifespan of PI cells may contribute to the protective effect of CTL that target the HIV-Gag protein.

EVOLUTIONARY DYNAMICS OF IMMUNE ESCAPE MUTATIONS IN HIV-1 HAPLOTYPES

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Human immunodeficiency virus type 1 (HIV-1) undergoes a transmission bottleneck when a few, or even a single, genome(s) establish the infection in a new individual. Following this bottleneck, the virus undergoes a phase of rapid expansion and adaptation inside the individual during the acute and early chronic phase of infection. During these stages strong immune selection is known to drive the evolution of HIV-1. Next generation sequencing technology has enabled us to study the low frequency HIV-1 variants existing inside an individual. However, given the variation in HIV-1, and the short reads lengths obtained from the next generation sequencing, only limited regions of the genome can be studied in detail. Using computational approaches, we study the evolution of early CD8+ T cell immune-escape variants by reconstructing HIV-1 whole genome haplotypes from the sequencing reads. This approach allows us to study evolution at the whole genome level instead of focusing on a limited region. We show how CD8+ T cell escape mutations evolve in HIV-1 haplotypes. The approach also allows us to study the temporal selection of HIV-1 haplotypes where beneficial CD8+ T cell immune-escape mutations lead to selective sweeps.
MODELING THE COURSE OF THE HCV EPIDEMIC AMONG HIV-POSITIVE MSM IN THE NETHERLANDS

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4. GGD
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6. AMC
7. AMC
8. AMC

Background: Since 2000, outbreaks of sexually transmitted Hepatitis C virus (HCV) infections among HIV-positive men who have sex with men (MSM) have been reported in several high income-countries, including the Netherlands. However, recently, the HCV incidence observed at STI clinics seems to be leveling off. Hence, we investigated the course of the HCV epidemic among HIV-positive MSM using incident HCV cases infected between 1994-2012 with a Bayesian statistical framework.

Methods: Of 64 HIV-positive MSM from the MOSAIC study the E2 fragment, including the HVR1 region, was sequenced from the first RNA positive sample and each follow up year per patient. Changes in the effective population size (Ne) through time were inferred with a Bayesian framework as implemented in BEAST v1.7.4. We employed the HKY+G+I evolutionary model and the skyline plot coalescent model under a relaxed uncorrelated lognormal molecular clock.

Results: At the first RNA positive time point 44 MSM were infected with genotype 1a, 4 MSM with 1b, 2 with 3a and 18 with 4d. Because of the small number of infections with genotypes 1b and 3a, the Ne could not be modeled for these genotypes. The skyline plot analyses of genotype 1a shows an exponential growth from 2002 onwards, peaking in 2008 in which the Ne increased with a 3-fold and after a slight decline, the epidemic continued with a constant size till 2012. Between 2001-2002, the Ne of genotype 4d increased exponentially with a 4-fold in size, from 2002-2008 the size was constant and from 2008 till 2012 a slight decline in the Ne is observed.

Conclusions: Our findings indicate that the HCV epidemic among HIV-positive MSM is not expanding currently, on contrary a slight decline was observed, which is in concordance with the data from the STI clinics. In addition, distinct genotypes follow a different epidemic course.
FROM IN VITRO TO IN VIVO QUANTIFICATION OF ANTIRETROVIRAL DRUGS EFFECTS BASED ON DYNAMICAL MODELS OF HIV.

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Population dynamics of HIV and CD4+ T cells can be modeled with Ordinary Differential Equations (ODE). We aim at quantifying the in vivo effect of combinations of antiretroviral drugs treatment (cARTs) by a function of the effects of the antiretroviral drugs (ARVs) in the combination. To estimate the ARVs effects we must have a large dataset and it is desirable to add external information to ensure identifiability. An adequate modeling of in vitro assays yields such information.

Recent single-round infectivity assays allowed quantifying the dose-response curves in vitro (Shen et al., Nat. Med., 2008): the instantaneous inhibitory potential (IIP) has been established as a measure of ARVs activity. The IIP of cARTs can be viewed as a function of ARV’s IIP based on known interactions (Jilek et al., Nat. Med., 2012). Bliss independence is a convenient assumption to build dynamical models. Random effects account for inter-individual variability of IIPs that may result from host and virus genomics (Sampah et al., PNAS, 2011). Finally, more flexibility is provided by estimating an in vitro to in vivo conversion factor. We used a Bayesian techniques for estimating the ARVs effects: cARTs effects follow by computation.

This approach is applied to a dataset of 350 patients from four clinical trials (ALBI, PUZZLE, PREDIZISTA, ZEPHIR). As a start, only the eight main ARVs in these trials are considered (AZT, 3TC, D4T, DDI, RTV, LPV, APV and DRV). First analysis show that we may rank cARTs in agreement with previously published studies results. We propose an extension of our method to the analysis of the effect of cARTs in HIV-1 infected patients followed in the Aquitaine cohort. Our approach opens the perspective of individualizing treatment, a step toward “personalized medicine”.

SIMULTANEOUSLY ESTIMATING VIRAL EVOLUTIONARY HISTORY AND PHYLOGENETIC TRAIT SIGNAL

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Phylogenetic signal quantifies the degree to which continuously valued traits resemble each other more for closely related species than for phylogenetically distant species. This measure has been of considerable interest in a wide range of ecological and evolutionary research areas, and it recently also gained traction in viral evolutionary studies. Here, we develop a novel inference method to simultaneously estimate evolutionary history and phylogenetic signal for multivariate traits. Our approach builds upon continuous diffusion models and represents a Bayesian estimator of Pagel's lambda parameter. We assume that continuous traits evolve according to a Brownian motion process and estimate the degree to which the underlying tree needs to be transformed to reflect the statistical dependence among traits. Our approach is implemented in BEAST and integrates over phylogenetic uncertainty as well as uncertainty in the trait evolutionary process. Using simulation analyses we demonstrate that the Bayesian estimator of phylogenetic signal outperforms the maximum likelihood estimator of Pagel's lambda as well as Blomberg's K estimator in terms of both bias and accuracy. We further illustrate the Bayesian estimator’s use by revisiting several recent examples of viral evolutionary trait analyses, including the 'heritability' of HIV infection traits.
Replication of Human Immunodeficiency Virus (HIV) genome is one of the most important steps in the infection process, and has been an important stage for drug targeting in anti-retroviral therapies. Reverse Transcriptase, encoded by HIV *pol* gene, has been an important drug target against HIV for last two decades. However, a major challenge has been the emergence of resistance owing to mutations in RT, thereby rendering these drugs useless. Unraveling the mechanisms of inhibition and resistance is an important area of research that contributes to both basic understanding of the processes and clinical applications. In this work we have attempted to study the structural basis of the mechanisms of inhibition and resistance in Reverse Transcriptase in HIV-1 using the network modeling approach of the “Protein Contact Networks” (PCN)

The PCNs of HIV-1 RT in inhibitor-bound and unbound states were developed in both coarse grained scale, and by considering the side chains of the constituent amino acids. The contact patterns for all cases were analysed along with their network parameters. Though inhibitor binding and resistance mutations in RT cause significant functional changes, they are known to cause only subtle changes in the protein structures. Our results show that they can be easily identified by changes in the contact patterns of the active site and inhibitor binding site residues. Analysis of different network parameters (e.g., shortest paths, cliques and communities) provide an insight into the communication pathways between the inhibitor binding region and the active site. Study of the allosteric communication pathways, considering the dynamics of the proteins shows that small conformational fluctuations cause important changes in the communication pathways, thus contributing to the overall effect of inhibition and resistance. These analyses can help in understanding the crucial residues involved, directly or indirectly in the mechanism of inhibition and evolution of resistance to drugs.

A typical HIV infection trajectory consists of three stages: an initial acute infection, a long asymptomatic period and a final increase in viral load with simultaneous loss in healthy CD4+T cell counts. What causes CD4+T cell loss and viral explosion is not known. A mathematical model may reveal the key mechanisms in HIV infection. However, the majority of existing mathematical models provide a good representation of either the first two stages or the last stage of the infection. Using macrophages as a long-term active reservoir, a deterministic model is used to explain the three stages of the infection including the progression to AIDS. Simulation results suggest that HIV dynamics might be divided in two coupled feedback paths. One path provides the fast dynamics presented in the early stages of infection as a result of a fast infection of CD4+T cells. The second feedback path sustains a slow but constant process of infection in macrophages. In this way, infected macrophages induce growth in the viral load in the last stages of the infection, and therefore drive the slow depletion of CD4+T cells.

In this work the macrophage population is essential for explaining the progression to AIDS. Nonetheless, other classes of reservoirs could be responsible. The progression to AIDS in HIV infection is still an open problem for discussion in clinical circles, where further examination of macrophages may either confirm or falsify the hypothesis presented with this model.
In spite of HAART, that can control HIV infection, the immune system of many patients does not restore correctly. Treatments based on interleukins may help the restoring process. Interleukin 7 (IL7) is a cytokine playing a key role in thymopoiesis, lymphocytic peripheral homeostasis and survival of CD4 and CD8 T cells. In the INSPIRE study, 126 patients were injected an artificial form of IL-7 (CYT107), in the hope to obtain an increase of CD4 T cells. We consider a system of non-linear differential equations for describing the dynamics of the populations of CD4 cells, distinguishing ki67- and ki67+ (being considered as non-proliferating and proliferating cells, respectively). The parameters of the model are biological parameters, such as the production, proliferation, and mortality rates. The model includes a feedback effect on the proliferation rate. We consider that IL7 can modify one or several of these biological parameters. Besides, some biological parameters in this mathematical model are modeled as the sum of fixed and random effects. All these parameters are estimated using maximum penalized likelihood, which can also be viewed as a Bayesian approach. This is possible by using a special program called NIMROD. After treatment of IL7, an increase of the CD4 T cells is observed and this can be relatively well fitted by our models.
T-CELL TURNOVER IN HIV-1 INFECTED PATIENTS ON ANTIRETROVIRAL THERAPY: DIFFERENCE BETWEEN IMMUNOLOGICAL RESPONDERS AND NON-RESPONDERS

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We have shown that in untreated chronic HIV-1 infection, the turnover of naive and memory CD4+ and CD8+ T cells is at least three-fold faster than in healthy individuals. Here, we investigated to what extent this increased T-cell turnover normalizes in patients whose virus is successfully suppressed on HAART, and distinguished between immunological responders (CD4+ count >500 cells/µL while at least one year on HAART) and non-responders (CD4+ count <350 cells/µL while at least two years on HAART, or CD4+ count <200 cells/µL while at least one year on HAART).

T-cell turnover rates were quantified by in vivo stable-isotope labeling. Patients drank deuterated water for 9 weeks, during and after which blood was withdrawn at 10 time points to determine label incorporation in naive and memory CD4+ and CD8+ T cells using GC/MS analysis. Turnover rates were estimated by fitting a mathematical model that accounts for kinetic heterogeneity within cell subsets to the resulting label enrichment data.

Despite successful viral suppression and reconstitution of CD4+ T-cell counts, the turnover rates of naive CD4+ and CD8+ T-cells in immunological responders were still increased compared to healthy individuals. In contrast, turnover rates of memory CD4+ and CD8+ T cells tended to normalize. Strikingly, in immunological non-responders, the turnover of all T-cell subsets was even faster than in untreated HIV-infected patients even though the virus was successfully suppressed. Only in immunological non-responders who received treatment intensification with a CCR5-inhibitor drug, the turnover rates of naive and memory CD4+ and CD8+ T cells became comparable to those in immunological responders.

Thus, successful immunological and virological response to antiretroviral therapy is associated with normalization of turnover rates of memory T-cells. By decreasing of naive T-cell turnover rates, CCR5-inhibitor intensification treatment for immunological non-responders may have beneficial effects in the long run.
HIV type 1 enters CD4+ cells using both CD4 and a chemokine co-receptor: CCR5 or CXCR4. HIV-1 is classed as either exclusively R5- or non-R5 depending on co-receptor usage. Determining tropism of HIV-1 is required before administration of CCR5-antagonists, which inhibit R5 HIV-1. Non-R5 virus is naturally insensitive to CCR5 antagonists. Currently genotypic and phenotypic methods are used to screen for tropism. A new and novel genotypic tropism prediction algorithm for HIV-1 subtype B, TroGen, is described. Based on the V3 loop sequence of the envelope gene and its structure, it classifies viral sequences as R5 or non-R5. The TroGen algorithm, together with the charge rule, PSSM and SVM algorithms, the methods most commonly applied to tropism prediction, were used in the evaluation, firstly using the phenotypic RLU measurements for clonal data from 20 patients, then the clinical outcomes at 8 weeks of 317 patients enrolled in maraviroc (MVC) studies MOTIVATE -1, -2 and A4001029. The baseline virus from these patients was classified by the three algorithms using the population sequence data and then tested to see whether this genotypic determination could be used to predict poor response (no suppression of viral load following eight weeks of MVC therapy). The TroGen algorithm was further refined by integrating an SVM predictor.

In classifying sequences relative to phenotypic RLU measurements, TroGen had sensitivity and specificity of 0.86/0.96, compared to 0.81/0.95 and 0.86/0.87 for WebPSSM and Geno2Pheno, respectively. Analysis of population sequence data for 317 patients called 230 patients as R5-tropic with a 2.3 median log reduction of RNA, and 74 as non-R5 with a 1.2 median log reduction of RNA. By combining TroGen with SVM, we achieved highly significant prediction of therapy outcome (no week-8 suppression: 46/323, 14.2%; p<0.001, Wilcoxon test), with sensitivity and specificity of 0.83/0.33.
PREDICTING OUTCOMES OF TREATMENTS TO ERADICATE THE HIV LATENT RESERVOIR

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Background

Latency reversing agents are investigational drugs that may specifically target cells with latent HIV infection for rapid activation or death, potentially reducing the size of the latent reservoir. This class includes the HDAC inhibitor vorinostat, the alcoholism drug disulfiram, the PKC activator prostratin, and quinoline derivatives. The hope is that administration of these drugs will permit patients to discontinue HAART with minimal risk of virologic rebound. Yet it is unclear how drug efficacy in vitro will translate to clinical outcomes. Mathematical models are needed to guide clinical trial design and interpret outcomes.

Methods

We use a stochastic model to investigate the dynamics of the latent reservoir and plasma viral load following administration of latency reversing agents. Parameters are calibrated based on clinical measurements and in vitro characterization of latent reservoir composition. We account for interpatient variation in these parameters and a range of potential drug efficacies.

Results

The model predicts patient outcomes measured as the time until virologic rebound following cessation of co-administered HAART and a latency reversing agent. We find that drugs which only decrease the latent reservoir by one or two orders of magnitude are unlikely to increase the rebound time by more than a few weeks, while four orders of magnitude are likely needed to delay rebound substantially or prevent it altogether. Analysis of the model reveals a critical threshold drug efficacy beyond which rebound time dramatically increases, owing to increasingly rare reactivation events. Interpatient variation in rebound time may be years, suggesting that extensive follow up and large trial populations will likely be needed to evaluate clinical efficacy of therapy with latency reversing agents.
INTEGRATING GENEALOGICAL AND DYNAMICAL MODELLING TO INFERENCE ESCAPE AND REVERSION RATES IN HIV EPITOPES

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The rates of escape and reversion in response to selection pressure arising from the host immune system, notably the cytotoxic T-lymphocyte (CTL) response, are key factors determining the evolution of HIV. Existing methods for estimating these parameters from cross-sectional population data using ordinary differential equations (ODE) ignore information about the genealogy of sampled HIV sequences, which has the potential to cause systematic bias and over-estimate certainty. Here, we describe an integrated approach, validated through extensive simulations, which combines genealogical inference and epidemiological modelling, to estimate rates of CTL escape and reversion in HIV epitopes.

We show that there is substantial uncertainty about rates of viral escape and reversion from cross-sectional data, which arises from the inherent stochasticity in the evolutionary process. By application to empirical data, we find that point estimates of rates from a previously published ODE model and the integrated approach presented here are often similar, but can also differ several-fold depending on the structure of the genealogy. The model-based approach we apply provides a framework for the statistical analysis of escape and reversion in population data and highlights the need for longitudinal and denser cross-sectional sampling to enable accurate estimate of these key parameters.

NON-B HIV TRANSMISSION NETWORKS IN THE UK

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Subtype B HIV transmission networks among men who have sex with men (MSM) in the UK have been suggested to grow through a process of preferential attachment. The degree distribution of these networks is best fitted by a Waring distribution. Non-B subtypes circulate mainly among heterosexuals (HET) and so we examined whether non-B HIV networks showed the same behaviour.

Maximum likelihood phylogenies were reconstructed separately for 10830 subtype C and 2083 subtype A pol sequences from the UK HIV Drug Resistance Database. Phylogenetic clusters supported by bootstraps ≥90% and genetic distances ≤4.5% were identified in the two trees. Clusters were sorted into one of four risk groups following a hierarchical classification procedure: injection drug users (IDU), cross-over, MSM or HET. Clusters were then time-resolved in BEAST under an SIR model in order to reconstruct networks representing possible HIV transmissions. Nodes in the network are linked together if sequences share a common ancestor within a certain amount of time in the time-resolved trees. Networks were thus inferred at different depths for each risk group and Pareto, negative binomial, Poisson log normal, Yule and Waring distributions were fitted to the network degree distributions each time.

160 subtype A and 647 subtype C clusters were identified although over 75% were pairs. 89/108 (82.4%) clusters with an assigned risk group were HET in subtype A, as were 486/525 (92.6%) in subtype C. 19 subtype A, and 39 subtype C clusters were classed as MSM, IDU or cross-over. On average, 90% of clustered sequences shared a common ancestor with at least one other sequence at a network depth of 5 years. Preliminary results suggest that in contrast to MSM transmission of subtype B HIV, heterosexual transmission of non-B subtypes may follow a negative binomial distribution, suggesting acquisition of partners at a constant rate.
GENERALIZED DYNAMICS OF DRUG RESISTANCE IN SUSCEPTIBLE-INFECTED-MULTIPLE TREATMENT MODELS

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While “drug resistance” is increasingly seen as a ubiquitous dynamic, it is difficult to find abstractions that usefully capture commonalities across all the varied (viral, bacterial, other pathogens, cancer) scenarios within which it arises. Here we focus on two: the speed of the pathogen's evolution relative to our species' ability to develop new drugs; and the effect of combination vs. mono-therapies. We use HIV as our canonical example. We build from a traditional SIR (susceptible / infected / recovered) differential equation model: The world population begins in a susceptible state $S$. Those infected by the virus $I$ die at a rate $v$ more virulent than baseline. Historical data for global, human population-wide statistics regarding HIV drug resistance are used to calibrate these key parameters. For HIV, the SIR's "recovered" state must be replaced with a "treated" state $T_{1}$ reflecting those infected that have been treated with some drug $D_{1}$, modulated by the number of treatments available $N$ and the effectiveness of these treatments. As we are centrally concerned with drug resistance, a fourth state $I_{1}$ reflects those treated by $D_{1}$ that develop resistance to it; they return to the more virulent death rate and become another source of infection for susceptibles. The subscripts on $T_{i}$ and $I_{i}$ anticipate the indexed case, in which we consider sets of treatments with each treatment $T_{i}$ giving rise to its own resistant subtype $I_{i}$. We call this a Susceptible-Infected-Multiple Treatment (SIMT) model. A resource allocation question arises, requiring a "strategy" to specify the distribution of total available treatments $N$ across each of the drugs $N_{i}$. We subject SIMT models to hypothetical drug treatment strategies, involving increasing drug supplies, innovation of new drugs, and potential interactions among the drugs, to analyze their effect on resulting infection rates. Across a broad range of parameter values, several distinctive patterns of drug resistance emergence are demonstrated.

PREDICTING THE IMPACT OF CD8+ T CELL DYSFUNCTIONALITY ON HIV DISEASE PROGRESSION

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CD8+ T lymphocytes mediate several different effector functions to fight viral infections and are essential to control infection by human immunodeficiency virus type 1 (HIV-1). Several studies showed that the quality of the CD8+ T cell response during the chronic phase of the infection correlates with disease progression. Individuals who rapidly progress to AIDS have less polyfunctional HIV-specific CD8+ T cells than non-progressors, while non-HIV related CD8+ T cells do not differ in their quality. However, it has not been determined yet if this dysfunctionality explains differences in the CD4+ T cell count and plasma viral load of HIV-progressors and non-progressors, and to which extent cytolytic and non-cytolytic effector functions of CD8+ T cells contribute to viral control. We use a mathematical model to study the influence of CD8+ T cell effector functions, such as (i) cytotoxicity, (ii) the impairment of viral replication, and (iii) the inhibition of viral entry to target cells, on the CD4+ T cell count and plasma viral load. Using in vitro experimental data on the efficacy of IFN-? and MIP-1β/RANTES against HIV to parameterize our model, we study how the polyfunctionality and the functional diversity of the CD8+ T cell response influence disease progression. Model predictions are compared to clinical data for HIV infection. We show that the dysfunctional CD8+ T cell response that is observed in HIV-progressors explains the low CD4+ T cell count in these individuals. Our model reveals that non-cytolytic effector functions contribute more to viral control than cytotoxicity. Thus, increasing and maintaining non-cytolytic effector functions in HIV-infected individuals by immuno-modulatory interventions shows the greatest promise in improving disease outcome.
LACK OF X4-TROPIC HIV PREVENTS VIRAL REBOUND POST CCR5-?-32 STEM CELL TRANSPLANTATION IN THE “BERLIN PATIENT”

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Background: The “Berlin patient” is the first patient functionally cured of HIV. He received stem cell transplantation (SCT) from a homozygote CCR5-?-32 donor as treatment for myeloid leukemia. These cells are resistant to infection with HIV variants utilizing CCR5 as co-receptor but are susceptible to CXCR4-using viruses. According to gp120-V3 deep-sequencing analysis, the patient harbored a minority (2.9%) of CXCR4-predicted viruses (geno2phenocoreceptor FPR 10%). It remains puzzling why these HIV variants did not rebound after transplantation and HAART discontinuation. We hypothesize that these CXCR4-predicted variants depend on CCR5 for replication. Methods: Patient-derived viral constructs were generated by cloning V3-sequences of the CXCR4-predicted viruses (pX1-pX7) and the dominant CCR5-predicted strain (pR5) into HXB2-?-V3. As controls V3-sequences of HXB2 (cHXB2; CXCR4-tropic) and BaL (cBaL; CCR5-tropic) were cloned. Co-receptor preference was investigated in U-373-MAGI cells expressing CD4+CCR5+ or CD4+CXCR4+, PBMCs from healthy donors and patient-derived post transplant CCR5-?-32 PBMCs. Results: One CXCR4-predicted strain had an amino acid substitution at position 25 (pX2) a glutamic acid for a lysine strongly associated with CXCR4-tropism. cHXB2 infected CD4+CXCR4+ MAGI-cells only and was fully inhibited by AMD-3100 (CXCR4-inhibitor) in donor PBMCs. Remarkably, the CXCR4-predicted viruses (pX1-pX5, FPR 2.7-9.3) depended on CCR5 for replication in MAGI-cells and were inhibited by Maraviroc (CCR5-inhibitor) in donor PBMCs similar to pR5 and cBaL. Furthermore, it was shown that the CXCR4-predicted strains pX2-pX5 could not replicate in the post transplant derived CCR5-?-32 PBMCs, whereas cHXB2 replication was observed. Conclusion: This study demonstrates that the minority population of CXCR4-predicted viral strains is fully dependent on CCR5 for replication in vitro, which could explain lack of rebound after discontinuation of HAART in the “Berlin patient”. This provides a strong rationale for the further development of CCR5-targeted gene therapy even in patients in which deep-sequencing + geno2phenocoreceptor predicts virus with an FPR >2.9 and <10.
CO-RECEPTOR AND PROLIFERATION MARKER EXPRESSION AND CHARACTERIZATION OF THE VIRAL RESERVOIR IN CD4+ T-CELLS AFTER LONG-TERM THERAPY

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Background: Compartmentalization of HIV occurs such that viral DNA is found in naïve cells, central memory cells and effector memory cell populations. We characterized co-receptor expression and proliferation markers in CD4 and CD8 cells as well as viral DNA in cellular subpopulations of patients undergoing long-term HAART therapy. Methods: Three patients who had plasma viral load <50 copies/mL during a median of 8 years of HAART donated a large volume of blood. Expression levels immunological markers Ki67, PD1, CD57 and co-receptors CCR5 and CXCR4 were measured by FACS. To characterize the viral reservoir, 1.5x10^8 PBMCs were sorted into CD4+ T-cell compartments: naïve CD27+CD45RO- (N); central memory CD27+CD45RO+ (CM), effector memory CD27-CD45RO+ (EM). Total viral-DNA was amplified and HIV V3 and RT regions were deep sequenced. Tropism was determined using g2p, FPR cut-off 3.5%. Results: Expression levels of Ki67 and PD1 increased in CM and in EM compared to CD4+ and CD8+ N T-cells, suggesting that EM cells proliferate at the highest rate. The levels of Ki67 and PD1 remained constant despite long-term successful HAART. The expression of CCR5 increased in further differentiated T-cells while CXCR4 levels decreased. Despite higher levels of CXCR4 than CCR5 expression in N and CM cells after long term suppression of viremia, HIV-DNA was predicted to be 100% R5 in all assessed compartments. In samples where input copy number was >10 molecules, identical V3 amino acid sequences was detected as the predominant species in CM compared to EM compartments within each patient. Conclusion: Proliferation and immunesenescence remained similar after 8 years of successful HAART with EM T-cells proliferating at the highest rate demonstrating ongoing immune activation. Despite higher levels of CXCR4 expression compared to CCR5 expression in the CD4+ N and CM T-cell compartments the tropism of the viral reservoirs was predicted as R5.
IS HIV SHORT-SIGHTED?

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It is remarkable that HIV appears to have evolved a level of virulence that maximises its transmission potential, thus maximising the between-host fitness of the virus. With a higher level of virulence the virus is more likely to be transmitted while the infection lasts, but death due to AIDS will be swifter, resulting in fewer onward infections. With a lower level of virulence the host will live longer, but the rate of onward transmission will be lowered, again reducing the number of onward infections. However, this observation sits uncomfortably with the concept of “short-sighted” evolution. During the course of long-term infections we should expect strains with a competitive advantage to sweep through the within-host population if and when they arise, regardless of whether this reduces the transmission potential of the current or subsequent infections. Evolution is ‘short-sighted’ because what is good for the virus in the short-term within the host is not necessarily what is good for the virus in the longer-term at the epidemiological level. We have developed a nested modelling approach allowing us to follow the evolution of pathogens at the epidemiological level by explicitly considering within-host evolutionary dynamics of multiple competing strains and the timing of transmission. We use the framework to investigate the impact of short-sighted within-host evolution on the evolution of virulence of HIV, and find that the topology of within-host adaptive landscape determines how virulence evolves at the epidemiological level. If viral replication rates increase significantly during the course of infection, the viral population will evolve a high level of virulence even though this will reduce the transmission potential of the virus. However, if replication rates increase more modestly, as data suggests, our model predicts that HIV virulence will be only marginally higher than the level that maximizes the transmission potential of the virus.

WITHIN-HOST AND BETWEEN-HOST EVOLUTIONARY RATES ACROSS THE HIV-1 GENOME

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HIV evolves rapidly at the epidemiological level but also at the within-host level. The virus’ within-host evolutionary rates have been argued to be much higher than its between-host evolutionary rates. However, this conclusion relies on analyses of a short portion of the virus envelope gene. Here, we study in detail these evolutionary rates across the HIV genome. We build phylogenies using a relaxed molecular clock assumption to estimate evolutionary rates in different regions of the HIV genome. We find that these rates vary strongly across the HIV genome, with higher rates in the envelope gene (env). Within-host evolutionary rates are consistently higher than between-host rate throughout the HIV genome. This difference is significantly more pronounced in env. Finally, we find only weak differences between overlapping and non-overlapping regions. These results provide a genome-wide overview of the differences in the HIV rates of molecular evolution at the within- and between-host levels. Contrary to hepatitis C virus, where this difference is only located in the envelope gene, within-host evolutionary rates are higher than between-host evolutionary rates across the whole HIV genome. This supports the hypothesis that HIV strains that are less adapted to the host have an advantage during transmission. The most likely mechanism for this is storage and then preferential transmission of viruses in latent T-cells. These results shed a new light on the role of the transmission bottleneck in the evolutionary dynamics of HIV.
Several studies have recently provided evidence that set point HIV load in donor and recipients are correlated arguing that virus load is at least partially under the genetic control of the virus (1-4). The observed high heritability of viral load, however, is difficult to reconcile with the following two relatively uncontroversial observations. First, set point virus load is relatively stable within a patient over prolonged periods during asymptomatic infection, but varies over several orders of magnitude crosssectionally between patients (5). Second, viral evolution within an infected patient is rapid as is evidenced for example by the fast rates of immune escape, the rapid evolution of resistance and the rapid accumulation of genetic diversity over the course of an infection (6). Thus, if we accept that virus load is heritable then the question arises as to how virus load can be stable over prolonged periods of time in a system that is known to have the capacity of rapid evolution. In other words, is it conceivable that virus load is a trait which is in part under the genetic control of the virus but that those factors that influence virus load are not linked to intra-host competitive ability? In an earlier paper some of us have argued that differences in virus load may arise through differences in target cell activation rates (5). Bartha et al (7) have argued that target cell activation rate may be under the partial control of the virus, and if so, that the factors responsible for target cell activation would be expected to evolve neutrally within the host. We present models of within and between host evolution in order to explore to what extent a hypothetical viral factor controlling target cell activation is compatible with the observed within patient stability, large cross-sectional variation and high heritability of virus load.

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THE PARADOX OF HIV-1 ADAPTATION TO NK-CELL-MEDIATED IMMUNE PRESSURE

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Recently, Alter et al (2011 - Nature 476:96) showed that NK cells exert selection pressure on HIV-1. They identified 22 positions in the HIV-1 genome at which amino-acid polymorphisms were significantly associated with the presence of a specific inhibitory or activating KIR gene and focused on 4 polymorphisms associated with KIR2DL2, an inhibitory NK receptor. A further in vitro investigation showed that higher viral replication was found in KIR2DL2+ donors infected with the variant polymorphism (V) compared to infection with WT virus. Alter et al suggested this might be due to an enhanced signalling through the inhibitory KIR2DL2-receptor in V infection and thus to an increased inhibition of NK cells towards V-infected cells. However, based on our understanding of NK cell function, the enrichment of the variant polymorphism in the KIR2DL2+ population, reported to be between 68-85%, is higher than expected. Inhibitory signalling is provided through KIR-specific HLA-ligands whose genes are on different chromosomes than the KIR-genes and therefore segregate independently. As a consequence, only in a fraction of the KIR2DL2+ individuals can peptides of the variant polymorphism be presented on the matching HLA and can NK cells thus exert selection pressure on the virus. Using mathematical tools we predicted this fraction and investigated if it is sufficient to predict the enrichment described in the Alter-study. We conclude that current knowledge about NK cell function is not sufficient to explain the high enrichment of variant polymorphism in KIR2DL2+ individuals presented by Alter et al and point out directions for further investigation.

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PRE-EXISTENCE AND EMERGENCE OF VIRAL DRUG RESISTANCE

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Highly effective antiviral drugs have been successful in reducing the burden of HIV, but emergence of drug resistance can lead to treatment failure. Although alleviated by combination therapy, resistance remains problematic for patients with poor access to drugs or low adherence. It is also an important consideration in designing novel treatments, including those for other viral diseases such as Hepatitis C. Understanding the source of resistance – whether from mutations occurring before treatment begins (pre-existing) or during treatment – thus remains an important question from both clinical and basic evolutionary perspectives. The limits of empirical approaches in detecting low-frequency variants have created a key role for mathematical models. Here we revisit the topic of pre-existence and emergence, with two novel questions. (1) How general are earlier conclusions, which attribute resistance predominantly to pre-existing mutations? In particular, are results sensitive to viral replication cycle and mode of drug action? This has implications for transferring our understanding to new scenarios, including new drug classes and other viruses. (2) What is the role of stochasticity, and does it affect the relative contribution of pre-existence? To address these questions, we extend the standard two-strain viral dynamics model to a more general replication cycle, and develop highly accurate analytical approximations to the stochastic dynamics. We find that details of the replication cycle, particularly the relationship between the most mutagenic step and the point of drug action, matter in several ways. The roles of parameters such as cost of resistance and infected cell lifetime can be more complex than found previously, but we elucidate a dual disadvantage of cost to pre-existing mutations. Finally, we see that taking into account stochastic loss of rare resistant mutants predicts a greater role for de novo mutations during treatment than previously suggested.
JOINT ASSOCIATION ANALYSIS OF GENOME-WIDE HUMAN AND HIV-1 VARIATION

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Background: Joint analysis of human genetic and HIV sequence variation has been largely limited to HLA alleles and viral mutations in corresponding epitopes. We performed an unbiased genome-wide survey of associations between human SNPs and variation across the HIV proteome. We hypothesized that this non-a priori genome-to-genome analysis would identify and map all host selective pressures on the viral genome.

Methods: Human genome-wide genotyping and HIV-1 full-length sequencing data were available for the study. Binary variables were created for each variable amino-acid positions, for every amino acid that was present in at least 3 HIV genomes. Human SNP imputation was performed using 1000 Genomes data as reference. Associations between all SNPs and HIV-1 amino acids were tested by logistic regression under an additive genetic model. For each amino acid that had genome-wide significant association, we searched for independently associated SNPs by iteratively conditioning on the most significant SNP.

Result: A total of 1071 patients of European ancestry from 7 cohorts and 5 countries were included in the study. After imputation, 6,889,656 SNPs were tested for association with more than 4000 different HIV residues. Highly significant associations (p < 1E-11) were observed between SNPs in the Major Histocompatibility Complex (MHC) and multiple amino acids in several HIV-1 proteins. SNPs tagging HLA class I alleles strongly associated with viral variation in CTL epitopes targeted by the corresponding alleles. No significant signals were identified outside the MHC.

Conclusion: A non a priori genome-to-genome approach maps associations between host SNPs and HIV genomic variation. We confirm the extensive evolutionary effect that MHC exerts on HIV Gag, Pol and Nef and the lack of common variants with strong effects elsewhere in the host genome on within-host viral evolution.
DIMINISHED TRANSMISSION OF DRUG RESISTANT HIV-1 VARIANTS WITH REDUCED REPLICATIVE CAPACITY IN A HUMAN TRANSMISSION MODEL

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Different patterns of drug resistance are observed in treated and therapy naïve HIV-1 infected populations. Especially the NRTI-related M184I/V variants, which are among the most frequently encountered mutations in treated patients, are underrepresented in the antiretroviral naïve population. M184I/V mutations are known to have a profound effect on viral replication and tend to revert over time in the new host. However it is debated whether a diminished transmission efficacy of HIV variants with a reduced replication capacity can also contribute to the observed discrepancy in genotypic patterns.

As dendritic cells (DCs) play a pivotal role in HIV-1 transmission, we used a model containing primary human Langerhans cells (LCs) and DCs to compare the transmission efficacy M184 variants (HIV-M184V/I/T) to HIV wild type (HIV-WT). As control, we used HIV harboring the NNRTI mutation K103N (HIV-K103N) which has a minor effect on replication and is found at a similar prevalence in treated and untreated patients.

In comparison to HIV-WT, the HIV-M184 variants were less efficiently transmitted to CCR5+ Jurkat T cells by both LCs and DCs. The transmission rate of HIV-K103N was slightly reduced to HIV-WT in LCs and even higher than HIV-WT in DC. Replication experiments in CCR5+ Jurkat T cells revealed no apparent differences in replicative capacity between the mutant viruses and HIV-WT. However, the infection rate of LCs and DCs was in concordance with the transmission results; infection by HIV-M184 variants was lower than infection by HIV-WT, and the level of infection by HIV-K103N was intermediate for LCs and higher than HIV-WT for DCs.

Our data demonstrate that drug resistant M184-variants display a reduced replicative capacity in LCs and DCs which directly impairs their transmission efficacy. As such, diminished transmission efficacy contributes to the lower prevalence of drug resistant variants in therapy naïve patients.
IDENTIFICATION OF SOURCES OF HIV-1 TRANSMITTED DRUG RESISTANCE USING PHYLOGENETICS

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Background: Phylogenetic analysis allows characterization of the spread and potential sources of transmitted drug resistance (TDR) mutations. The Swiss HIV Cohort Study (SHCS) and associated drug resistance database (DRDB) provide a highly representative study population for analyzing patterns of TDR.

Methods: ART-naïve MSMs with estimated infection dates between 2001 and 2007 were chosen as a surveillance population for detecting TDR in HIV-1 subtype B (n=852). We focused on this group as the SHCS-DRDB contains 73% of all MSM diagnosed between these dates. To find sources of TDR, pol-sequences from these patients were pooled with all subtype-B sequences from the SHCS-DRDB (9213 sequences) and a phylogeny was inferred. Well-supported clusters containing TDR were examined for potential sources of TDR (sequences clustering with and sharing ≥1 resistance mutation with a TDR sequence).

Results: We found that 67/852 (7.9%) surveillance patients carried TDR mutations and 38 (56.7%) of these were assigned to clusters. Potential sources of TDR were found in 33 cases (87%). Most (89.5%) potential sources were ART-naïve. 28/33 patients were in clusters with more than one surveillance TDR sequence (range 1-7). Some clusters showed evidence of reversion, which may obscure detection of drug-resistance transmission. We also found a trend toward transmission earlier in infection for transmitters of TDR (mean 347 vs. 498 days; p-value: 0.059) versus transmitters of sensitive virus. By contrast, we did not find overrepresentation of primary infections among potential TDR sources.

Conclusions: More than half the analyzed TDR individuals belonged to Swiss transmission clusters, implying a large role for domestic transmission for subtype-B TDR in Switzerland. Most surveillance TDR in clusters could be linked to a source, implying that near complete surveillance of domestic TDR transmission is possible. ART-naïve patients composed most transmitters of TDR. Along with the presence of long TDR transmission chains, this suggests that, once established in a treated patient, resistance mutations are frequently transmitted among untreated individuals.
Background
We aimed to get insight in the poorly understood HIV-1 transmission dynamics in Curaçao, a Caribbean island within the Kingdom of the Netherlands.

Methods
As of June 2011 the ATHENA database contained polymerase sequences from 5852 subtype B infected patients in the Netherlands of whom 219 were in care in Curaçao. A selection was made of the 10 most similar sequences to every Curaçao sequence within the Netherlands and of the 10 most similar sequences available online at Genbank. Of the sequence selection a phylogenetic tree was built in Fasttree. Significant clusters with ≥5 Curaçao sequences were studied in a dated phylogeny obtained using the BEAST package.

Results
Seven transmission clusters were identified encompassing 96 (44%) of sequences from patients in Curaçao and 102 sequences from patients in the Netherlands of whom 53 (52%) were born in the former Dutch Antilles. All 7 clusters were a mixture of sequences from both the Netherlands and Curaçao, and from both heterosexual men and women and men having sex with men, but ratios differ significantly. Five clusters were dated between 1985-1990. Two clusters were introduced in the early 1990s, one of which was embedded in a cluster of sequences from Honduras, with a possible intermediate link via the Netherlands. The other 123 sequences pertained 79 clusters, 32 with sequences from patients in the Netherlands.

Conclusion
Three conclusions can be drawn from this study: 1) the transmission links found between the Netherlands and Curaçao show that the epidemic in Curaçao should not be studied as an isolated epidemic, 2) the clustering of sequences in the phylogenetic tree indicate that it took about 86 introductions of the virus in Curaçao to establish 7 on-going transmission networks; and 3) 7 networks established on Curaçao had spread amongst all risk groups.
HIV SUPERINFECTION DOES NOT CONTRIBUTE TO TRANSMitted DRUG RESISTANCE

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Background: Superinfection can facilitate the evolution of HIV by allowing for the recombination of distinct viral lineages.

In addition, drug resistant viruses may be able to infect individuals with drug-sensitive strains who have previously responded well to therapy. Superinfection may therefore potentially contribute to the transmission of drug resistance (TDR). To investigate the importance of this potential route to TDR, we analyzed routinely collected genotyping data from a large European collaborative HIV database to estimate the frequency of superinfection and to assess its contribution to TDR.

Methods: We used sequence data from routine genotypic tests spanning the protease and partial reverse transcriptase regions in the Virolab and EuResist databases. 4653 mostly (82%) drug experienced patients (gender: male=3139, female=1361, unknown=153; riskgroup: heterosexual=1176, IDU=1072, MSM=951, other=158, unknown=1296) had at least two sequences in the database, with a total of 14222 distinct sequence entries (of which 86% belonged to subtype B). Superinfection was indicated when sequences of a patient failed to cluster together in phylogenetic trees constructed with selected sets of background sequences. Several cases of putative superinfection were then further investigated by resequencing the POL and ENV region from the original samples.

Results: We identified 109 patients with sequences clustering robustly into two distinct lineages (indicative of superinfection) in maximum likelihood phylogenies. 12 of these cases were validated by sample resequencing: 8 cases showed evidence for sample mix-up, while only 2 cases were verified as superinfections. Resistance to drugs used at the time of strain replacement did not change in these two patients.

Conclusions: Routine genotyping data are informative for the detection of HIV superinfection; however, the majority of putative superinfections may arise from sample mix-up, which emphasizes the importance of validation by re-sequencing. Superinfection was rare in our treatment experienced cohort, and we found no evidence of TDR by this route.
Background
HCV co-infections in HIV infected patients in the Netherlands are mostly found amongst intra-venous drug users and men having sex with men. However in the prospective cohort of HIV infected patients we also find heterosexual patients having tested positive for HCV. We wanted to investigate the HIV transmission networks these patients pertained using polymerase sequences available from resistance testing.

Methods
A phylogenetic tree was built in Fasttree with the first HIV-1 subtype B polymerase sequence available from 5852 persons. Phylogenetic clusters were selected using PhyloPart. All clusters with a sequence from a heterosexual HCV co-infected patient were studied.

Results
1430 HIV-1 infected patients were co-infected with HCV; 9% (142) infected heterosexually; 48% MSM; and 28% drug users. In total 488 patients have a HIV-1 subtype B polymerase sequence available; 65% MSM; 22% drug users; and 22 (5%) were infected heterosexually of whom 10 female and 50% was of Dutch origin. Other 14 heterosexuals had a non-subtype B sequence available, of whom 86% was from non-Dutch origin. In the subtype B phylogenetic tree we found the 22 heterosexual patients to pertain 10 phylogenetic clusters. Five clusters were dominated by (≥70%) MSM (n= 75;31;10;18;32), 2 by heterosexually infected patients (n= 39;5), 1 forms a mix of MSM and heterosexuals (n=36), and 2 formed a mix of MSM, drug users and heterosexuals (n= 348;9). The largest cluster of 348 sequences, pertained 143 (41%) drug users, 130 (37%) heterosexuals, and 31 (9%) MSM. In this cluster 105 (30%) of the patients was also found to have been infected with HCV; 59% drug users; 12 (15%) heterosexuals; and 13% MSM.

Conclusion
Half of the HCV co-infected heterosexually HIV-1 subtype B infected patients was found to be part of a large HIV transmission network that was dominated by drug users and heterosexual HIV infected patients.
In evolutionary ecology, "tolerance" is defined as an evolutionary response of a host population against pathogen pressure, which is characterized by the lack of pathogenesis despite high pathogen loads. In the context of immunodeficiency viruses, the best known example of tolerant hosts are sooty mangabeys that remain healthy despite high virus loads. We studied if there is variation in tolerance to HIV in humans linked to HLA genotype.

We used data from the Swiss HIV Cohort Study. For each HLA-typed individual for whom sufficient data were available (n=923), we calculated viral set-points and the rate of CD4 cell decline before treatment start. We then analyzed if the relationship between viral set-point and CD4 cell decline differs with HLA genotype. Such a difference would be indicative of variation in tolerance. Investigating the variation in relationship across HLA-B alleles goes beyond standard analysis of the effect of HLA-B alleles on the viral set-point or the rate of disease progression alone.

We found that the relationship between viral set-point, spVL, and CD4 cell decline, ΔCD4, is described by: ΔCD4=α*\log_{10}(spVL)^2, where α=0.0118±0.0004 is a measure of tolerance. Grouping individuals into those who carry protective HLA-B alleles (n=416) and those that do not (n=507), revealed that α does not differ significantly between those two groups (ANOVA, p=0.4). Furthermore, we asked if there is variation in tolerance, irrespective of the protectiveness of HLA-B alleles. Using a mixed-effects model with HLA-B genotype as a random effect, we find small but significant variation in α linked to HLA-B genotype (ANOVA, p=0.0002).

We conclude that protective HLA-B alleles do not confer partial tolerance. The deceleration of disease progression these alleles induce can be fully attributed to the extent of virus load reduction in their carriers. However, we have evidence that other, non-protective HLA-B alleles may confer partial tolerance.
Background: The global phylogeography of HIV is characterized by compartmentalized local epidemics that are typically dominated by a single subtype, which indicates strong founder effects. We hypothesized that the competition of viral strains at the epidemic level may be characterized by an advantage of the “resident” strain that was the first to colonize a population. Such an effect would slow down the invasion of new strains and thus also the diversification of the epidemic. Methods: We developed a stochastic modelling framework to simulate HIV epidemics over dynamic contact networks. We simulated epidemics in which the second strain was introduced into the population with varying time lags after the first, and assessed whether and on what time scale the second strain was able to spread in the population. Simulations were parameterized based on empirical data; we tested scenarios with varying levels of overall prevalence, and varying differences in the transmission efficiency of both strains. Results: With strains of equal transmission efficiency, the second strain was unable to invade on a time scale relevant for the history of the HIV pandemic. To become dominant over a time scale of decades, the second strain needed considerable (>10%) advantage in transmission efficiency over the resident strain. Importantly, the inhibition effect was strong even with short time lags (second strain introduced in the early exponential phase of the first). Finally, we tested how possible mechanisms of interference between the strains contributed to the inhibition effect. Conclusions: Our simulation confirmed asymmetrical competition dynamics of HIV at the population level, with an advantage of the first successful strain in the population. This effect may explain the global phylogeography of the virus. The modelling framework also allows us to make predictions on the future evolution of the pandemic.
IDENTIFYING HIV-1 TRANSMISSION RISK FACTORS AND THE SOURCE OF TRANSMISSION FROM MOLECULAR DATA

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Introduction: Epidemiological dynamics leave an imprint on the molecular diversity of HIV. We have shown how mathematical epidemiological models can be used to estimate transmission rates by clinical, behavioral and demographic attributes from molecular sequence data. Here, we demonstrate how these models allow us to estimate the distribution of unobserved transmission events along each branch of a phylogeny, and hence the probability that a given patient is the source of infection for any other patient in the phylogeny.

Methods: A posterior sample of time-resolved phylogenies were obtained from 437 HIV-1 subtype B partial polsequences collected from men who have sex with men in Detroit, MI, USA. We fitted a multicompartmental model of HIV transmission to these phylogenies, which generates expressions for the probability that one individual infected another. Multivariate models were used to relate this probability to characteristics of the individuals in the potential transmission pair. We quantified non-random transmission patterns using the coefficient of assortativity, a measure of correlation between discrete traits, in transmission pairs.

Results: Identifying HIV-1 transmission pairs with phylogenetic data is subject to large error from incomplete sampling. Only 5% of patients in the sample were estimated to have an infector that was also in the sample. Nevertheless, estimated infector probabilities are useful for characterizing epidemiological transmission patterns. Being identified as an infector was highly correlated with age, AIDS diagnosis, viral load and CD4 count at the time of diagnosis. Transmission patterns were highly assortative by race (41.7%) and age (21.6%).

Conclusions: By taking a probabilistic approach, we are able to separate out true transmission pairs from clustered sequences; while this approach is not reliable at the level of individual pairs, it provides a rich source of information at the population level.
Background Progressive loss of CD4+ T-cells is the hallmark of HIV-1 infection. CD4 counts fall more rapidly in advanced disease when CCR5-tropic viral strains, which dominate early in disease, tend to be replaced by X4-tropic viruses. We hypothesized: (i) that the early dominance of CCR5-tropic viruses results from faster turnover rates of CCR5+ cells, and (ii) that X4-tropic strains exert greater pathogenicity by preferentially increasing turnover rates within the CXCR4+ compartment. Methods To test these hypotheses we measured in vivo turnover rates of CD4+ T-cell subpopulations sorted by chemokine receptor expression, using in vivo deuterium-glucose labeling. Deuterium enrichment was modeled to derive in vivo proliferation (p) and disappearance (d*) rates which were related to viral tropism data. Results 13 healthy controls and 13 treatment-naive HIV-1-infected subjects (CD4 143-569 cells/ul) participated. CCR5 expression defined a CD4+ subpopulation with accelerated in vivo proliferation rates (p=2.50 vs 1.60 %/d, CCR5+ vs CCR5-; healthy controls; P< 0.01); phenotypically, such cells were predominantly CD45R0+ memory cells. Conversely, CXCR4 expressing CD4+ T-cells (predominantly CD45RA+ naive cells) had low turnover rates, whether control or infected subjects. The dominant effect of HIV infection was accelerated turnover of CCR5+CD45R0+CD4+ memory T-cells (p=5.16 vs 2.50 %/d, HIV vs controls; P<0.05). Naïve cells were relatively unaffected. Similar patterns were observed whether the dominant circulating HIV-1 strain was R5-tropic (n=9) or X4-tropic (n=4). Although numbers were small, X4-tropic viruses did not appear to specifically drive turnover of CXCR4-expressing cells (p=0.54 vs 0.72 vs 0.44 %/d in control, R5-tropic, and X4-tropic groups respectively). Conclusions Our data are most consistent with models in which CD4+ T-cell loss is primarily driven by non-specific immune activation.
CO-EVOLUTION OF A BROADLY NEUTRALIZING HIV-1 ANTIBODY AND FOUNDER VIRUS FROM TIME OF INFECTION

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Current HIV-1 vaccines elicit strain-specific neutralizing antibodies (nAbs). Because broadly cross-reactive nAbs arise in about 20% of HIV-infected individuals, understanding conditions for nAb development would provide insight for effective vaccination strategies. Here we describe coevolution of autologous nAbs and HIV env populations in an African donor followed from acute infection established by a single founder virus. The mature antibody, CH103, neutralized ~55% of HIV-1 isolates, and its co-crystal structure with gp120 revealed a novel loop-based mechanism of CD4-binding site recognition. Virus and antibody sequencing revealed virus evolution with concomitant antibody affinity maturation. Notably, the unmutated common ancestor of the CH103 lineage avidly bound the transmitted/founder HIV-1 envelope glycoprotein, and development of antibody neutralization breadth followed extensive viral diversification among contacts in the CH103 footprint. Recurrent mutations appeared within four weeks post-infection in Loop D of the CD4 binding pocket. Positive selection was evident among CH103 contacts by week 20. A selective sweep occurred in the virus population between weeks 30 and 52 post-infection, whereby early forms of the antibody lost potency as the virus population escaped from immune selection. This reduced neutralization potency was accompanied by increased length, gain of N-linked glycosylation sites, and negative net charges in the V1 and V5 hypervariable loops. Unlike 16 other subjects followed from acute viremia over the first year of infection, neutralization breadth in CH505 is preceded by extensive Env diversity in CD4 binding-site contacts by six months post-infection. However, by one year post-infection, nearly half of 18 study subjects exhibited similar diversity in CD4 binding-site contacts. This suggests that acquisition of breadth in the neutralizing antibody response follows, rather than precedes, viral epitope diversification. These findings elucidate virus and antibody coevolution resulting in a lineage of broadly neutralizing antibodies against HIV-1, and suggest approaches for immunogens to elicit similar antibodies via vaccination.

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Neutralizing antibodies (nAbs) develop late in response to HIV infection and selection for resistant viruses can rapidly drive immune escape. However, their ability to bind diverse viral variants and prevent experimental transmission in nonhuman primate models suggests potential for broadly cross-reactive nAbs in vaccine development. To understand better the genetic basis of nAb epitope variation on tier-2 strains of HIV, we studied neutralization assays from a panel of 219 envelope pseudoviruses representing acute through chronic infection and 205 chronic sera, together representing five HIV-1 M-group subtypes (A,B,C,D,G) and three CRFs (01,02,07), plus various URFs. We analyzed neutralization titers from TZM-bl luciferase assays and Env sequences isolated from each sample, and compared neutralization profiles with monoclonal antibodies tested against the same panel of pseudoviruses. Neutralization was significantly more potent within than between clades. Viruses from early infection were more resistant to sera from other clades than from the same clade. The originating clade and duration of regional epidemic (diversity, quantified by sequence divergence) were both significantly associated with neutralization titers. Within-clade sensitivity was greatest for more recent CRF07 and CRF01 infections from China and Thailand, respectively. Virus resistance to neutralization was partially explained by lengths of the V1/V2 hypervariable loops and their net charge. Overall susceptibility of an Envelope and overall potency of a serum were highly predictive of the outcome of any given interaction. Monoclonal antibodies shared some neutralization patterns with sera, suggesting approaches to identify antibody specificities in polyclonal antisera. Clustering analyses identified a distinctive serological profile shared by 14 CRF01 infections and 2 B-clade infections from Thailand, which did not share specificity with monoclonal antibodies. These findings suggest ways to use neutralization profiles for immunogen design, e.g. vaccine antigens should favor short V1/V2 loops and have nearly neutral net charge, and to discover novel antibody specificities.

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HIV-1 EVOLUTION IN CAMEROON: 1995-2012

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Abstract

Background: HIV-1 has evolved to classifiable Groups (M, N, O and P), subtypes, circulating recombinant forms (CRF) and unique recombinant forms (URF) worldwide. The broad HIV-1 genetic variability has numerous implications on pathogenesis, diagnostics, treatment and vaccine development. In Central Africa, coincidentally where Cameroon is situated, the level of HIV-1 diversity is highest. Understanding the dynamics of the virus, as reported here would guide vaccine development, treatment strategies, clinical management, surveillance and monitoring of new viral variants and development of new diagnostic assays. Method: HIV-1 pol sequences isolated from Cameroon were downloaded from the HIV Sequence database of the Los Alamos National Library (LANL). Subtyping was performed using phylogenetic analysis (Simplot and MEGA) of 601 and 882 HIV-1 pol sequences from Group 1 (1995-2003) and Group 2 (2004-2012), respectively. Results: In Group 1, (n=601), we identified 4 (0.7%) HIV-1 Subtype B, and 597 (99.3%) non-B variants. Of these non-B variants, 235 (39.3%) were of Subtype A, 240 (40.2%) were CRF02_AG, 116 (19.4%) were non-CRF02_AG, and 6 (~1%) were URF. However, in Group 2 (n=882), 2 (0.23%) were Subtype B and 880 (99.77%) were non-B variants. Among the non-B variants, 188 (21.4%) were pure non-B variants with subtypes A, G, F and D predominating, 545 (61.9%) were CRF02_AG, 127 (14.4%) were non-CRF02_AG, 20 (2.3%) were URF and ?1% of HIV-1 Group P. Conclusion: Pol sequences were used because they are targets for first and second line antiretroviral treatment in Cameroon. Recombinant strains predominate more so after the introduction of HAART in the Treatment Programme in Cameroon in 2004. Monitoring HIV evolution is therefore important in guiding treatment strategies and choosing diagnostic assays. In addition, data on the viral genetic landscape are useful in the choice of populations and sites for vaccine efficacy trials. Full Correspondence Address for the presenting author Name: BIMELA JUDE SABER Address: P. O. Box 5141 Yaounde, Cameroon. Telephone N°: (237) 7400 1637 Email: bimelajude@yahoo.com.au
A MULTIPLE-ALIGNMENT BASED PRIMER DESIGN ALGORITHM FOR GENETICALLY HIGHLY VARIABLE DNA TARGETS

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Primer design for highly variable DNA sequences is difficult, and experimental success requires attention to many interacting constraints. The advent of next-generation sequencing methods allows the investigation of rare variants otherwise hidden deep in large populations, but requires awareness to population diversity and primer localization in relatively conserved regions, in addition to recognized constraints typically considered in primer design. Such constraints include degenerate sites to maximize population coverage, matching of melting temperatures, optimizing de novo sequence length, finding optimal bio-barcodes to allow efficient downstream analyses, and minimizing risk of dimerization. To facilitate primer design addressing these (and more) constraints, we have created a novel computer program (PrimerDesign) that automates this complex design procedure.

The overall software workflow proceeds through inter-connected steps: 1) the target locations for primers are determined, guided by sequence entropy estimates and complexity, 2) primer melting temperatures are optimized, 3) bio-barcodes and adaptors are added, and finally 4) dimerization risks are estimated. Each inter-connected step informs the subsequent steps; if, as is possible, previous steps have to be re-optimized, this occurs automatically. Thus, each step considers both user requirements and automatic parameters used within the algorithm.

PrimerDesign is useful for researchers who want to design DNA primers and probes for analyzing highly variable DNA populations. It can be used to design primers for PCR, RT-PCR, Sanger sequencing, next-generation sequencing, and other experimental protocols targeting highly variable DNA samples.

The software is platform independent and available as a web application at http://www.hiv.lanl.gov/content/sequence/HIV/HIVTools.html
It is widely recognized that cytotoxic T lymphocytes (CTLs) are a major factor in the control of HIV replication. CTLs arise early in acute infection causing escape mutations in many viral epitopes to spread rapidly through the population of infected cells. It is unknown how mutation cost and CTL-mediated selection pressure decide the rate of escape and the order in which epitopes escape. Furthermore, it is not currently known what causes the complex patterns of escape that are observed in most epitopes in which escape is observed. In the majority of epitopes, several escape sequences are observed transiently, with earlier mutations replaced by a mutation at another site ("leapfrog pattern"). To determine which parameters determine rates and patterns of escape, we design a mathematical model of HIV evolution under dynamical selection pressure from multiple CTL clones. Based on recent experimental findings, we assume that mutations arising in CTL epitopes cause, in addition to a decrease in viral replication rate, partial loss of CTL recognition. Our analysis shows that these two factors, as well as the number of immunodominant CTL clones, determine whether an epitope escapes, whether the first escape variant is stable over time, and the rate of fixation of escape variants. When recognition loss and fitness loss caused by a mutation correlate positively among sites in an epitope, the leapfrog pattern of escape may occur. Our analysis stresses the need to measure recognition losses, as well as breadth of CTL response, to determine fitness costs.

Although there is clear and compelling evidence that the gut is a major site of CD4 T cell depletion during acute SIV and HIV infection, it is less clear that this is where majority of viral replication in fact originates. We use deep sequencing data of a CTL escape region of SIV Gag in multiple compartments in early infection to estimate the maximum possible contribution of virus originating in the gut to the plasma virus load based on mutations at a common CTL epitope in Mamu A01+ monkeys. We conclude that the median highest possible post-peak (day 28) contribution is <10% of total plasma virus originating from gut, and that limited peak data tell a similar story. We use mathematical modeling to further justify our conclusion for different possible escape mechanisms.
UP TO 20% OF SUBSTITUTION MUTATIONS DURING REVERSE TRANSCRIPTION OCCUR IN ASSOCIATION WITH RECOMBINATION.

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HIV reverse transcriptase induces a high rate of mutation and recombination during reverse transcription. However, it is unclear whether these events are mechanistically linked (for example recombination induces mutation), or whether these events occur independently. We have developed a system of synonymous marker mutations in HIV-1 gag and pol of pDRNL(AD8) to identify recombination. Using a single round of infection of PBL, combined with a high-throughput sequencing and mathematical modeling approach, we directly estimate the viral recombination and mutation rates. From >7 million nt we observed 4801 recombination events and 859 substitution mutations (≈1.51 and 0.12 events per 1000 nt respectively). We use experimental controls for PCR-induced recombination and sequencing error, and find the net virus-induced mutation rate is 0.046 per 1000 nt after correction. Individual regions can then be sorted into recombinant and non-recombinant sequences, which have total [ie: PCR + virus-induced] mutation rates of 0.181 and 0.117 respectively (p=0.003, Fisher’s exact). We use a permutation approach to eliminate a number of potential confounding factors and confirm that mutation occurs around the site of recombination, and is not simply co-located in the genome.

Thus, mutation rates are significantly higher in recombinant regions. Combining the difference in mutation rates in recombinant regions with the recombination rate we find that recombination-associated mutations account for 15-20% of all mutations occurring during reverse transcription. This method does not allow us to assign the direction of causality, but suggests that recombination-associated mutation is an important contributor to overall HIV mutation.
The factors shaping HIV-1 dispersal remain poorly understood due to the lack of a statistical framework for phylogenetic hypothesis testing and the availability of large, unbiased, geo-referenced nucleotide datasets. Here we apply a novel Bayesian framework to elucidate the factors underlying HIV-1 spread at a regional scale, while accounting for sampling bias and statistical uncertainty both at the phylogenetic and dispersal level.

To scrutinize viral dispersal in East Africa, we collected 1,035 nucleotide sequences and added 3,037 publicly available data from 17 locations. In our phylogeographic approach, we parameterize rates of dispersal as a generalized linear model to simultaneously test and quantify the contribution of candidate factors shaping viral spread. We considered spatial accessibility data, pairwise geographic distances, train transportation network, population changes, distances to the North and Central highway corridors and sample sizes as potential predictors in the model.

We found that distance to the North highway corridor, and not regional accessibility, spatial distances nor population changes, was the single best predictor of subtype dispersal in East Africa, with substantial statistical support in favour of including this predictor in the process model and a negative log conditional effective size. This implies that individuals living more closely to the North highway corridor, a key axis of transport in this region, will be involved in more intense virus exchange, while individuals living in regions with no connectivity to the highway will experience less intense viral migration. This scenario finds support irrespective of including sample sizes, suggesting that sampling bias does not affect this conclusion.

Our findings show a significant role for the North highway corridor in determining dispersal of HIV-1 in East Africa, supporting that HIV-1 prevention and monitoring strategies in this region should preferentially target populations that travel or live along or in the vicinity of this transport infrastructure.
Antibodies against human immunodeficiency (HIV) with the potential to prevent the virus from infecting host cells are considered a crucial component of a successful anti-HIV vaccine. Virologists typically characterize the neutralizing strength of a monoclonal antibody with the antibody concentrations at which 50%, 70% or 90% of the infection in the probed assay system is inhibited, commonly referred to as IC50, IC70 and IC90, respectively. These values allow an easy comparison of antibody activities across different virus isolates and/or assay systems. However, by focusing on one value only, potentially important information included in the shape and the final plateau of the inhibition curve is lost. We hypothesize that thoroughly analyzing these curves provide information on the mechanism of neutralization and ideally on the evolutionary consequences of the antibody response to the virus. In our study we combine a theoretical analysis of neutralization titration curves obtained for a broad range of monoclonal antibodies tested against a panel of HIV isolates. With the theoretical framework employed here, we are able to demonstrate that considering the titration curve slope in combination with the IC50 is a more meaningful measure of antibody potency than the IC50 alone. Viral escape exerted by antibody induced selection pressure can have different consequences for the IC50 and the inhibition curve slope: (i) the IC50 can increase, (ii) the slope can decrease or (iii) both phenomena arise at the same time. With our theoretical framework we analyzed our data set to identify these patterns across different monoclonal antibodies. A successful anti-HIV vaccine should not only elicit a potent neutralizing antibody response but also an antibody response which the virus cannot evade quickly. Our framework will make it possible to identify these candidates.
54 PROPERTIES OF MHC CLASS I PRESENTED PEPTIDES THAT ENHANCE IMMUNOGENICITY

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T-cells have to recognize peptides presented on MHC molecules to be activated and elicit their effector functions. Some peptides are recognized better than others and are therefore more likely to be epitopes. We set out to determine which properties of a presented peptide cause a difference in T-cell recognition. By careful selection and analysis of a large set of data describing the T-cell recognition of various pMHCs, we could determine which properties influence T-cell recognition. First, T-cell recognition was observed to depend on preferences for certain amino acids, especially large and aromatic residues. Second, the positions in a presented peptide with a larger influence on T-cell recognition were identified. We combined these findings on T-cell preferences in a simple prediction model that can predict the immunogenicity of a presented peptide. This model was validated with data from two independent epitope discovery studies. Interestingly, with this model we could show that T-cells are equipped to better recognize viral than human (self) peptides. After the past successful elucidation of different steps in the MHC-I presentation pathway, the identification of variables that influence immunogenicity will be an important next step in the investigation of T-cell epitopes and our understanding of cellular immune responses towards pathogens such as HIV-1.
55 VIRAL GENOTYPE IN SUBTYPE C SIGNIFICANTLY INFLUENCES PLASMA VIRAL LOAD

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Set-point plasma viral load (pVL), an important predictor of disease progression, is influenced by many factors. However, the influence of the viral genome on pVL is poorly understood. Previous studies, based on transmission pairs and phylogenetic analysis in small numbers of individuals, have estimated the heritability of pVL at between 20-60%. In order to avoid the possible confounding effects of transmission pair studies and analyse as many individuals as possible, we have adapted quantitative genetics methodology and analysed the viral phylogeny as a pedigree. Using this method on subtype B, we estimated the heritability of pVL to be 5%. Here, we apply this method to the C subtype. Viral phylogenies were produced in RAxML using 1,821 resistance-site stripped C subtype sequences, and bootstrapped 100 times. The phylogenies were then linked to pVL, taken as the first available viral load before ART, and analysed in ASReml to obtain an overall heritability estimate and genetic effect estimates for each node, controlling for factors such as age, sex, ethnicity, country of origin, year of diagnosis, and time since diagnosis. To investigate the effect of poorly-supported structure, nodes with bootstrap-support values of less than 90% were collapsed and the data re-analysed. The mean heritability for the C subtype was estimated to be 29.7% (CI 14.7-44.7%), which was highly significant. The mean rose slightly to 31.2% after collapsing poorly-supported nodes (CI 18.9-43.5%). This is much higher than the previous estimate in subtype B of 5.4% (2.7-8.6%), and the confidence intervals do not overlap. The distribution of the genetic effect estimates in the C subtype also has a much wider spread (s.d.=0.1695) than the B subtype (s.d.=0.0708). These findings suggest that heritability of pVL differs across subtypes, and that subtype C particularly may have more viral control of disease progression than previously believed.
Immune responses during early infection are thought to be an important factor influencing disease outcome. In particular, immune pressure from Cytotoxic T-lymphocytes (CTLs) selects for viral mutants that confer escape from CTL recognition. CTL escape has been well documented but measurements of time to escape have ranged from days to years and the effects of anti-retroviral therapy (ART) on viral evolution are not fully understood.

The SPARTAC (Short Pulse Anti-Retroviral Therapy at HIV Seroconversion) clinical trial involved the randomisation of 120 treatment naïve HIV-1B infected patients within 6 months of seroconversion onto one of three trial arms – short course (12 week) ART, long course (48 week) ART or no therapy. Primary endpoint was time to CD4 count < 350 cells/mm<sup>3</sup> or initiation of long term ART. Patient HLA-type, longitudinal CD8 ELISpot responses and HIV gag sequence data were used to address the following questions:

(a) Across 120 patients, how common is CTL escape very early in infection
(b) Do measurable CD8 ELISpot responses predict viral escape?
(c) Does treatment slow the rate of viral evolution within CTL epitopes?

Results showed that within-epitope sequence variation at the first time point was more often consistent with mutations being transmitted in the infecting viral strain than with escape arising within the first few weeks of infection. Statistical analyses found a highly significant relationship between measurable CD8 response at one time point and escape in the epitope at the next time point (p<0.0001 when 'escape' was taken to be 'variation in previously documented escape sites', other definitions of escape were tested and yielded similar results). Patients on long course therapy had slower viral evolution in the first year than those not on therapy but the same effect was not seen for short course therapy.

Recent data suggests that, in SIV and HIV-1 infection, CD8+ T cells mediate anti-viral control predominantly via non-cytolytic mechanisms. This is in apparent conflict with the observation that SIV and HIV-1 variants that escape CD8+ T cell surveillance are frequently and reproducibly selected. Whilst it is clear that a variant that has escaped a lytic response can have a fitness advantage compared to the wild-type, it is less obvious that this holds in the face of non-lytic control where both wild-type and variant-infected cells would be affected by soluble factors. In particular, the high motility of T cells in lymphoid tissue would be expected to rapidly destroy local effects making selection of escape variants by non-lytic responses unlikely. We use a cellular automata model that describes spatial and temporal HIV-1 dynamics to address the question "is the observation of escape variants evidence that CD8+ T cells kill HIV-1 infected cells?"
IMPACT OF THE GENETIC BACKGROUND AND POPULATION SIZE ON THE EVOLUTION OF RALTEGRAVIR RESISTANCE

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Background: Emergence of resistance against integrase inhibitor raltegravir is generally associated with selection of either Y143C/R, Q148K/H/R or N155H, representing three distinct resistance pathways. The mechanisms that drive selection of a specific pathway are still poorly understood. We investigated the impact of the genetic background and population dynamics on the emergence of raltegravir resistance.

Methods: We analyzed the integrase genes in longitudinal samples from five patients who initiated raltegravir plus optimized background therapy at viral loads >5000 copies/ml. Population dynamics were assessed using deep-sequencing data. To investigate the role of the genetic background, we created recombinant viruses containing the viral integrase genes from pre-raltegravir samples from patients in whom raltegravir resistance developed through different pathways. The in vitro selections performed with these viruses mimicked population bottlenecks.

Results: In three patients, raltegravir containing HAART resulted in a >2 log decrease in HIV-1 RNA while the other patients only demonstrated a 1 log decrease in HIV-1 RNA. Deep-sequencing revealed the presence of multiple signature mutations during treatment in 4/5 patients. Evolution during continued raltegravir pressure resulted in selection of variants with a fitness advantage, often representing a different resistance pathway. Interestingly, the resistant population could consist of one resistant variant that completely dominated the population but also of multiple variants from different resistance pathways that coexisted in the viral population. Multiple in vitro selections revealed that a single virus was able to select different resistance pathways, although, typically only one resistance pathway emerged in each individual culture.

Conclusions: The generation and selection of the raltegravir resistance variants is not determined by the genetic background of the viral integrase. Multiple resistance pathways are typically selected in the early phases of therapy failure during which the sequence space is explored and frequently results in a switch of signature mutations towards the fittest variant.
MEASURING EMERGENCE AND TRANSMISSION OF HIV DRUG RESISTANCE USING PHYLOGENETICS

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Introduction
The emergence and spread of drug resistant HIV is a major challenge facing efforts to prevent AIDS and new infections through widespread drug treatment. While much work has focused on the patterns of treatment failure and evolution of drug resistance in infected individuals, this does not directly correspond to how drug resistance evolves at a population level.

Methods
HIV sequences from infected individuals can be used to identify drug resistance mutations, and also to reconstruct phylogenetic relationships between the viruses, which allows inference of the transmission chain (i.e. the phylogeny). The inferred phylogeny and drug-resistant status of each individual is fitted to our new two-strain birth-death model which quantifies the fitness cost in transmission of drug-resistant strains. We use HIV pol data from the Swiss HIV Cohort Study to estimate the relative rates of emergence and transmission of drug resistant virus.

Results
Initially the methods have been tested by simulating the transmission chains, and then attempting to re-estimate these values. Substantial correlation between the parameters present challenges to their estimation, and indicate that the methods require further development. However, initial results from the data suggest that the basic reproductive number of resistant virus in this cohort is substantially lower than that of the drug sensitive virus. The evolution of virus from sensitive to resistant is a much more common event than reversion to the sensitive type.

Conclusion
Drug resistant virus appears to spread too slowly to produce a self-sustaining epidemic, and is instead continually seeded from an ongoing drug sensitive epidemic. However, further refinement of the methods are needed to justify this conclusion. A future key step is to investigate the spread of resistant viruses in sub-Saharan Africa where drug treatment becomes more and more common, but drug regimes are different from Western world treatment.
In vitro studies permit the approach to cell culture conditions of complex in vivo situations and to study, for example, the effects of population fluctuations in HIV-1 evolution and fitness. In this project, we subjected 6 viral clones, derived from the same population, to 15 serial plaque to plaque passages and to 30 recovery passages. The plaque to plaque passages resulted in drastic fitness losses of the viruses. Two clones of these final debilitated viral populations were obtained and subjected to 31 large population passages to recover their fitness. After the passages, there was an overall fitness recovery, but each virus was evolving following its individual pathway.

The sequencing of the complete genomes, at the global level, permitted the identification of mutations associated for the fitness changes. To study the role of the different mutations appearing from these serial passages, they were analyzed individually by in vitro mutagenesis. A complete viral characterization of these viruses was carried out including fitness estimation, viral protein production, viral titration and the effect on cell viability. During the bottleneck passages, mutations accumulated, depending on the viral lineage, mainly in the viral LTR (G379A) and in pol gene (G196E, V189I or E194K in RT). The change in the LTR produced a structural change in the RNA structure resulting in the augmentation of the free energy. Mutations in the RT protein, which mapped close to the catalytic pocket, induced changes in the polarity of the protein, disturbed residues interactions producing important fitness and viral titer losses.

Regarding mutations arising during the recovery passages they mapped in the LTR (A586G, A587G and G593T), in the RT (E194K, E196K, in the V3 loop in the envelope protein (S314K) and also a convergent mutation (V35I) in p17 (detected in different lineages). All these individual mutations produced, in general, fitness and viral titer losses, and different levels of the p24 production.

In summary, although differences exist between the distinct mutations, all individual mutations resulted in less fit viruses (except the S314K mutant in the envelope). In general, mutations, arising during the plaque to plaque passages, resulted in strong decreasing effects in the characteristics of the viruses. Mutations detected during the recovery passages, because of the mapping in the same regions than deleterious mutations from the Muller ratchet and its consequences in the viral characteristics, suggested a compensating effect in the viruses.
Various characteristics of the HIV-1 envelope glycoprotein determine the binding affinity and preference of the virus to the target cell chemokine receptors (coreceptors). These features include the presence/absence of specific amino acids, V3 loop net charge, as well as the extent of glycosylation. Further, glycans play a vital role in the susceptibility to neutralising antibodies. HIV-1 gp120 is heavily glycosylated, however the challenges associated with crystallising glycosylated proteins means that most of the structural investigations have used a non-glycosylated HIV-1 gp120 model. In this study we applied a molecular dynamics approach to determine the influence of glycans on the mobility of different regions of HIV-1 gp120. High-mannose glycans (Man-9) were attached to the N-linked glycosylation sites of two unique gp120 structures modelled on 2B4C. The underlying amino acid sequence of each structure had been selected from a panel of viruses for which the tropism had been phenotypically determined, and comprised a CCR5-tropic (R5) and CXCR4-tropic (X4) virus. We used GROMACS to simulate the movement of atoms across time, and compared the dynamics of various regions of gp120 between the three structures by calculating the root mean square fluctuation (RMSF). In pairwise comparisons between the structures, there was no significant difference in the RMSF values covering the entire HIV-1 gp120. For specific regions, however, we found that the RMSF values for X4 were significantly different from that of R5. The most evident of these differences was for the tip of the V3 loop, the domain previously suggested to be responsible for determining coreceptor tropism. Thus, these results suggest that the interplay between the sequence characteristics and the N-linked glycosylation of HIV-1 gp120 plays an important role in determining coreceptor tropism.
Background: South Africa has the largest worldwide HIV/AIDS population with approximately 5.6 million people infected. A unique characteristic of HIV-1 is its extreme genetic diversity. Heterosexually transmitted HIV-1 subtype C is the major subtype circulating in South Africa. A minor subtype B epidemic, initially detected in the homosexual population, is also present. Methods: The study consists of 86 female patients attending 12 different HIV clinics in Cape Town between 2008 and 2010. The HIV-1 subtype distribution of these patients were characterized through standard HIV-1 genotyping methods using gag p24 and pol PR and RT fragments. The pol sequences were also used to analyse HIV-1 drug resistance and transmitted drug resistance. In addition to phylogenetic analysis, all sequences were screened with the online HIV-1 subtyping tools: REGA 3.0, RIP 3.0, jumping profile Hidden Markov Models (jpHMM) and SCUEAL. Results: A total of 74 (86%) of the 86 samples could be successfully sequenced. HIV-1 subtype C predominates with a prevalence of 91.9% (n = 68). We also show the presence of circulating BC subtypes in at least 4 (5.4%) of our sequences. In addition 1 subtype B and 1 subtype D sequence was also characterized. With SCUEAL analyses we could show 6 (8.10%) sequences having intersubtype breakpoints between subtype C and another subtype, while 7 (9.46%) sequences had intra subtype C breakpoints. We also detected transmitted resistance associated mutations in 3 (4.0%) of the analysed sequences. Conclusion: With tourism and migration rates to South Africa currently very high, we are detecting more HIV-1 CRFs and URFs. It is still unclear what role these rare subtypes will play in terms of long term antiretroviral treatment and vaccine development challenges. It remains vitally important to monitor the HIV-1 diversity worldwide as the face of the epidemic is continually changing.
CO-EXPRESSION OF HLA-B27, BUT NOT B57, RESULTS IN ENHANCED RESPONSIVENESS OF HIV-SPECIFIC CTL RESTRICTED THROUGH OTHER HLA-ALLELES

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HLA-B27 and B57 are associated with relatively slow progression to AIDS. Here, we investigated various possible mechanisms via which these HLA-alleles may exert a protective effect. We compared CTL-responses from 24 HIV infected individuals after ex vivo stimulation with a broad panel of HIV-1 derived peptides presented via the protective HLA-alleles HLA-B27 or B57 or via the non-protective HLA-A2-allele. In 46% of our study population, CTL responses restricted by the protective HLA alleles B57 and B27 and by non-protective HLA alleles could be compared within the same host, such that the effects of slow or rapid progression are not interfering with our read-out. Thereby we could show that i) neither immunodominance, nor breadth, magnitude, or affinity of the response are a general mechanism of protection. Moreover, even though HLA-B27 and B57 are thought to target the most conserved parts of HIV, we found that during disease progression, CTL responses restricted by HLA-B27 and B57 were lost at least as fast as CTL responses restricted by HLA-A2. Strikingly, we found that individuals co-expressing HLA-B27 and HLA-A2 had a significantly higher and broader HLA-A2 restricted CTL response compared to individuals expressing HLA-A2 without HLA-B27. Also the half-life of the interaction between the HLA-A2-peptide-complex and the TCR was significantly higher in individuals co-expressing HLA-B27. Individuals co-expressing HLA-A2 and B57 on the other hand responded to less HLA-A2 restricted peptides and with lower magnitude. These data suggest that there is not a general mechanism of protection for HLA-B27 and B57. While HLA-B57 restricted responses are of exceptionally high affinity and down-regulate CTL responses restricted through other HLA molecules, HLA-B27 restricted responses are not exceptionally high, broad, or of high affinity, but have a clear beneficial effect on CTL responses restricted by other HLA molecules of the host.
MOLECULAR EPIDEMIOLOGY OF FELINE IMMUNODEFICIENCY VIRUS (FIV) IN THE KRUGER NATIONAL PARK (KNP), SOUTH AFRICA

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FIV has been detected in a number of nondomestic feline species including the lion (*Panthera leo*). The Kruger National Park (KNP) is home to roughly 2000 African lions representing one of the largest subpopulations in Africa. Even though species-specific FIV strains share a conserved genome organization, differences exist and FIV in African lions (FIVPle) has diverged into at least 6 genetically distinct subtypes (A-F). Some of these subtypes have distinct geographic areas and only FIVPle subtypes A and D have been isolated in South African lion populations. The aim of this study is to assess the molecular epidemiology of FIVPle and to identify the subtypes present in the Kruger National Park lion population.

Blood samples have been collected from 172 individually identified free-ranging lions in the KNP. Proviral DNA was isolated and a 570 bp region of the *pol* gene was amplified and directly sequenced. Multiple sequence alignments were done using CLUSTAL and manually adjusted. Phylogenetic trees were constructed using both NJ and ML methods, implemented in MEGA v 5. The 172 samples represented 35 prides across the KNP: North (n=13), Central (n=10) and South (n=35). Phylogenetic analysis of the *pol* RT region has confirmed the presence of subtype A (Southern African and East African Isolate), subtype D (South African Isolate) and subtype E (Botswana Isolate) in KNP lions. In addition 4 individual lions, from across the reserve, cluster more closely with FIVPpa (Leopard) and FIVAju (Cheetah) than with any of the FIVPle isolates. In free ranging felids cross-species transmission is a rare occurrence and this needs to be further investigated.

CHALLENGES WITH USING PRIMER ID’S TO IMPROVE ACCURACY OF NEXT GENERATION SEQUENCING

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Similar to some other investigators, we have developed a novel next-generation sequencing method which aims to reduce sequencing errors by molecular barcoding and resequencing of template molecules. Our barcode consisted of a stretch of eleven randomized nucleotides in the primer for cDNA synthesis giving approximately 4 million combinations (Primer ID’s).

In this pilot study the methodology was applied to a 167-base pair fragment of the HIV-1 pol gene using ultra-deep 454 pyrosequencing (UDPS). Data were generated from a clone (HIV-1 SG3?env) and plasma samples from five HIV-1 patients with transmitted drug resistance (TDR). UDPS data were analyzed with and without considerations of the Primer ID’s. Consensus template sequences (CTS) were generated from reads with Primer ID’s observed at least three times. A total of 273,167 UDPS reads were obtained and provided a total of 2,963 CTS, which was lower than expected. This was due to a highly uneven resampling of template molecules (range 3 – >7,000). Furthermore, we observed “false” CTS, which were generated by PCR errors in the Primer ID’s after the templates had been barcoded. Finally, a hotspot for UDPS errors (a deletion in homopolymer stretch of five A’s) induced erratic CTS from the clone when more than 50% of the constituent reads had this deletion. After bioinformatic management of the “problems” above, the error rate of Primer ID UDPS was reduced by at least an order of magnitude compared with standard UDPS. Additionally, we found that low levels of M184V/I could be detected in some patients with TDR and that these levels could be overestimated with standard UDPS.

The Primer ID methodology is promising, but some challenges remain. In particular, we aim to modify our experimental protocol to obtain more even resampling of templates, which would also reduce the risk of “false” CTS due to PCR errors in the Primer ID’s.
Undertaking drug resistance genotyping using the high number of sequence reads generated by next-generation sequencing approaches requires mapping of reads to a reference sequence both rapidly and accurately. RAMICS (Rapid Amplicon Mapping in Codon Space) is a novel tool developed for this purpose. RAMICS uses hidden Markov models to map sequences in codon space, accounting for both the nucleotide and the amino acid at each position in the reference sequence, as well as the relative likelihood of insertions, deletions and mutations at each position. This method allows the tool to distinguish between genuine mutations and spurious indels, meaning RAMICS correctly calls drug resistant mutations near potentially error-rife homopolymer regions such as K65R and K103N. Hidden Markov models are also trainable to a given dataset, which allows RAMICS to discover the most accurate mapping to the HXB2 reference sequence, even when the query sequence set is not subtype B and is very divergent from HXB2. Alignments of subtype C HIV V3 loop sequences produce Cline Shift Scores averaging 0.994 against reference alignments, in comparison with MUSCLE’s score of 0.880 on the same datasets. Next-generation sequencing enables cost-effective, high-throughput drug resistance testing, capable of detecting low abundance variants in a viral population. RAMICS leverages the computational power of graphics processing units (GPUs) to rapidly process the high data volumes generated by NGS, at rates in excess of 32,000 reads per minute. In addition, RAMICS’ algorithm is uniquely placed to cope with the increased error rates and ambiguous bases common in NGS reads. RAMICS is currently being used to map NGS reads for the Seq2Res HIV drug resistance testing pipeline, a new next-generation data analysis pipeline developed at the South African National Bioinformatics Institute.

Supported by a grant from amfAR to attend this program.
Background: Despite prolonged antiretroviral therapy (ART) residual HIV-1 replication is still ongoing. However, it is not yet fully understood where the residual virus is produced in the blood compartment or in peripheral tissues as well.

Methods: We studied three individuals with anal intraepithelial neoplasia (AIN-3). A biopsy was taken from the anal mucosa with AIN 3 and one from the neighboring healthy tissue. Blood was drawn the same day. We investigated virus activity in the blood compartment and in sampled tissue using sensitive HIV-1 RNA/DNA amplification methods. The virus envelope from the lymphocytes, the monocytes, and the tissue biopsies was sequenced.

Results: All three individuals had received ART for up to 15 years with plasma RNA loads < 40 c/ml however with our assay all three individuals had detectable viral RNA in both mucosa tissue and two out of three had viral RNA in plasma. Furthermore, higher levels of viral RNA were found in the neoplastic tissue compared to the healthy mucosa. Sequence analysis of the V1V3 region of the envelope showed a varied picture with one individual carrying two different virus strains in the two mucosa samples indicating variation between adjacent mucosa loci. Another individual harbored CCR5 using virus in the monocytes while the virus found in the lymphocytes was a CXCR4 using strain.

Conclusion: The complex interaction between numerous pathogens at the anal mucosa may play a critical role in influencing HIV-1 replication and transmission. Anal neoplasia lesions demonstrated heightened HIV-1 infection which may provide for an increased HIV-1 transmission. Sequence analyses of the virus envelope showed strain differences between neighboring healthy and neoplastic mucosa suggesting a dynamic localized alteration of the viral reservoirs, even during successful ART.
Drug resistance to antiretroviral therapy threatens our best methods to control and prevent HIV infection. Current drug resistance genotyping methods are costly and optimized for subtype B viruses. We designed a universal primer pair to PCR amplify multiple subtypes of group M viruses for simultaneous drug resistance genotyping of reverse transcriptase, protease, and integrase genes using the Illumina MiSeq platform. Additional advantages of this system include high capacity for multiplexing, frequency information about drug resistance mutations and capacity for detection of low frequency variants. Furthermore, without the need for sequencing primers to match patient virus, this method is less likely to fail during sequencing than current FDA-approved genotyping systems for non-subtype B samples. We used this genotyping method to characterize drug resistance in a cohort of patients exhibiting treatment failure after incarceration. We sequenced 32 patients with paired samples including one time point prior to treatment failure, and one after treatment failure. We successfully amplified and sequenced 98% of the samples. We identified the emergence of new drug resistance mutations in 72% of samples following drug failure. 36% of samples contained major drug resistance mutations based on the Stanford drug resistance database. Half of the major mutations were found at a frequency above the current FDA-approved genotyping limit of detection (20%) and half were found between 2% and 20%, indicating that we are detecting many mutations missed by the current approach. While the main cohort genotyped using this method were subtype B samples from the US, we are currently confirming in silico predictions of primer binding with a panel of subtype A, C, D, CRF01_AE and CRF02_AG viruses. The feasibility of one-amplicon drug resistance genotyping by deep sequencing suggests that methods like this may improve drug resistance genotyping in resource-constrained settings. Dawn Dudley 585 Science Drive Madison, WI 53711 608-890-8047 (phone) 608-265-8084 (fax)
USING AN EPIDEMIOLOGICAL MODEL FOR PHYLOGENETIC INFEREN
REVEALS DENSITY-DEPENDENCE IN HIV TRANSMISSION

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The control, prediction and understanding of epidemiological processes requires insight into how infectious pathogens transmit in a population. The chain of transmission can in principle be reconstructed with phylogenetic methods which analyse the evolutionary history using pathogen sequence data. The quality of the reconstruction, however, crucially depends on the underlying epidemiological model used in phylogenetic inference. Until now, only simple epidemiological models have been used, which make limiting assumptions such as constant rate parameters, infinite total population size, or deterministically changing population size of infected individuals. I will present a novel phylogenetic method to infer parameters based on a classical epidemiological model. Specifically we use the susceptible-infected (SI) model, which accounts for density-dependent transmission rates and finite total population size, leading to a stochastically changing infected population size. We first validated our method by estimating epidemic parameters for simulated data and then applied it to transmission clusters from the Swiss HIV epidemic. We showed that our estimates of the basic reproductive number $R_0$ for the considered Swiss HIV transmission clusters are significantly higher than previous estimates, which were derived assuming infinite population size. This difference in key parameter estimates highlights the importance of careful model choice when doing phylogenetic inference.
We examined the relationship between viral evolutionary history and transmission history using a Bayesian coalescent approach. For 11 patients in a previously described heterosexual HIV-1 transmission chain, we obtained at least one sample and performed amplification, cloning and sequencing for two gene regions. Timed evolutionary histories were inferred from the serially sampled sequences and a population genetic perspective was adopted to evaluate their compatibility with the timed history of infection events. We show that particular clustering patterns in the pol and env genealogies result in divergence times incompatible with the transmission history. For the earliest transmission event, with an unknown direction, we were unable to determine a clear population genetic preference for a transmission direction. We further demonstrate how to constrain the viral evolutionary history to be compatible with the transmission history using a new transmission prior that also includes a coalescent model to infer within-host population dynamics. Crucially, the demographic history is shared between patients to achieve estimation accuracy for demographic parameters for which the uncertainty can be high due to sparse within host sampling schemes. We highlight the potential of this model in estimating the loss in genetic diversity associated with HIV-1 transmission. We also apply it to the transmission chain data to obtain a direct comparison of the within and between host evolutionary rate of epidemiologically linked patients. Our results indicate that multiple transmission bottlenecks are required before the intra- and inter-host evolutionary rates differ substantially.
QUANTIFYING THE TURNOVER OF TRANSCRIPTIONAL SUBCLASSES OF HIV-1-INFECTED CELLS

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HIV-1-infected cells in peripheral blood can be grouped into different transcriptional subclasses. Quantifying the turnover of these cellular subclasses can provide important insight into the viral life cycle and the generation and maintenance of latently infected cells. We used previously published data from five patients chronically infected with HIV-1 that initiate cART. Patient-matched PCR for unspliced and multiply spliced viral RNAs combined with limiting dilution analysis provided measurements of transcriptional profiles at the single cell level. Furthermore, measurement of intracellular transcripts and extracellular virion-enclosed HIV-1 RNA allowed to distinguish productive from non-productive cells. We developed a mathematical model describing the decay dynamics of plasma virus and the transcriptional subclasses of HIV-1-infected cells. Fitting the model to the data allowed us to better understand the phenotype of different transcriptional subclasses and their contribution to the overall turnover of HIV-1 before and during cART. We find that the majority of newly infected cells (~60%) become latently or defectively infected. The numbers of activated, virus-producing cells in peripheral blood are small during chronic infection (~10 per ml). Assuming that the infection is homogenous throughout the body, we estimate the in vivo viral burst size to be on the order of 105 virions per cell. The model predicts that the pool of latently infected cells is fully established one year after infection. Early initiation of cART during the acute phase could limit the number of latently infected cells about 10-fold which should be considered for the evaluation of eradication strategies.

T-CELL REPERTOIRE DYNAMICS DURING CHRONIC HIV-1 INFECTION

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Multiple studies have implicated the CD8+ T-cell repertoire in determining HIV-1 disease outcome. This has been attributed to either T-cell receptor (TCR) diversity or cross-reactivity of CTL populations, both of which allow for the recognition of viral escape mutations. To increase our understanding of T-cell repertoire dynamics during HIV infection, we isolated 32 HIV-specific CTL populations against several HIV-1 epitopes (A*02-SL9, A*02-IV9, B*08-FL8, B*08-EI8, and B*27-KK10) on two time points in eight HIV-1 infected, treatment naïve individuals. In addition, we analyzed the sequence of circulating autologous viral variants. Interestingly, whereas some T-cell populations showed significant shifts in clonotype hierarchy over time, others remained much more conserved. Samples that showed little variation in TCR usage over time often displayed little viral evolution, indicating that viral quasispecies can shape the TCR profile of antigen-specific CTL. Notably, an increase in the magnitude of response was often accompanied by a decrease in TCR diversity, suggesting that an increase in CTL response is not caused by proliferation of the T-cell repertoire as a whole, but is rather the consequence of proliferative bursts of single clonotypes. These clonotypes may react to alterations in the viral milieu. Collectively, these data shed light on host-pathogen interactions and will be relevant when exploring novel T-cell based regimens against HIV.
Guinea-Bissau is the epicentre of HIV-2, but HIV-2 halved in prevalence in this country between 1990 and 2007 and it is now almost absent amongst young adults. We developed a transmission model to explain observed changes in age-stratified incidence and prevalence of HIV-2 in Caió - a village in Guinea-Bissau. Using this model we predict that HIV-2 will continue to decline in prevalence such that only 0.1% of the population will be infected in 2056 (95% confidence interval (CI) 2041-2078) and extinction will occur in Caió in 2074 (95% CI: 2056-2106). HIV-2 epidemics are behaving in a very similar way elsewhere making wider scale extinction of HIV-2 probable in the future.
THE APPLICATION OF SEQ2RES TO FACILITATE LARGE-SCALE, COST-EFFECTIVE HIV DRUG RESISTANCE GENOTYPING USING 454 PYROSEQUENCING.

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The development of high-throughput, sensitive and cost-effective HIV drug resistance (HIVDR) genotyping approaches is critical to the long-term success of ART programmes in resource-limited countries with high burdens of HIV who will need to perform high volumes of HIVDR testing. Next-generation sequencing (NGS) coupled with pooling of samples from multiple patients using a multiplexing protocol has the potential to facilitate such an approach.

We have recently developed Seq2Res, a high-throughput, sensitive, web-based tool that interprets HIV drug resistance data generated using NGS. The utilisation of hidden markov models to map sequence reads in codon space enables the identification and correction of sequencing and PCR induced errors. Seq2Res employs graphics processing units (GPUs) and is capable of processing a 454/Roche Junior plate in approximately six minutes.

Here, we present the application of Seq2Res to the analysis of 480 participant samples from the CIPRA-SA study, a randomised-controlled trial of antiretroviral treatment and laboratory monitoring strategies in South Africa. Three overlapping amplicons covering PR and RT tagged with 48 multiplex identifiers were generated per sample, and sequenced using the 454/Roche Junior platform (10 runs). Sequencing was successful for 475 samples with an average of 711 full-length sequences (encompassing all three amplicons) demultiplexed and analysed for each patient.

The resistance profile for each patient was evaluated using the Stanford HIVdb algorithm and compared, where available, with the corresponding result obtained using conventional Sanger sequencing. Strong correlation between Sanger and NGS sequencing was observed when drug resistance was identified. NGS sequencing, however, predicted resistance in a higher number of individuals, most likely as a result of the ability of NGS sequencing approaches to identify low abundance drug resistant viral variants.

We propose that the application of Seq2Res to NGS sequencing data provides an easy-to-use, viable and sensitive analytical approach for supporting high volume, affordable HIVDR.
Background: HIV-1 RNA level during persistent low-level viremia (LLV) while taking antiretroviral treatment (ART) was positively associated with development of new drug resistant mutations (DRMs). These analyses focused on whether or not a DRM in reverse transcriptase (RT) or protease (PR) was detected. Here we analyze the magnitude of HIV-1 genetic evolution.

Methods: 54 subjects from two ACTG studies had HIV-1 sequencing of RT and PR pre-ART and during LLV (≥2 HIV-1 RNAs >50 and <1000 cp/mL in 24-week period on ART). Hamming distance (modified for mixtures) quantified the percentage mismatch in amino acids pre-ART versus LLV. We interrogated 329 amino acids (PR codon 1-99, RT codon 1-230) including 46 DRM sites (15 in PR, 31 in RT; IAS-USA-2011). Rank-sum tests compared genetic distances between subjects with and without new DRM. Spearman coefficients (r) correlated genetic distances to metrics of HIV-1 viremia.

Results: Subjects with new DRM during LLV (20/54, 37%) had greater HIV-1 evolution (median [Q1, Q3] genetic distance: 1.6% [1.0%, 2.0%]) from pre-ART to final sequence of LLV compared to subjects without DRM (1.0% [0.3%, 1.3%]) across the full sequence (P=0.001). Genetic changes were greater over DRM sites (4.3% [2.2%, 6.0%] for those with DRM). Evolution over non-DRM sites was similar between groups (1.2% [0.7%, 1.8%] vs. 1.0% [0.4%, 1.4%], P=0.25). Higher degree of genetic evolution across the full sequence, and over DRM sites, was positively associated with higher HIV-1 RNA levels during LLV (first, minimum, maximum and HIV-1 RNA area under the curve, all P<0.001, r=[0.44-0.60]). Restricting to non-DRM sites, evolution also positively correlated with HIV-1 RNA levels during LLV (P<0.01 for each metric, r=[0.39-0.48]).

Conclusions: The positive correlation between HIV-1 evolution during low-level viremic ART and HIV-1 viral load levels emphasizes the importance of maximal viral suppression on ART to reduce the development of drug resistance.
INTERPLAY BETWEEN LONG-LIVED ANTIBODY RESPONSES AND SHORT-LIVED CD8+ T CELL RESPONSES CAN EXPLAIN THE MAINTENANCE AND LOSS OF HIV-1 CONTROL

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Despite vast increases in our knowledge of immune responses against HIV-1 and its evolution within the host, it remains unclear why the control of viraemia eventually breaks down. Cytotoxic CD8+ T cell (CTL) responses are thought to be responsible for bringing viraemia under control and establishing the initial set-point in acute infection, with a continuing role in controlling viral replication thereafter. Contrastingly, neutralising antibody responses emerge much later and the plasticity of the viral envelope protein means that their utility in controlling infection is uncertain. Here, we propose a new, mathematical, model for the pathogenesis of HIV-1 in which the transition to AIDS is primarily linked to the gradual loss of the ability to make antibody responses rather than escape from or degeneration of CTL responses. In our model, the control of viraemia in the chronic phase is achieved by a combination of short-lived CTL responses directed at epitopes of limited variability and long-lived antibody responses directed at more malleable epitopes; the effects of CD4+ loss on the humoral arm of the immune response leads to a sudden transition to a different stable state where the virus is controlled at much higher levels by CTL alone. In this model, pathogenesis is caused by the attenuation of the antibody response, but this in turn increases the chances of CTL escape, which further acts to accelerate the progression to disease. Our results suggest that the CTL response may still be effective in late-stage disease and that the key to improving the prognosis of infection is the preservation of the antibody response.

EXTRACTING EPIDEMIC DYNAMICS FROM VARYING HIV-1 EVOLUTIONARY RATES

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The mapping of genetic distance onto calendar time is not straightforward. Heterogeneities in immune response, viral phenotype, and transmission dynamics can all lead to highly variable evolutionary rates that complicate genetic distance relationships. A standard approach to this problem is to assign rates to branches from an assumed population distribution in a maximum likelihood framework. This approach has proved to be very powerful in refining the epidemiology of HIV on multiple scales. However, treating evolutionary rate heterogeneity in a phenomenological way prevents us from making inferences to the causes of rate variation that may be informative to the dynamics of HIV in a given population. In this talk, I will discuss how we used a partially observed Markov model of rate variation along branches to estimate the length of chains of acute transmission and the relative contribution of acute to chronic stage transmission. The data are a sample of 239 HIV-1 A1 p17 and V3 isolates from a large, actively surveyed outbreak among Latvian intravenous drug users (IDU) and heterosexuals. Previous analyses have shown that evolutionary rates are as much as 8.5 times slower in rapidly spreading epidemics. First, we developed a Bayesian statistic based on data augmentation showing that two simultaneously operating evolutionary rates better describe this epidemic, where the two evolutionary rates relate to slow and fast spread, respectively. Our second approach involves maximum likelihood estimation based iterated filtering technique to fit partially observed Markov models of rate variation to an inferred phylogenetic trees. This method naturally handles phylogenetic uncertainty though Monte Carlo integration over a population of trees weighted by their likelihood. Even among IDUs, who are known to spread HIV rapidly, we observed a 4-fold difference in evolutionary rates, suggesting a more complex pattern of transmissions than is implied by the incidence curves alone. We argue that the definition of the state space of the Markov model and its parameters can be used to paint a picture of the underlying population dynamics.
HIV-1 exists in its hosts as large ensembles of related mutants. Genetic diversity, generated by mutation and recombination, is essential for HIV-1 to survive in changing environments and it is a key factor for disease progression and treatment success. Today, next-generation sequencing experiments allow for probing the diversity of virus populations at an unprecedented level of detail. Error corrections, single nucleotide variant detection and local haplotype reconstruction can be sufficiently accomplished, however, global haplotype reconstruction based on short reads has still not been achieved.

RNA was isolated from a mix of 5 molecular HIV-1 viral strains. Full-length HIV-1 genomes were amplified and sequenced by 454/Roche (~500bp read length, amplicon approach) and Illumina (150 versus 250 bp read length, shotgun approach) achieving uniform coverages of 9,878±4,209 and 21,985±7,249 (mean±SD) per nucleotide, respectively. Global haplotype reconstruction was performed using the software packages PredictHaplo and QuasiRecomb.

Global haplotype reconstruction of all 5 virus variants was feasible using 454/Roche reads spanning HIV-1 gag to vif (~4,300 bp). Despite higher coverage using the Illumina technology and a read length of 250 bp, global haplotype reconstruction of all 5 virus variants was possible for gag (~1,500 bp). When using reads that are shorter than 150 bp, global haplotype reconstruction was not achievable due to insufficient diversity within very short overlaps. In addition to the successful global haplotype reconstruction, estimated frequencies of the five virus variants derived from both NGS technologies were similar to frequencies obtained by single genome amplification of 472 clones of the same virus strain mix: HIV-189.6 10.0-16.1%, HIV-1HXB2 9.2-19.0%, HIV-1JRCSF 19.4-24.0%, HIV-1NL4-3 29.6-38.4%, and HIV-1YU2 10.4-16.1%.

Haplotype reconstruction of HIV-1 genomes is feasible and depends critically on read length, uniformity of coverage, and error correction. Depth of coverage cannot compensate short read length, however, becomes an important issue in reconstructing low-abundant viral haplotypes.
ORIGIN AND PHYLOGENETIC RELATIONSHIPS OF AN HIV-1 SUBTYPE F1 TRANSMISSION CLUSTER RAPIDLY EXPANDING IN MEN WHO HAVE SEX WITH MEN IN SPAIN


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We recently reported a HIV-1 subtype F outbreak among men who have sex with men (MSM) in Galicia, Northwest Spain, with viruses forming a monophyletic cluster in protease-reverse transcriptase. Here we update this outbreak, analyze the V3 region and full-length genomes and estimate its geographic and temporal origin. Segments in PR-RT, and V3 region and near full-length genomes were amplified from plasma RNA by RT-PCR. Phylogenetic analyses were performed via maximum likelihood. Recombination was analyzed by bootscanning. Most probable locations at nodes and times of most recent common ancestors (tMRCA) were estimated with a Bayesian Markov chain Monte Carlo method.

From March 2009 through December 2012, 86 HIV-1 subsubtype F1 infections grouping in a monophyletic cluster were diagnosed in Spain. Of them, 81 (94%) were diagnosed in Galicia. Four additional infections were closely related to the Galician subtype F cluster (GFC), branching basally to it. All individuals were sexually-infected men and at least 64 were MSM. Of 14 full-length genomes analyzed, 13 were uniformly of subtype F and 1 was BF recombinant. Through BLAST searches and phylogenetic analyses, 11 viruses from 4 Western European countries [Switzerland (n=4), France (n=3), Belgium (n=2), and Great Britain (n=2)] and 1 from Brazil closely related to the GFC, branching basally to it, were identified. Of these, at least 8 were from recently transmitted infections, and 5 of 6 with reported transmission routes were from MSM. Phylogeographic analyses estimated the most probable origin of the GFC in A Coruña, Galicia. Depending on the analyzed segment, tMRCA were estimated in 2005-2007 and 1997-2002 for the GFC and the European cluster, respectively. In conclusion, a recent HIV-1 subtype F outbreak among MSM in Spain derives from a subtype F variant with a wide geographical circulation among MSM in Western Europe originated in late 1990s to early 2000s.
THE EVOLUTION OF BROADLY NEUTRALIZING ANTIBODIES: WHAT CAN DEEP SEQUENCING TELL US?

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BACKGROUND
Broadly neutralizing antibodies (bNabs) against HIV are consistently highly divergent from germline alleles, suggesting a long history of selection. If a vaccine were to give rise to similar bNabs, then we would first need understand the evolutionary trajectories followed by naturally-elicited bNabs.

METHODS
We developed a high-throughput pipeline for the analysis of next generation sequencing (NGS) of light and heavy chains of immunoglobulin transcripts (IgSCUEAL, http://www.github.com/spond/IGScueal). IgSCUEAL applies: (i) codon-based alignment algorithms to correct common sequencing errors; (ii) model-based phylogenetic procedure to assign each read to a germline rearrangement with statistical confidence; (iii) phylogeny-based clustering algorithm to appoint each read to a unique clonotype. Finally, a weighted graph is constructed between germline and observed bNabs, and all evolutionary paths traversing the space of sampled clonotypes are identified. Clonotypes lying on the shortest (under an appropriately chosen non-linear distance) path between germline and the target are inferred to be the evolutionary intermediaries.

RESULTS
IgSCUEAL analyzes a typical 454 library in 2-10 hours on 128 cluster compute nodes, mapping 80-95% of NGS reads to productive rearrangements. On average, 5-10 transcripts map to a clonotype. Some bNabs have unusually long CDR3 regions (top 1%), but not are unusually abundant (<1% of the total). Path analyses reveal between 3 and 12 intermediate variants from germline to a mature bNab; however, in all cases there are large “gaps” in the evolutionary history. These gaps are most commonly associated with the introduction of long insertions at the junction region of an antibody.

DISCUSSION
We developed a bioinformatics solution for the analysis of NGS immunoglobulin libraries, and for extracting the evolutionary trajectory of the antibodies. While 454-based NGS recovers hundreds of thousands of clonotypes from one sample and some of the putative NAb intermediaries, there are many “missing links”. This currently precludes us from creating a credible evolutionary reconstruction of bNab development. Higher resolution platforms or serially sampled data may address this shortcoming soon.
ANALYSIS OF THE HETEROLOGOUS NEUTRALIZATION CAPACITY OF
PLASMA FROM PATIENTS SUPERINFECTED WITH CONCORDANT SUBTYPE
STRAINS OF HIV-1.

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Introduction:
Recent studies have demonstrated that infection by discordant strains of HIV-1 enhances the
neutralizing antibody response against heterologous viruses compared to patients only infected with a
single virus. However, no study has examined the potential effect of a second infection with subtype
concordant strains on the neutralizing antibody response.

Objective:
To study the effect of superinfection by subtype concordant HIV-1 virus strains on the potency and
breadth of the neutralizing antibody responses and compare it with that of discordantly superinfected
and singly infected patients.

Materials and Methods:
Sequential plasma samples over a time span of 10 years from 3 intrasubtype-superinfected subjects,
obtained before and after superinfection, were tested against 4 heterologous viruses (2 primary isolates
and 2 Tier 1 viruses) representing subtypes B, F2, and G in a TZMbl cell neutralization assay.
Additionally, 12 singly-infected control subjects matched for disease stage, CD4 counts, and time
between samples were studied. Plasma was assayed at 1:80 dilution to compare each plasma pair;
plasma samples that exhibited neutralization capacity >50% were further assessed for magnitude and
specificity of neutralization at serial dilutions of 1:20–1:5120.

Results:
At 1:80 plasma dilution, 1 of the 3 intrasubtype-superinfected subjects’ plasma (33%) obtained after
superinfection exhibited significantly increased neutralization compared to initial plasma. However,
plasma from the other two superinfected patients did not neutralize any of the heterologous viruses but
did neutralize the Tier 1 viruses at lower levels (15-65% neutralization). Seven of the twelve singly
infected control plasma (57%) exhibited a significant increase in neutralization over time whilst plasma
from the remaining 5 singly infected patients showed no neutralization against heterologous primary
isolates.

Conclusions:
These data suggest that re-infection with a subtype-concordant virus, in contrast to superinfection
with subtype-discordant strains may not broaden the anti-HIV-1 immune response over time.
OVERESTIMATED HETEROSEXUAL TRANSMISSION OF HIV-1 SUBTYPE B IN THE UK

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Background: HIV-1 subtype B infections are strongly associated with men who have sex with men (MSM) in the United Kingdom (UK), yet around 13% of patients infected with this subtype report heterosexual contact as route of transmission. We characterised heterosexual transmissions of HIV-1 subtype B in more detail.

Method: 22,481 anonymised HIV-1 subtype B partial pol gene sequences from the UK HIV Drug Resistance database were analysed by phylogenetic means. Sequences were sampled between 1996 and 2008, and associated to the patients’ demographic information. The phylogeny of the sampled population was reconstructed and transmission clusters identified as phylogenetic clades of =2 sequences with a maximum patristic genetic distance of 4.5% and a local branch support =95%. The characteristics of the patients involved in transmission clusters containing at least one heterosexual were analysed. The timeframe of these transmission chains was reconstructed using Bayesian statistical inference.

Results: 13,699 patients (61%) were epidemiologically linked to at least one other individual, including 1,207/13,699 (9%) who only reported heterosexual intercourse as their risk factor. 355 of these (29%) clustered with MSM only: they were twice as likely to be male (n=246; 24% of the studied male heterosexuals) as female (n=109; 10% of the studied female heterosexuals). Dated phylogenies indicated that this gender discrepancy was consistent when looking at HIV transmissions occurring within a 5 years timespan: 9% and 4% of the male and females giving heterosexual contact as their risk factor were linked to MSM only. This is suggestive of misreporting in up to 13% of the total number of heterosexual subtype B infections studied.

Conclusion: Non-disclosure of homosexual contact occurs among heterosexual males in the UK and phylogenetics techniques can be used to validate and adjust miscoding of HIV-1 risk categories.
Antiretroviral drugs vary widely in their ability to penetrate different anatomical compartments, implying that drug concentrations are spatially heterogeneous during combination therapy. While for single drugs, suboptimal concentrations are known to facilitate the evolution of resistance by allowing viral replication and subsequent selection of resistance mutations, less is known about the role of drug penetration in the evolution of multi-drug resistance. Here, we present a mathematical model showing that discordant drug penetration, defined as the presence of compartments where only some drugs in a combination regimen exist at therapeutic levels, greatly speeds up the evolution of multi-drug resistance. We find that discordant drug penetration creates regions of effective monotherapy and allows resistance mutations to arise in a step-wise – instead of concurrent – manner. These results offer a possible explanation for why viral strains resistant to only one drug are sometimes observed to grow during a course of combination therapy. We compare our model results to clinical trials to identify cases where discordant penetration may have led to the stepwise acquisition of mutations. Overall, our results suggest that use of combination regimens containing drugs with similar penetration profiles may prevent the evolution of multi-drug resistance.

Often in early HIV infection, multiple epitope-specific CD8+ T cell responses are observed to drive viral escape mutations that sequentially go to fixation. The later an escape mutation emerges, the slower it goes to fixation. This pattern of escape rate decline could indicate waning strength of CD8+ T cell responses over time, substantial variation in fitness costs of escape mutations, or could also be due to interference among different escape strains. We studied the effects of selective interference on the decrease of successive escape rates. We developed and used a novel mathematical multi-epitope model of HIV dynamics accounting for stochastic effects, recombination, and mutation. Averaged linkage disequilibrium measures were utilized to quantify the amount of selective interference present in a simulation run. We found that nearly synchronous, similarly strong immune responses in systems with high stochasticity enhance the generation of selective interference. Selective interference combined with densely spaced sampling times at the beginning of infection in turn lead to decreasing successive escape rates, even though there were no selection differences. When the virus population is under pressure from multiple CD8+ T cell responses, the rate of escape may not simply reflect the fitness difference between wildtype and escape mutant, as is often assumed when interpreting such data. In such systems, new estimation methods are required that account for interference effects.
Approximately 30% HIV-1 infections in Portugal are caused by a monophyletic subtype G variant, which also circulates at lower prevalences in Spain, mainly in the Northwestern region of Galicia, with sporadic cases in other European countries. Here we analyze, by using a Bayesian method, the origin of this variant (Iberian G or GIB) and its temporal-spatial propagation dynamics. Through BLAST searches using protease-reverse transcriptase sequences and phylogenetic maximum likelihood analyses, we identified 4 Cameroonian viruses closely related to GIB. These were included in the alignment used for the Bayesian analysis, together with other subtype G viruses from Africa (n=65, from 8 countries), Portugal (n=25) and Spain (n=23). SRD06 codon position substitution model, lognormal relaxed clock, and Bayesian skyride demographic growth model priors were used. Diffusion pathways were analyzed through a Bayesian stochastic search variable selection (BSSVS) approach. The posterior distribution of trees, summarized in a maximum clade credibility tree, showed that viruses from Portugal and Spain formed a clade with a posterior probability (PP) of 1, in which Portuguese and Spanish viruses were interspersed. The 4 above-mentioned Cameroonian viruses formed a sister clade of GIB, joining it with PP=1, with 10 other Cameroonian viruses being the next African isolates most closely related to GIB. The time of the most recent common ancestor of GIB was estimated in 1987 and its split with the Cameroonian clade in 1984. The Cameroonian ancestry of GIB was supported with PP=0.82 and its origin in Portugal with PP=0.84. BSSVS analysis supported two separate introductions from Portugal to Spain, with the Galician city of Vigo (near the Portuguese border) being the main center of GIB dispersion in Spain. The results therefore indicate that GIB derives from a Cameroonian strain, and that it originated in Portugal in the mid-1980s, with subsequent diffusion to Spain through multiple introductions.
ANALYSIS OF PCR FOUNDER EFFECT AND SEQUENCING ERRORS IN ILLUMINA SEQUENCING

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Background: Biased PCR amplification (founder effect) results in inaccurate analyses of HIV populations in vivo leading to inaccurate measures of virus diversity, evolution, and drug resistance. Here we analyze in detail PCR bias and other error types in illumina sequencing and compare them to other deep sequencing methods.

Methods: We synthesized cDNA from mixtures of wild-type and drug resistant HIV-1 (BH10) pol transcripts (100% to 0.1% mutant containing ~ 100,000 RNA molecules) using a gene-specific primer containing 10 random nucleotide sequences (dogtag), meant to label each cDNA molecule with a unique sequence. The cDNA was sequenced using paired-end Illumina MiSeq technology. The resulting sequencing data were used to build consensus sequences from all reads that shared the identical dogtag, indicating that they were amplified from the same cDNA molecule. We analyzed the data to 1) investigate PCR bias in illumina sample preparations, and 2) determine the error types and rates for sequencing HIV populations by illumina Miseq technology.

Results: In one sample (100% wild type), we obtained 759,501 sequences, but there were only 11,896 unique dogtags indicating that only about 12% of the starting RNA molecules were copied and amplified. We also found significant PCR bias. Approximately 50% of the unique dogtags were found only once, while others were present nearly 3,000 times. Together, these data demonstrate a high degree of bias in PCR amplification. We also identified errors in cDNA synthesis at 0.007%, and in early cycles of PCR at 0.06%. Late PCR/sequencing errors, while often observed, did not affect the consensus sequences generated from each dogtag set. Also observed were indel errors (0.03%) of 3 types: upstream insertion induced deletions, upstream deletion induced insertions, and stand-alone indels.

Conclusions: Our results show that PCR founder effects in the preparation of samples for Illumina sequencing limit its use for evaluating HIV population structure and for detecting minor drug resistance variants. New methods that reduce PCR bias are clearly needed. However, PCR errors were much reduced by inclusion of dogtags and were greatly reduced by Illumina compared to 454 sequencing.
DETAILED PHYLOGENETIC AND PHYLOGEOGRAPHIC ANALYSIS OF HIV-1 IN THE SCANDINAVIAN REGION

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The HIV-1 epidemic in the Scandinavian region is characterized by multiple subtypes and CRFs. Frequent travelling within Scandinavia may affect the migration patterns of HIV-1 and lead to outbreaks that span country borders. Here, we analysed 4,003 pol sequences (approximately 1,000 bp) from Sweden, Denmark and Finland collected 1982-2012 to determine the detailed molecular epidemiology of HIV-1 in Scandinavia. The dominating forms of HIV-1 were subtypes B (57%) and C (10%); and CRF01_AE (13%) followed by subtypes A, D, G and CRF02_AG, which were selected for further study. The most common transmission route was homo/bisexual transmission (74%) for subtype B, and heterosexual transmission for subtypes A (82%), C (83%), D (86%), G (81%), CRF01_AE (67%) and CRF06_cpx (58%).

To be able to identify local Scandinavian transmission clusters we used a Blast-approach to construct a reference sequence dataset consisting of (1) 5,788 patient-unique reference sequences from Genbank and (2) 2,268 non-overlapping, patient-unique reference sequences from a database within the SPREAD Programme, which contains 5,289 sequences that have been representatively obtained from patients in European countries during 2002-2010. Maximum-likelihood and Bayesian phylogenetic methods will be used to investigate phylogenetic clustering and phylogeography of HIV-1 in Scandinavia. Specifically, we will study (1) the number of HIV-1 introductions into Scandinavia, (2) the number of past and active transmission clusters and their demographic patterns within Scandinavia, and (3) HIV-1 spread within and between the Scandinavian countries. Preliminary data have shown small and larger Scandinavian clusters, especially among MSM and intravenous drug users. Some clusters involve patients in several countries. The results will be important for predicting the future HIV-1 spread in this region and therefore for HIV prevention.
A COMPREHENSIVE, ACCURATE, FAST, CROSS-PLATFORM AND USER-FRIENDLY PIPELINE FOR THE ANALYSIS OF HIV-1 NEXT GENERATION SEQUENCING DATA

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Background

Next generation sequencing (NGS) is rapidly becoming the standard approach for characterizing HIV-1 genotypic diversity. Despite several years of intense research activity, there is no simple comprehensive informatics solution that is accessible to the average user on commonly available hardware. We have developed such a solution, leveraging open-source software components and algorithmic expertise from academic and commercial groups, for the analysis of HIV-1 NGS data produced by any of the commonly used sequencing platforms.

Methods

Because no single reference genome can be used for all HIV-1 samples, a sample-specific reference (consensus) is reconstructed using a version of the Quiver algorithm, (PacBio), which uses a hidden Markov model based approach to iteratively refine an initial consensus generated by partial order alignment (POA). This algorithm is fast and achieves accuracy greater than 99.99% on samples of known composition. Common sequencing errors (e.g. spurious indels, homopolymer length miscalls, etc) are corrected using a codon-aware read-mapping algorithm (UCSD). Individual minority variants are called significant based on a binomial mixture model (UCSD), and phased into haplotypes using a POA approach (PacBio).

Results

We demonstrate the performance of the software on amplicon and whole-genome data sets, generated using 454 FLX and PacBio RS instruments. Our algorithm is fast (typical analysis time of <10 minutes on a 64 CPU cluster), produces consistent results between platforms and technical replicates, and generates directly interpretable outputs. Outputs include a statistical confidence that a particular mutation is not due to a sequencing error, phylogenetic trees representing viral diversity, the inferred number and frequency of haplotypes, etc.

Discussion

Our pipeline is easy to install, configure, and use. It does not depend on other complex software, such as genome assemblers, is provided under open-source licenses, and is usable through a public web-based interface (datamonkey.org).
null
CHARACTERIZATION OF HIV-1 GENOMES FROM 24 ACUTELY INFECTED SUBJECTS

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The RV217 cohort was established to identify 150 acute infections in East Africa and Thailand in order to better define prevalence, risk behavior, incidence and retention in high risk individuals. Our goal is to characterize viruses from acutely infected subjects and to follow HIV-1 evolution over time. We have sequenced 375 HIV-1 genomes from 24 subjects, with an average of ten genomes per time point. Bi-weekly testing for HIV-1 RNA allowed to identify subjects during the Fiebig stage 1 of infection: plasma samples for sequencing were obtained at a median of 5 days after the first positive test, which occurred at a median of 4 days after the last HIV-1 negative visit. For these 24 subjects, peak viral load occurred 13 days after the first positive test with an average of 3,14 million viral copies per ml (IQR: 1,93- 27,23). Infections were established by a single HIV-1 variant in 18 of the 24 subjects. Among infections with multiple founders, two variants were identified in four individuals and complex viral populations with recombination were seen in two subjects. Sequences from subject 40100 showed that a variant that was present in less than 3% of the viral population at the first time point was found to dominate the viral population six months later. During the first 6 months of infection, viral genome diversity increased across all individuals at a yearly rate of 0.60% for all nucleotide sites. As expected, the average rates of diversification were lower in gag (0.53%) than in gp120 (1.61%). Contrary to previous results, our results do not indicate that the number of variants establishing infection differs between homosexual and heterosexual HIV-1 transmission. Bi-weekly follow up allows us to better define HIV-1 genetics and evolution as a function of viral load kinetics in each subject.
100 SPATIAL DISTRIBUTION AND PHYLOGEOGRAPHY OF THE HIV TRANSMISSION NETWORK IN SAN DIEGO, CALIFORNIA

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Introduction: A HIV transmission network was previous reconstructed from a densely sampled San Diego (SD) HIV epidemic. Here, we examine whether geographical location of subjects informs molecular linkage.

Methods: Between 1996-2012, HIV-1 pol sequences were collected from 1023 infected individuals in San Diego, California and Tijuana, Mexico. Molecular linkage was inferred when sequences were less than 1.5% distant (Tamura-Nei 93 model), and each subject’s connectivity was defined by their network degree (the number of sequences linked to their sequence). For 565 of these individuals, sociodemographic information and ZIP code of residence was available. Using this information, we defined the geographic epicenter of the San Diego epidemic, and compared how network properties differed between it and the surrounding areas.

Results: 41.4% (241/565) of subjects were connected to at least one other subject (clustered), consistent with previous estimates for the area; 39.2% (227/565) of subjects resided within the two ZIP codes that comprise the Hillcrest neighborhood of SD, defined as the epicenter of the epidemic. Subjects living outside of Hillcrest were more likely to be younger (34.08 vs. 35.76 p=0.04), Hispanic (40.1% vs. 29.7% p=0.027), heterosexual or bisexual (18.2% vs. 3.2%, p<0.001, and have a non-homosexual risk factor (17.8% vs. 4.6%, p<0.001). However, even though significant demographic differences were present, there was no difference in the mean degree or the distribution of network hubs (high degree nodes) between the epicenter and the periphery.

Discussion: Network analysis of the local HIV epidemic revealed demographic and risk behavior differences depending on geographic location. However, the mean network degree and distribution of the nodes in the epicenter and the periphery could not be distinguished, suggesting either that all acquisition of infection occurs predominantly within the epicenter, or that the transmission dynamics are similar between those within and outside the epicenter.
A model for HIV-1 molecular evolution during the disease course has been presented by Shankarappa et al. The results showed that the asymptomatic stage of infection displayed phases with variable patterns of viral divergence and diversity. Our objective was to investigate if evolutionary dynamics confirmed a similar pattern also for HIV-1 non-subtypes B. To study the HIV-1 evolution over the natural course of infection, we focused on HIV-1 seroprevalent individuals of an occupational cohort in Guinea-Bissau (West Africa) from whom we had estimates of seroconversion dates among individuals infected with the circulation recombinant form (CRF)-02_AG. Among individuals infected with CRF02_AG (n=20), from which we were able to amplify cDNA obtained from plasma samples of 3 or more time-points, the time between seroconversion and development of AIDS was used to classify them according to disease progression rate. PCR products were cloned and 10-12 clones per time-point were sequenced. Analysis of a subset of the individuals showed that HIV-1 of six of eight individuals had a decline in diversity 3-6 years after seroconversion, while it continued to increase in the other two. Among those that showed a decline in viral diversity, four individuals developed AIDS between 1-2 years after the diversity peak. Our preliminary results suggest that diversity and divergence of HIV-1 CRF02_AG follow the same tendency as reported for HIV-1 subtype B in previous studies. Studies are underway to include the remaining longitudinal samples in the analysis. The result of our longitudinal study will shed light of the molecular evolution of CRF02_AG in relation to disease progression rate. This has not been reported for HIV-1 non-subtype B.
Background
Mother-To-Child Transmission (MTCT) of the Human Immunodeficiency Virus type 1 (HIV-1) is responsible for most of pediatric HIV-1 infections worldwide. MTCT may occur during breastfeeding, labor or pregnancy. So far, the timing of vertical transmission has not been studied from the perspective of the molecular evolution of viruses. However, time series of viral sequences can provide powerful insights into evolutionary and other biological processes.

Methods
Molecular variation accumulates with time and provides thus information about the age of viral populations. In this study, we analyzed time series of sequence data of the env gene from nine HIV-1 infected mother-and-child pairs using random genealogy based models (coalescence) within a Bayesian statistical framework to estimate MTCT timing. Samples were obtained from participants enrolled in a clinical trial for the prevention of MTCT in Thailand.

Results
A mean value of six sequential samples per infant were analyzed (n = 2-8). The mean follow-up period was 664 days (277-1034 days). All were infected by CRF01_AE strains. Infection in four children was detected during pregnancy and in four more children at delivery. For eight of nine pairs, these results were consistent with the empirical transmission periods as assessed from PCR assays. The discordance in the remaining case supports co-infection at delivery by simultaneous introduction of multiple maternal viral strains.

Conclusion
This study has benefited from the temporal anchoring of HIV sequences to infer transmission timing and evolutionary rates in mother-infant pairs. Most notably, these results illustrate the power of such population genetics approach to draw inference about viral evolution and offer the opportunity to validate the Bayesian coalescent framework on a documented case.

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HIV-1 evolves under two levels of selection. At the within-host level, the virus acquires mutations in epitopes which inhibit HLA-binding and CTL-recognition. Because of the massive heterogeneity with respect to HLA haplotypes in the human population, these adaptive mutations are likely not beneficial in future hosts. Furthermore, immune-escape mutations often come with a fitness cost. Within-host viral fitness relates via virus load to virulence and infectiousness and it has been suggested that intermediate virus loads optimize the basic reproduction number ($R_0$). Furthermore, observed set-point virus loads are centered around a predicted optimum. Ignoring within-host adaptations, these observations are likely due to population-level adaptation, that is, optimization of the basic reproduction number. The aim of our research is to investigate the implications of the above described intertwined selection mechanisms. We developed a variety of mathematical and simulation models and found that for realistic mutation rates, the effect of selection for $R_0$ is minimal. We do however find positive heritability of set-point virus load. Also our models predict that due to HLA-polymorphism, HIV-1 can accumulate many deleterious mutations, resulting in intermediate virus loads.
HIV-1 uses the coreceptors CCR5 and/or CXCR4 for cell entry. R5 strains, using CCR5 as coreceptor, are often seen early in the infection while X4 strains, using CXCR4, may appear during AIDS. CXCR4 use may consist of both monotropic (X4) and dualtropic (R5X4) viruses. The viral phenotype is important in evaluating the response to CCR5 inhibitors, a new class of antiviral drugs.

Methods: The coreceptor use of HIV-1 was investigated in patients from Botswana, carrying HIV-1 subtype C and failing antiretroviral treatment. DNA of the \textit{env} V3 region of the HIV-1 gp120 was sequenced from 24 patients. Sequences from 26 untreated patients were controls. Single genome PCR was used to discern small populations of HIV-1 and the Geno2pheno method, with the cutoff recommended by the European Guidelines, to predict the coreceptor use phenotype from these nucleotide sequences. The glycan-charge model adjusted for subtype C was also used for phenotype prediction.

Results: The viral phenotype was predicted from the population sequences of 24/24 patients, of whom eight (33%) were predicted to harbor CXCR4-using strains as compared to 2/26 treatment naïve patients (P=0.03), when using the Geno2pheno method. Single genome sequencing generated 295 clones in total, ranging 4-23 clones/patient in patients failing treatment. Altogether 90/295 (31%) putative CXCR4-using clones were identified. In 10/24 (42%) treated patients at least one clone was predicted to be able to use CXCR4, as compared with the 33% by population sequencing.

Conclusions: These numbers of CXCR4-using strains in treated patients were large for subtype C, which is associated with relatively few X4 strains, suggesting perhaps that CCR5 inhibitors are less suitable in this patient group as opposed to in treatment naive patients.
LYMPHATIC TISSUES SHOW PERSISTENT REPLICATION, EVOLUTION AND DISPERSAL OF HIV-1 DESPITE ANTIRETROVIRAL THERAPY

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Antiretroviral therapy (ART) can reduce HIV-1 to undetectable levels in peripheral blood, but the effectiveness of treatment in suppressing replication in lymphoid tissue reservoirs has not been determined. Here, we use 454 deep-sequencing to characterize viral genetic diversity in 4 patients undergoing ART, under which plasma viremia decreased below the limits of detection in peripheral blood samples. We examine viral diversity in these patients in plasma RNA, in proviral DNA in peripheral blood mononuclear cells (PBMC) and in proviral DNA in lymph nodes (LN) at 0 months, 3 months and 6 months after the initiation of ART. The p17 and p24 regions of the gag gene and the PR and RT regions of the pol gene were sequenced for each patient / tissue / time point combination using 454 Life Sciences' GS-FLX pyrosequencing system, and the resulting sequencing reads denoised and aligned. We use these alignments to analyze evolutionary dynamics across tissues and time. We find that sequence divergence increases with time in both PBMC and LN tissues, showing an average increase of 0.028 substitutions per site per year. Additionally, we find that Bayesian phylogenetic analysis using the software package BEAST gives a similar rate estimate, while incorporating an explicit model of platform noise. Across these datasets, we observe mutations first appearing in LN samples, before spreading to PBMC, coincident with an evolutionary reservoir in LN tissues. Thus, we find evidence that viral replication and evolution has proceeded despite treatment, and that new sequence variants first emerge in LN, suggestive of a persistent viral reservoir.
107 THE COLOMBIAN EPIDEMIC IS DOMINATED BY HIV-1 SUBTYPE B OVER TIME: A MOLECULAR EPIDEMIOLOGY AND PHYLODYNAMICS STUDY

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Background: Although HIV-1 subtype B predominates in Latin America, little is known about the molecular epidemiology in Colombia. This study aimed to shed light on the viral diversity and spatial dynamics of the HIV-1 epidemic in this country. Methods: 610 pol sequences sampled from 2002-2007 were obtained from 7 distinct regions: Bogotá (275), Cali (77), Medellín (64), Coffee region (42), Santander (26), Caribbean (18), and others (108). For each Colombian sequence, the 10 most similar sequences BLASTn were selected. All sequences were subtyped using REGA, and COMET. In case of discordant classification, manual phylogenetic analysis was used. We used a non-reversible phylogeographic model implemented in BEAST to infer a time-calibrated phylogenetic history and applied a Bayesian Stochastic Search Variable Selection procedure to estimate the most significant pathways of viral dispersal within Colombian and between several other regions as defined by the BLASTn outcome.

Results: The Colombian epidemic remains dominated by subtype B (99.8%). However, one subtype F (1/610, 0.2%) was found in Bogotá. The most similar sequences retrieved by BLASTn were mainly from Spain (32%), followed by other countries of southern Europe (17%) and other European regions (20%), North America (13%), Central-South America excluding Colombia (12%), and other countries (6%). We found strong support for epidemiological links between Spain and Central-South America and between the latter region and Bogotá. Within Colombia, we found evidence for bi-directional flow between Bogotá and Medellín, with these regions, together with Cali, being the main contributors of HIV-1 dispersal at the country-level.

Conclusions: This is the first study using a large sample, which shows the predominance of Subtype B in Colombia. We show that viral populations in Bogotá, Medellín and Cali play a central role in shaping the HIV-1 epidemic within Colombia, which has most likely been seeded from other Central and South American countries.

108 TRANSMISSION AND EARLY EVOLUTION OF HIV-1

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There is an ongoing discussion of stochastic versus selective determinants in the transmission of HIV-1. There are likely to be elements of both, and selective pressures may influence the probability of transmission, not act as a gatekeeper. These pressures may vary by route of transmission or even the circumstances of transmission. Thus we should expect to see the influences of selective pressure as weak signals within the transmitted virus population. We have confirmed that the transmitted virus in heterosexual transmission is slightly under-glycosylated relative to a population of viruses from chronically infected subjects. In addition, transmitted viruses are less likely to use an alternative conformation of CCR5 that is resistant to inhibition by an entry inhibitor. Both of these features of the transmitted virus hint at steps in the transmission event where interaction with the host helps shape the transmitted virus population. Transmitted viruses are known to be predominantly R5, we have now measured their CD4 dependence and found that they require high levels of CD4 to enter cells, indicating that the target cell for transmission is a CD4+ T cell and not a macrophage or dendritic cell, which have only low levels of CD4 on their surface. We have observed in both vertical and horizontal transmission the sequestration of either a transmitted virus or an early recombinant within the CNS, as measured by compartmentalization in comparing virus in the blood and the CSF. Finally, using deep sequencing of a V1-V3 amplicon, we easily observe X4 viruses as distinct lineages in late-stage subjects. There is only a weak and scattered genotypic signal for X4 viruses among the R5 lineage of both these late-stage subjects and subjects early in infection (1-2 years), suggesting that this weak genotypic signal is likely to be artifactual and biologically irrelevant.
109 MANIPULATION OF IMMUNDOMINANCE WITH A CONSERVED ELEMENT-BASED HIV-1 VACCINE

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HIV sequence diversity is a major hurdle for development of AIDS vaccines. Our Conserved Elements (CE) vaccine design approach seeks to circumvent this problem by generating immunogens encompassing critical elements of the viral proteome while excluding regions capable of mutating without destroying or limiting virus viability, including potential immunodominant (i.e., “decoy”) epitopes. A prototype Group M HIV-1 p24gag (p24CE) was constructed and evaluated in several ways: The criticality to viral function was assessed in competitive fitness assays. Vaccine component peptides were recognized in HIV infection and high avidity responses against them are associated with virologic control. De novo induction of human T cell responses was shown by exposure to CE-expressing autologous dendritic cells, inducing CD4 and CD8 responses at levels similar to full-length Gag. Immunization of mice and macaques elicited strong, long-lasting, cross-clade cellular and humoral responses. In contrast, vaccination with full-length, p55gag DNA elicited responses to epitopes only outside of the CE. Boosting of p24CE-primed macaques with p55gag DNA greatly augmented CE-specific cellular as well as humoral responses. In addition, the immunodominant responses elicited by p55gag DNA were substantially lost. Thus, the combination of a p24CE followed by p55gag DNA vaccine strategy led to strong responses and to the redirection of Gag immunodominance hierarchy in macaques. These results provide a novel and effective strategy to avoid eliciting immunodominant responses against decoy epitopes while focusing responses to critical features of the virus for which few viable escape pathways have been found by HIV evolution to date.

110 MODELING HCV INFECTION AND TREATMENT

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Hepatitis C virus (HCV) infects about 170 million people worldwide and is the leading cause of liver transplantation. Drug therapy is undergoing rapid development and current protocols can cure this disease in a majority of patients – making HCV the first chronic viral disease that is curable. Here I will discuss recent modeling work on HCV that required the use of multiscale dynamic models in order to understand the response to therapy with an NS5A inhibitor daclatasvir. The use of this model led to new interpretations of the first and second phases of viral decline as well as a new estimate of the HCV half-life of 45 minutes.

111 IMMUNOGENETIC FACTORS THAT IMPACT THE COURSE OF HIV INFECTION

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Variation in the HLA class I genes have the greatest influence on outcome after HIV infection relative to the rest of the genome. While allelic effects of these genes have been well-appreciated for over two decades, more recently it has become evident that other polymorphisms within the region also contribute to HIV control, such as those involved in regulation of HLA-C cell surface expression. The HLA class I loci function as ligands for the killer immunoglobulin-like receptors (KIR) and the leukocyte immunoglobulin-like receptors (LILR), and variation in these genes in combination with that at the HLA loci can have an impact on host defense against the virus. I will present data that emphasizes the multiple ways in which variation within/around the HLA class I loci affects their interaction with other genes, and how these interactions in turn modulate HIV control.
Adaptive immune responses by dendritic cells (DCs) are controlled by pattern recognition receptors such as Toll-like receptors (TLRs) and C-type lectins. C-type lectins interact with carbohydrate structures on pathogens. Upon pathogen binding, C-type lectins trigger signaling pathways that induce specific cytokines to dictate T cell polarization. Thus, C-type lectins are crucial in tailoring immune responses to pathogens. Recent data demonstrate that C-type lectin signaling seems to control NF-κB activation, either by enhancing or inactivating transcriptional activity of specific NF-κB subunits. Dissecting the signaling pathways induced by C-type lectins and their effects on host immunity is essential to understand the molecular mechanisms leading to adaptive immune responses. Here I will discuss the molecular signaling pathways induced by C-type lectin DC-SIGN that are involved in adaptive immunity to fungi, mycobacteria and HIV-1. Our data show that DC-SIGN induces carbohydrate-specific signaling; DC-SIGN binding to mannose-expressing pathogens induces pro-inflammatory cytokines, whereas fucose-expressing pathogens suppress pro-inflammatory cytokines through DC-SIGN. Notably, HIV-1 hijacks the signaling by DC-SIGN and TLR8 for its own replication and transmission. Recent data strongly suggest that innate signaling triggered by HIV-1 is also required for efficient fusion and integration into the host genome. The subversion of these crucial immune signaling pathways by HIV-1 and the consequences for HIV-1 infection will be discussed.

Since 2005, the Spanish clinical laboratories introduced the sequencing in naïve patients. Now the number of available HIV nucleotide sequences lets us know with more accuracy the molecular epidemiology of HIV in local areas. Moreover the huge number of sequences in Genbank with information about the geographical origin will be used to understand the traceability of the low prevalent variants in our environment. Our propose were to compare the molecular epidemiology in two period of time among the different regions with different immigration rates. 10.877 nucleotide sequences of pol gene were recovered from seven Spanish regions during the periods 2005-2007 (2,858 sequences) and 2008-2011 (8,019). All sequences were subtyping using PhML methods and the RDP3 program. Globally, the percentage of non-B variants was continuously increased from 8.4% to 14.2% reaching 20% in some regions, and the number of variants was increased until 7 subtypes, 27 CRFs and 10 URFs in at least three independent cases. However, the diversity reached the maximum value in 2007 (0.87), which was maintained during the following years. Those sequences identified as non-B subtypes were reanalyzed (PhML o RxML) using download sequences from Los Alamos database with information about their geographical origins. Although, the percentage was similar among the different regions without correlation with immigration rates, the phylogeographical patterns showed interesting differences. Region near Portugal has high prevalence of subtype G. In Madrid, the prevalence of non-B subtype is majority due to CRF02_AG from Africa. In Northwest region the subtype C circulating is related to African sequences while subtype C in Centre and South regions was related to South American sequences. Subtype F sequences related to outbreak described in North of Spain was present in practically all regions now. So the HIV epidemiology must be described at local level, attending to distribution and social, geographical, cultural and economic factors.
Introduction: The source of persistent HIV viremia during suppressive antiretroviral therapy is unknown. To investigate its origin, we have been carrying out detailed analysis of the HIV RNA and DNA populations in a cohort of patients followed for more than 10 years. We focus here on one individual who developed persistent low level viremia >50 c/ml after more than a 11 years of suppressive ART.

Methods: Pretherapy and on therapy samples were analyzed at frequent intervals for levels of HIV plasma virion RNA in this cohort, and genetic relationship of plasma virus RNA populations to one another was assessed phylogenetically. The focus patient had 2 cART interruptions accompanied by high levels of viremia during this time, but plasma HIV RNA returned to <50 c/ml until he developed viremia of 200-300 c/ml persisting > 6 months during which oral squamous cell carcinoma (SCC) was diagnosed. We analyzed HIV populations in stored plasma obtained prior to cART, during suppressive cART, following cART interruption, and after switch in regimen, using single genome sequencing.

Results: In all patients, there was a dramatic difference in the kinetics of decay of HIV RNA and DNA following initiation of cART, with the 3-4 log initial decline in RNA levels accompanied by only a 20-fold average drop to a steady state level in HIV DNA in PBMCs. RNA sequences did not exhibit signs of ongoing replication, but did tend to simplify with the appearance over time of clonal sequences. In the focus patient (HIV RNA=238,000 c/ml, CD4=22 cells/µl at therapy initiation) HIV RNA remained genetically diverse with no drug resistance mutations detected by SGS. Low-level viremia (330 c/ml) emerged after 11 y on cART (consisting of ABC+3TC+EFV); SGS revealed that the rebound HIV comprised 2 clades, one wildtype (WT) and one drug resistant. The WT population consisted largely of multiple identical sequences; the resistant population comprised diverse variants all encoding K103N and M184V. Switch to TDF+FTC+RTG (directly observed) produced a 10-fold reduction of the drug-resistant variants, but only a 2-fold change in the WT variants.

Conclusions: Wildtype, drug sensitive viremia >50 c/ml, consisting of identical sequences, can arise and persist even after clonal, wildtype viremia on cART and its insensitivity to a switch to cART implies that the source of viremia was an expanded clone of HIV-infected cells and/or increased HIV production from such a clone.
Cytotoxic T-lymphocytes (CTLs) recognize viral protein fragments displayed by MHC molecules on the surface of virally infected cells and generate an anti-viral response that can kill the infected cell. Virus variants whose protein fragments are not efficiently presented on infected cells or whose fragments are presented but not recognized by CTLs therefore have a competitive advantage and spread rapidly through the population. We present a method that allows a more robust estimation of these escape rates from serially sampled sequence data. The proposed method accounts for competition between multiple escapes by explicitly modeling the accumulation of escape mutations and the stochastic effects of rare multiple mutants. Applying our method to serially sampled HIV sequence data, we estimate rates of HIV escape that are substantially larger than those previously reported. The method can be extended to complex escapes that require compensatory mutations. We expect our method to be applicable in other contexts such as cancer evolution where time series data is also available.
ESTIMATING THE EFFICACY OF LATENCY REVERSING AGENTS FROM RESIDUAL VIREMIA MEASUREMENTS

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Antiretroviral drugs are currently unable to cure HIV infection due the presence of a reservoir of latently infected resting CD4+ T cells. A new class of drugs, which we term “latency reversing agents,” is being developed to target this reservoir for eradication. These drugs are designed to act by specifically reactivating viral gene expression from cells with integrated HIV DNA, with the hope that infected cells will then be cleared by viral cytopathic or immune effects. A critical parameter for determining the potential success of these drugs is their efficacy, defined as the log-reduction in the size of the reservoir after a course of treatment. Our modeling work shows how this parameter determines both the time to rebound following treatment interruption and the probability that the infection is completely cleared without rebound (see abstract by DIS Rosenbloom). However, it is difficult to measure this efficacy directly, due to limitations of both the cell culture systems for evaluating drugs in vitro and the sensitivity of assays used on patient samples. Here we present an alternate method for assessing the efficacy of treatment with latency reversing agents, by monitoring changes in residual viral load while these drugs are administered to patients on fully suppressive HAART. Using a model of viral dynamics, we show that drugs with any hope of reducing the reservoir generally cause observable transient elevations in residual viral load. By measuring the size and timing of this peak, one may estimate treatment efficacy. We also show how the viral peak depends on differences between drug-reactivated and naturally antigen-reactivated cells: Slower virion production and shorter cell lifespan in the drug-reactivated cells imply that a smaller peak is produced. The model presented may aid interpretation of clinical trials of latency reversing agents.
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