24th International

HIV Dynamics & Evolution

Promoting discussion between HIV specialists

May 23-26, 2017
Sabhal Mòr Ostaig, Sleat, Isle of Skye, Scotland

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Andrew Leigh Brown
Angela McLean
Emma Hodcroft

Organizing Committee
Jan Albert
Marcia Kalish
Thomas Leitner
Bette Korber
Jim Mullins
Sergei Kosakovsky Pond
Morgane Rolland
Steven Wolinsky
Michael Worobey
Tuesday/Dimàirt, May 23, 2017

5:00pm Registration
6:30pm Dinner
8:00pm Welcome Reception

Wednesday/Dìciadaín, May 24, 2017

7:30am Breakfast
8:30am Fàilte/Welcome

Andrew Leigh Brown

Session I – Latency
Session Chairs: Jim Mullins & Sergei Kosakovky Pond

8.35am NO DIFFERENCE IN CLONAL HIV POPULATIONS IN BLOOD AND LYMPH NODE IN DONORS ON SUPPRESSIVE ART, William R. McManus 1

9.00am MODELING THE DYNAMICS OF HIV REBOUND FOLLOWING TLR7-AGONIST TREATMENT

Jeffrey Gerold 2

9.25am PREDICTING TREATMENT OUTCOMES WITH LONG-ACTING ANTIRETROVIRAL THERAPY

Alison Hill 3

9.50am HIV PROVIRAL LANDSCAPE AND EXPRESSION PATTERNS DISCERNED FOLLOWING EX VIVO EXPANSION, Michael Dapp 4

10.15am General Discussion
10:30am Break

Session II - Latency 2
Session Chairs: Jim Mullins & Sergei Kosakovky Pond

11.00am ESTIMATING THE CONTRIBUTION OF LYMPHOCYTE PROLIFERATION TO HIV RESERVOIR PERSISTENCE, Jeffrey Gerold 5

11.25am META-ANALYSIS OF HIV INTEGRATION SITES REVEALS SIMILAR INTEGRATION PATTERNS IN VITRO AND IN VIVO, AND A STRONG PROVIRUS ORIENTATION BIAS IN VIVO SIMILAR TO THAT OF RETROTRANSPOSONS AND ENDOGENOUS RETROVIRUS ELEMENTS, James Mullins 6

11.50am FOUNDER IDENTIFICATION PIPELINE: AN AUTOMATED TOOL TOWARDS ENHANCED VACCINE EFFICACY ASSESSMENT, Raayya Rossenkhan 7

12.15pm General Discussion
12:30pm Lunch
1:30pm Plenary Presentation: REAL-TIME VIRAL GENOME SEQUENCING FOR OUTBREAK AND EPIDEMIC RESPONSE, Professor Andrew Rambaut

Moderator: Angela McLean

Session III - Epidemiology: patterns
Session Chairs: Marcia Kalish & Stéphane Hué

2.00pm A MODEL-BASED GENETIC CLUSTERING METHOD FOR DETECTING VARIATION IN HIV TRANSMISSION RATES, Art Poon 8
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<td>2.25pm</td>
<td>DETAILED ANALYSIS OF HIV TRANSMISSION CHAINS: INPUT OF ULTRA-DEEP SEQUENCING</td>
<td>Stéphane Hué</td>
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<td>2.50pm</td>
<td>MOLECULAR EPIDEMIOLOGY OF HIV-1 SUBTYPE A IN FORMER SOVIET UNION COUNTRIES</td>
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<td>3.02pm</td>
<td>PHYLOGEOGRAPHY OF THE HIV-1 SUBTYPE G IBERIAN VARIANT: ANCESTRY IN CAMEROON AND SPREAD FROM THE IBERIAN PENINSULA INTO CAPE VERDE AND WESTERN AND EASTERN EUROPE</td>
<td>Michael Thomson</td>
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<td>Break</td>
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<td>4.00pm</td>
<td>NONDISCLOSED MSM LINK TOGETHER IN HIV TRANSMISSION NETWORKS</td>
<td>Manon Raggonnet-Cronin</td>
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<td>4.25pm</td>
<td>IDENTIFYING THE SOURCES OF RECENT HIV INFECTION IN A UK COHORT,</td>
<td>Larissa Mulka</td>
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<td>Nicholos Hbosa</td>
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<td>4.50pm</td>
<td>HIV TYPE 1 GENETIC DIVERSITY, TRANSMISSION NETWORKS AND PHYLOGEOGRAPHIC ANALYSIS OF VIRAL SPREAD IN FISHING COMMUNITIES OF LAKE VICTORIA AND THE NEIGHBOURING GENERAL POPULATION IN UGANDA,</td>
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<td>RECONSTRUCTION OF HIV TRANSMISSION CLUSTERS USING PHYLOSCANNER</td>
<td>Matthew Hall</td>
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<td>Posters with Whisky Tasting</td>
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**Thursday/Diardaoin, May 25, 2017**

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<td>8.30am</td>
<td>DEVELOPMENT OF SIMILAR BNAg SPECIFICITIES IN TWO SUBJECTS INFECTED WITH CLOSELY RELATED STRAINS OF HIV,</td>
<td>Ruchi Newman</td>
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<td>TWO SUBJECTS INFECTED WITH CLOSELY RELATED STRAINS OF HIV,</td>
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<td></td>
<td>Ruchi Newman</td>
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<td>8.55am</td>
<td>SHIV CH505 REPLICATION IN INDIAN Rhesus Macaques and CONVERGENT EARLY ESCAPE FROM AUTOLOGOUS NEUTRALIZING ANTIBODIES,</td>
<td>Peter Hraber</td>
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<td>Peter Hraber</td>
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<tr>
<td>9.20am</td>
<td>IMPUTING HUMAN GENOTYPES FROM VIRAL SEQUENCES,</td>
<td>Jonathan Carlson</td>
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<td>Jonathan Carlson</td>
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<td>9.45am</td>
<td>USING MOLECULAR DYNAMICS TO ILLUSTRATE THE CHANGES IN THE GLYCAN SHIELDS OF TWO HIV-1 ENVELOPE TRIMERS AFTER THE LOSS OF A GLYCAN,</td>
<td>Natasha Wood</td>
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<td>11.00am</td>
<td>GLYCOSYLATION AND DIVERSIFICATION OF HIV-1 ENVELOPE IMPACTS THE EARLY B-CELL LANDSCAPE AND SUBSEQUENT DEVELOPMENT OF NEUTRALIZING ACTIVITY</td>
<td>Abigail Smith</td>
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<td>11.25am</td>
<td>HIV POPULATION-LEVEL ADAPTATION CAN RAPIDLY DIMINISH THE IMPACT OF A PARTIALLY EFFECTIVE VACCINE, Joshua Herbeck</td>
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<td>NEUTRALIZING ANTIBODY SIGNATURES IN HIV-1 ENV AND APPLICATIONS FOR VACCINE DESIGN AND PREDICTING ANTIBODY SENSITIVITY PROFILES, Bette Korber</td>
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<td>12.15pm</td>
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<td>12.30pm</td>
<td>Lunch</td>
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<tr>
<td>1:30pm</td>
<td>Plenary presentation: PrePORTUNITY THAT EUROPE IS IGNORING</td>
<td>Professor Sheena McCormack, Moderator: Andrew Leigh Brown</td>
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**Session VII - Treatment and Public Health**

*Session Chairs: Emma Hodcroft & Jan Albert*

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<td>CLUSTER GROWTH DYNAMICS SUGGEST STRATEGY FOR TARGETED INTERVENTION IN NEW YORK CITY PUBLIC HEALTH HIV-1 SURVEILLANCE REGISTRY, Joel Wertheim</td>
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<td>UNDIAGNOSED HIV-1-CASES IN SWEDEN CLOSE TO 10% UNAIDS TARGET BASED ON A MULTIPLE BIOMARKER ESTIMATE OF INFECTION TIMES, Jan Albert</td>
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<td>ASSESSING THE DANGER OF SELF-SUSTAINED HIV EPIDEMICS FROM PHYLOGENETIC CLUSTER ANALYSIS, Teja Turk</td>
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**Session VIII - Treatment and Public Health 2**

*Session Chairs: Simon Frost & Joel Wertheim*

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<td>PHYLODYNAMIC ANALYSIS TO GUIDE ALLOCATION OF PRE-EXPOSURE PROPHYLAXIS IN THE UNITED KINGDOM, Erik Volz</td>
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<td>VALIDATING THE DSPS-HIV, A HIGHLY-CUSTOMIZABLE AGENT-BASED HIV EPIDEMIC SIMULATOR, AGAINST THE UK HIV EPIDEMIC, Emma Hodcroft</td>
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<td>5.15pm</td>
<td>CHARACTERIZING HIV TRANSMISSION CLUSTERS WITHIN A MIDDLE TENNESSEE CLINICAL COHORT, Ann M. Dennis</td>
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**Friday/Dihaine, May 26, 2017**

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**Session IX - Within-host dynamics and evolution**

*Session Chairs: Angela McLean & Roland Regoes*

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<td>ACCOUNTING FOR DONOR VIRAL DIVERSITY GIVES HIGH ESTIMATES OF THE NUMBER OF HIV FOUNDER VIRIONS IN RECIPIENTS, Robin Thompson</td>
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<td>8.55am</td>
<td>IN VIVO MUTATION RATES AND THE LANDSCAPE OF FITNESS COSTS OF HIV-1 IN CHRONIC INFECTION, Richard Neher</td>
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<td>9.20am</td>
<td>SEX, DRUGS, AND PHYLOGENY: THE ABCS OF A SCIENTIFIC SOAP OPERA, <em>Ethan Romero-Severson</em></td>
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<td>META-ANALYSIS OF PHYLOGENETIC RECONSTRUCTION OF HIV-1 TRANSMISSION IN DOCUMENTED DONOR-RECIPIENT CASES, <em>Thomas Leitner</em></td>
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Session X - Within-host dynamics and evolution
*Session Chairs: Ethan Romero-Severson & Thomas Leitner*

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<td>DUAL HIV-1 INFECTION IN SEROCONVERTERS: PREVALENCE, DETERMINANTS AND BIOLOGICAL EFFECT, <em>Christophe Fraser</em></td>
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<td>RE-EVALUATING THE ROLE OF LATENCY IN HIV EVOLUTION AT THE WITHIN-HOST AND EPIDEMIOLOGICAL LEVELS, <em>Taina Immonen</em></td>
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<td>11.50am</td>
<td>DISSECTING HIV VIRULENCE: HERITABILITY OF SETPOINT VIRAL LOAD, CD4+ T CELL DECLINE AND PER-PARASITE PATHOGENICITY, <em>Roland Regoes</em></td>
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<td>Lunch</td>
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<td>PANGEA-HIV SATELLITE SYMPOSIUM (Everyone welcome)</td>
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<td>HEPATOTOXICITY AND ANEMIA CO MORBIDITY IN TREATED HIV PATIENTS IN FUNDON SUBDIVISION IN THE NORTHWEST REGION OF CAMEROON, <em>Lem Abongwa</em></td>
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<td>EPIDEMIOLOGY OF HIV AMONG YOUNG PEOPLE IN SOUTH KIVU PROVINCE: IMPLICATION FOR PREVENTION, <em>Bita Samuel Alvine</em></td>
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<td>DIVERGENT VARIANTS IN PURE AND RECOMBINANT CONTEMPORARY HIV 1 LINEAGES FROM THE DEMOCRATIC REPUBLIC OF CONGO REVEAL AN OLDER ORIGIN AND A HIGHER DIVERSITY DURING EARLY EPIDEMIC HISTORY OF SUBTYPE C</td>
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<td>NEAR WHOLE GENOME SEQUENCING OF NOVEL HIV 1 RECOMBINANTS FROM CAMEROON, <em>Andrew N. Banin</em></td>
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<td>MODELLING EBOLA VIRUS DYNAMICS: IMPLICATIONS FOR THERAPY, <em>Shingo Iwami</em></td>
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<td>QUANTIFYING THE FITNESS COST OF DRUG RESISTANCE MUTATIONS IN THE SWISS HIV COHORT STUDY, <em>Denise Kühnert</em></td>
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<td>MODELLING DRUG RESISTANCE EMERGENCE AND TRANSMISSION IN HIV 1, <em>Anna ZHUKOVA</em></td>
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<td>OBSERVING EVOLUTION IN HIV 1 INFECTION: PHYLOGENETICS AND MUTANT SELECTION WINDOWS (PHYLOMSW) TO INFER THE INFLUENCE OF THE NATURAL ANTIBODY RESPONSE ON THE VIRAL QUASISPECIES, <em>Carsten Magnus</em></td>
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<td>MULTISCALE, MECHANISTIC PIPELINE TO ASSESS THE PROPHYLACTIC EFFICACY OF HIV COMPOUNDS, <em>Max von Kleist</em></td>
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<td>A HIGHER FRACTION OF DRUG RESISTANT PROVIRUSES EXPRESS UNSPLICED HIV RNA THAN THEIR WILD TYPE PREDECESSORS, <em>Andrew Musick</em></td>
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<td>EMERGENCE OF MUTATIONS IN HIV 1 CRF02_AG ASSOCIATED TO RESISTANCE TO FIRST LINE ANTIRETROVIRAL THERAPY OF REVERSE TRANSCRIPTASE INHIBITORS IN PRE AND POST HAART ERA IN CAMEROON, <em>Judith Torimiro</em></td>
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16 NGS PRESENTS MORE RECENT DIFFICULTIES IN INFERRING VIRAL DIVERSITY
*Matthew Bendall

17 HIV 1M SUBTYPES DISPLAY STRIKING DIFFERENCES IN THE LOCATIONS OF GENOMIC SITES THAT THEY CONTRIBUTE TO RECOMBINANTS, *Marcel Tongo

18 THE PHYLOSCANNER METHOD: PHYLOGENETICS BETWEEN AND WITHIN HOSTS, ALL ALONG THE GENOME, SHOWS TRANSMISSION, DUAL INFECTION, RECOMBINATION AND CONTAMINATION, *Chris Wymant

19 INFRINGEMENT OF DIRECT EPISTATIC INTERACTIONS IN THE HIV 1 GENOME, *Maureen Smith

20 INFERRING DIRECTION OF HIV TRANSMISSION USING NEXT GENERATION SEQUENCING
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21 RAPID AND RECENT TRANSMISSION OF HIV AMONG PEOPLE WHO INJECT DRUGS IN GLASGOW, SCOTLAND REVEALED THROUGH PHYLOGENETIC ANALYSIS
*Manon Ragonnet

22 SOURCE OF HIV 1 DRUG RESISTANT MINORITY VARIANTS IN PEOPLE WHO ARE RECENTLY INFECTED, *Jean Mbisa

23 A BIOINFORMATIC APPROACH TO DETERMINING HIV 1 DRUG RESISTANCE PROFILES IN NEXT GENERATION SEQUENCING DATASETS, *Wei Shao

24 ASSESSING THE ROBUSTNESS OF PHYLOGENETIC MODELS TO CHANGES IN SELECTION PRESSURES OVER TIME: A SIMULATION STUDY, *Hassan Sadiq

25 LACK OF HCV MOLECULAR CLOCK CAUTIONS AGAINST USING GENETIC DISTANCE AS A MARKER OF TIME, *Oliver Laeyendecker

26 HIV 1 SEQUENCES FROM EARLY INFECTION PREDICT THE AGE OF THE INFECTION
*Christopher Owen

27 ASSESSMENT OF NEIGHBOR JOINING AND BAYESIAN METHODS FOR USE IN PHYLOGENETIC ANALYSES OF INTRA PATIENT HIV 1 POPULATIONS, *Michael Bale

28 DECONVOLUTING SEQUENCING ERROR FROM TRUE WITHIN HOST VIRAL DIVERSITY THROUGH PHYLOGENETIC COMPARISON OF ILLUMINA AND NANOPORE SEQUENCE DATA OF HEPATITIS C *David Bonsall

29 EXPLORING MINION NANOPORE SEQUENCING TO INFER THE WITHIN HOST VIRAL DYNAMICS FROM CLINICAL HIV 1 AND HCV SAMPLES, *Damien Tully

30 PRESERVING INTRA PATIENT VARIANCE IMPROVES PHYLOGENETIC INERENCE OF HIV TRANSMISSION NETWORKS, *August Guang

31 A PROFOUND TRANSMISSION BOTTLENECK BETWEEN THE FEMALE GENITAL MUCOSA AND THE BLOOD LEADS TO A HOMOGENOUS SYSTEMIC HIV 1 INFECTION, *Eric Arts
32 ARE HIV PHYLOGENETIC CLUSTERS ENRICHED WITH TRANSMITTING INDIVIDUALS?  
*Tiago Gräf  
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33 LIFE CYCLE SYNCHRONIZATION IS A VIRAL DRUG RESISTANCE MECHANISM  
*Alison L Hill  
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34 COMPARISON OF MICROBIOTA MEDIATED METABOLIC CHANGES IN HIV INFECTION  
*Shinji Nakaoka  
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REAL-TIME VIRAL GENOME SEQUENCING FOR OUTBREAK AND EPIDEMIC RESPONSE

Prof Andrew Rambaut, University of Edinburgh

The 2013-2016 epidemic of Ebola virus disease in West Africa was of unprecedented magnitude, duration and impact. It was also the first large epidemic of an acute disease to be comprehensively studied by virus genome sequencing. More than 1600 Ebola virus genomes, representing over 5% of known cases, were sequenced over the course of the epidemic from nearly all the affected areas. Retrospective analysis of these reveals the factors driving the dispersal, proliferation and decline of Ebola virus throughout the region.

We tested the association of geography, climate and demography with viral movement among administrative regions, inferring a classic ‘gravity' model, with intense dispersal between larger and closer populations. The majority of these genomes were sequenced by the traditional approach of shipping batches of samples to sequencing centers, restricting the use of these to post hoc analyses of the epidemic. However, towards the end of the epidemic, portable genome sequencing instruments were deployed in Sierra Leone and Guinea resulting in genomes being sequenced within days of the diagnostic sample being taken. These sequences allowed information about connections between cases to be extracted in a time frame where it was still relevant, informing the epidemiological response tackling the final, lingering, chains of transmission. This experience points towards a new approach to virus genome sequencing, where molecular epidemiology directly informs outbreak response in real-time.
PREPORTUNITY THAT EUROPE IS IGNORING

Prof Sheena McCormack, University College London

Within Europe, there are countries (or at least cities) that have achieved the 90:90:90 targets, including London. However, there has been little impact on the number of new HIV diagnoses.

The first randomised placebo-controlled pre-exposure prophylaxis (PrEP) trials reported in 2010 – one evaluated tenofovir (TFV) vaginal gel applied before and after sex, the second evaluated oral tenofovir disoproxil fumarate combined with emtricitabine (TDF/FTC). Both observed modest benefit (39-44% reduction in HIV incidence), but subsequent trials observed much higher levels of effectiveness and today biological failure of PrEP is sufficiently rare to warrant an oral presentation at an international conference. PrEP is the missing piece that makes a risk reduction strategy comprehensive as it is an intervention to offer to those at risk of catching HIV who are inconsistent in their use of condoms.

The US FDA was the first regulatory agency to approve Truvada for use as PrEP in July 2012, and implementation commenced. Five years later only three European nations, including Scotland, have implemented PrEP despite approval by the European regulatory agency, evidence of increasing HIV incidence amongst men who have sex with men (MSM), and two European trials showing an 86% reduction in HIV incidence in this key population (PROUD and IPERGAY).

Why is Europe dithering? The simple answer is that the drug is too expensive and the public purse is squeezed, but discussions at the national and European level have revealed a number of practical challenges that have financial implications, and concerns about managing public opinion. Chronic underfunding of civil society has weakened advocacy and activism, but the French have shown how effective the combination of a national community network (AIDES) with a clinical research network (ANRS/INSERM) can be, and the National AIDS Trust succeeded in their legal challenge to establish that the National Health Service of England could be the funding agency for PrEP.

Although there was no PrEP programme in England, MSM started to buy generic drug from online pharmacies in increasing numbers in 2015. Sexual health clinics formally recognised this from September 2015 and in 2016 five central London clinics observed a 42% drop in new HIV infections in MSM (from 928 in 2015 representing 1 in 3 diagnoses in the UK to 540 in 2016). This suggests that we finally have all the necessary tools to control the epidemic, an opportunity that no-one can ignore, and it is hoped that the map of PrEP implementation in Europe will become increasingly green over 2017.
NO DIFFERENCE IN CLONAL HIV POPULATIONS IN BLOOD AND LYMPH NODE IN DONORS ON SUPPRESSIVE ART


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Background: To better understand mechanisms of HIV persistence, we characterized HIV proviral populations, their levels of expression, and their sites of host integration in paired lymph node (LNMC) and peripheral blood (PBMC) samples collected after long-term ART.

Methods: PBMC and LNMC samples were obtained from two donors: Donor 1 initiated ART in chronic infection and had viremia suppression for 5 years; Donor 2 initiated ART in acute infection and had suppression for 13 years. Proviral populations and expression were characterized by single-genome P6-PR-RT sequencing of HIV DNA and cell-associated RNA extracted from aliquots containing one to a few HIV RNA expressing cells. Populations were compared phylogenetically and using a test for panmixia. Infected clones were identified by Integration Sites Assay (ISA) in Donor 1; HIV DNA in Donor 2 was too low for ISA (<1 copy/million cells).

Results: In Donor 1, proviruses in LNMC and PBMC were genetically similar (p=0.7). Similar proviruses, including identical sequences, were also expressed in both locations but at higher levels in LNMC (6% of cells with >10 HIV RNA copies in LNMC vs. 0.4% in PBMC (p=0.003)). Forty different infected clones were detected by ISA without evidence of compartmentalization by location (p=0.8). In Donor 2, hypermutant proviruses were more frequent in LNMC (75% vs. 16% in PBMC; p=0.0007). After excluding hypermutants, proviral populations were completely homogenous, indicating lack of divergence from the founder virus in either location. Both hypermutant and founder proviruses were expressed in LNMC and PBMC.

Conclusions: Comparison of proviral populations, including expanded clones, and their expression in LNMC and PBMC revealed no evidence of compartmentalization. There was also no evidence for divergence from the founder virus in PBMC or LNMC after ART initiation in acute infection. These findings are not consistent with continued viral replication and evolution during suppressive ART in either PBMC or LNMC.
MODELING THE DYNAMICS OF HIV REBOUND FOLLOWING TLR7-AGONIST TREATMENT

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New therapies under investigation, including latent virus reactivation and modulation of immune responses, may delay or prevent HIV rebound when antiretroviral therapy (ART) is stopped. Here we present analyses of viral dynamics in two studies in which SIV-infected macaques were treated with an immunomodulatory compound that stimulates the innate immune receptor TLR7.

In the first study, animals on long-term ART were concomitantly treated with a TLR7 agonist. TLR7 therapy led to large viral blips despite ART, and subsequently reduced reservoirs. After ART interruption, a subset of animals did not rebound, while the majority rebounded after a typical delay. We used a series of mathematical models to interpret these results. First, we determined the reservoir activation rate during TLR7-agonist therapy and estimated the reservoir size before treatment interruption. Analysis of rebound dynamics in treated and control animals revealed that despite similar timing of detectable rebound in both groups, the paths to rebound were significantly different. We found evidence that TLR7-agonist therapy reduced the reservoir even in animals who did rebound but led to more rapid viral growth during rebound. In the treated animals who did not rebound, we estimated the likely reservoir reduction.

In the second study, animals on long-term ART were administered a TLR7 agonist, a therapeutic vaccine, or both. All animals rebounded after ART cessation, but animals treated with the vaccine had reduced viral loads and a subset treated with the vaccine/TLR7-agonist combination completely suppressed viremia after transient rebound. We found that existing mathematical models of HIV/SIV dynamics could not explain the diversity of responses in this study, and we developed a new model of the adaptive immune response. Using the model to analyze these outcomes, we determined the relative contribution of reservoir-reduction and early and late immune responses in the effects of the immunotherapy.
PREDICTING TREATMENT OUTCOMES WITH LONG-ACTING ANTIRETROVIRAL THERAPY

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Long-acting antiretroviral therapy (ART) is currently in development to ease pill burden and increase adherence. This includes injectables, subdermal implants, nanoparticle formulations, and, as recently developed by our group, orally-delivered systems with prolonged gastric residence. Despite the promise, there are many uncertainties including the ideal administration route and release rate, the optimal dose level and frequency, and whether such therapies would increase the risk of developing drug resistance.

To address these challenges, we used mathematical models to simulate ART within individual patients. The pharmacodynamic component relates instantaneous drug levels to suppression of viral replication using ex vivo measurements of efficacy. The viral dynamics component tracks active and latent infection, with drug-level-dependent infectivity rates. Drug-sensitive and resistant viral strains interact by mutation and selection, and are defined by existing in vitro or in vivo data identification of resistance pathways. To model pre-exposure prophylaxis (PrEP), we also developed a mechanistic model of HIV transmission, calibrated to cohort data. Any pattern of patient adherence can be modeled.

We model hypothetical weekly oral long-acting ART with dolutegravir (DTG) or rilpivirine (RPV). Models predict that long-acting DTG and RPV given as maintenance monotherapy are similarly effective as the daily formulations, for a range of adherence levels, and substantially more effective if individuals who missed scheduled doses can decide to restart any day of the week. The fraction of treatment failures accompanied by resistance increases with long-acting RPV but not DTG. For PrEP, our model reproduces the dose-dependent efficacy of tenofovir-based regimens, and predicts that DTG could be highly-effective as PrEP, with over 95% relative-risk-reduction in transmission with perfect adherence and over 50% up to 10 days after the last dose.

This work provides a framework to quantify the risks-benefit trade-off of long-acting ART, provide recommendations during pre-clinical development, and prioritize dose regimens for clinical evaluation.
HIV PROVIRAL LANDSCAPE AND EXPRESSION PATTERNS DISCERNED FOLLOWING EX VIVO EXPANSION


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The standard assay to estimate latent HIV reservoirs in PBMC is the quantitative viral outgrowth assay (QVOA). QVOA underestimates reservoir size due to the presence of intact proviruses (IPs) resistant to activation by one or more mitogens and latency reversal agents. In contrast, viral DNA load assays overestimate reservoir size, as most infected cells harbor defective proviruses. IPs have been variously estimated to correspond to 3-11% of provirus populations, however, we argue that these are overestimates due to the PCR methods employed. More accurate measurements are therefore needed to define reservoirs and to assess the efficacy of cure strategies. We used two newly developed assays to assess the composition and functionality of HIV reservoirs – the infected cell expansion (ICE) assay, that employs culturing infected cells at limiting dilution with PHA, IL2 and antiretroviral drugs to block virus spread, followed a 3- or 6-probe viral ORF detection assay (VODA). These assays permit an accurate view of complete proviral structures and integration sites, although they too introduce potential bias by requiring cell outgrowth and survival in cell culture prior to analysis. We found that <1% of infected cells from 4 individuals that initiated long-term ART early in infection harbor IPs. Proviruses from ICE cultures derived from the same proliferating cell population in vivo had the same genome structure, and similar transcription and DNA methylation patterns, indicating provirus stability in culture. The frequencies of clonal cell populations detected by ICE are similar to the frequencies observed in vivo, suggesting a lack of selective loss of these cells during ICE. Indeed, cells found to be proliferating in vivo were often overrepresented relative to uninfected cells in ICE cultures, suggesting that cells found to be proliferating in vivo have a growth advantage in vitro. The vast majority of cells we examined harbored non-infectious proviruses, most commonly resulting from deletions between the 5’LTR and gag. Targeted analysis of RNA transcripts at proviral loci showed that deletions in the 5’LTR was associated with 3’LTR-initiated transcription and read-through into cellular sequences. Our results suggest that QVOA comes closer to estimating the true size of the reservoir in blood cells than currently thought. ICE and VODA are likely to broadly assist further investigation and understanding of latent reservoirs as well as the possible role of defective proviruses influencing T-cell proliferation and functionality.
Estimating the Contribution of Lymphocyte Proliferation to HIV Reservoir Persistence

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Despite suppressive antiretroviral therapy (ART), HIV persists in infected individuals as provirus integrated into the genomes of resting memory CD4+ T cells. Long-term studies have shown that the replication-competent viral reservoir is remarkably stable during ART, decaying with a half-life of approximately 4 years. The relative importance of intrinsic longevity of individual resting memory lymphocytes versus homeostatic proliferation of provirus-containing cells in maintaining this stability remains unknown. However, the mechanism of persistence has important implications for therapy. For example, the efficacy of strategies designed to bias the turnover of latently infected cells towards extinction depends critically on this population’s intrinsic dynamics. Recent investigations of HIV integration sites provide a window into the dynamics of latent infection. HIV is known to integrate randomly into the human genome, making the frequency of multiple integrations at the same site extremely low. Yet in samples of hundreds-to-thousands of latently infected cells, the same integration site can be found multiple times. Here, we develop a method to use the observed distribution of proviral integration sites to infer a quantitative estimate for the proliferation rate of latently infected cells. Using a branching process model and a Bayesian statistical framework, we inferred proliferation and death rates of cells tagged by HIV integration. Data from the Retrovirus Integration Database yielded median a posteriori estimates of proliferation rates in the range of 1.5-5.3 divisions per year, significantly larger than the net rate of latent reservoir decay (~0.2/year). We also tested consistency of the data with our model of homogenous dynamics versus models that allowed a distribution of proliferation rates between different clones (due to, perhaps, integration into genes related to cell survival). Our results suggest that a reduction in proliferation of latently infected cells could substantially decrease the half-life of the reservoir.
META-ANALYSIS OF HIV INTEGRATION SITES REVEALS SIMILAR INTEGRATION PATTERNS IN VITRO AND IN VIVO, AND A STRONG PROVIRUS ORIENTATION BIAS IN VIVO SIMILAR TO THAT OF RETROTRANSPOSONS AND ENDOGENOUS RETROVIRUS ELEMENTS


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Recent studies have shown that HIV provirus integration sites (IS) are associated with the proliferation of infected cells during ART. We compared the IS landscape from cell culture (in vitro) infections (N=56,637) to cells from individuals on ART (in vivo) (N=5,935).

For both datasets:
1) The distributions of IS were similar across chromosomes and proportional to gene density.
2) Most IS were found within genes (86.3% of all IS), and in introns (76.5%) relative to exons.

The in vivo dataset was unique in showing biases for IS in:
1) Genes and gene pathways associated with cancer, T-regulatory cell function, and T cell activation and function,
2) reverse proviral orientation with respect to the transcriptional orientation of the genes (60% of IS, p=0.005), and in
3) BACH2 (and a limited number of other genes). BACH2 had the most frequently observed IS in vivo, but all were in the same orientation as the gene.

Reverse orientation biases have previously been reported for endogenous retrovirus (ERVs) and retrotransposon integrations. Reverse orientations are less likely to disrupt the cellular gene since those in the same orientation insert polyadenylation signals and splice sites. Hence, the orientation bias we observed in vivo can be explained most simply by selection for maintenance of gene function at the IS locus and thus cell survival.

That proviral integration sites contribute to cell proliferation is strongly suggested by the biases observed for IS in genes associated with T cell survival and proliferation described here, our past studies (Wagner, Science, 345:570, 2014), and the preliminary observation that cells found to be proliferating in vivo have a growth advantage in cell culture (see abstract by K. Kim et. al.). Nonetheless, our studies to date do not rule out that some proliferating cells result from specific antigenic stimuli and/or homeostatic proliferation, regardless of the site of viral integration.
FOUNDER IDENTIFICATION PIPELINE: AN AUTOMATED TOOL TOWARDS ENHANCED VACCINE EFFICACY ASSESSMENT


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Analysis of sequences sampled from HIV-infected trial participants is essential for assessing the endpoints of ongoing clinical trials to evaluate both vaccine and antibody interventions. Sequence-informed estimates of the date of infection are especially important for evaluating time-varying correlates of protection, such as blood antibody concentration. Comparative sequence analysis across trial arms (“sieve analysis”) typically requires prior estimation of the ancestral sequence(s) of each participant’s circulating viral population. A reproducible, standardized and calibrated method for estimating three key unknowns: multiplicity, founder sequences and infection time is critical to elucidate the molecular basis of HIV-1 transmission and for improving vaccine design.

We built a pipeline that provides estimates for each of the three previously mentioned unknowns. The required input is a codon-aligned nucleotide FASTA file from a single host at an early time-point, 1-6 months post-infection. Hypermutated and recombinant sequences are removed automatically. A synonymous-only and within-cluster variant of Poisson Fitter is applied to obtain a timing estimate. Multiplicity and founder sequences are estimated from a phylogeny that is reconstructed using maximum likelihood. Results include an output table containing statistics for founder multiplicity, both nucleotide and amino acid founder (ancestral) sequences, and time since infection estimate.

An extensive calibration process, reported previously, was used to select optimal methodologies and to inform the selection of default settings that minimize prediction error. Optionally, viral loads and infection timing bounds, ascertained from diagnostic test algorithms, can be supplied for higher accuracy. When applied to Illumina sequences from the envelope V3 region of 21 South African women from the CAPRISA 002 study (sampled approximately 6 months post infection), the area under the ROC curve for the multiplicity estimation was 0.99 and the time since infection was estimated with a RMSE of 9 days.
A MODEL-BASED GENETIC CLUSTERING METHOD FOR DETECTING VARIATION IN HIV TRANSMISSION RATES

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Genetic clustering has become a popular technique for identifying potential outbreaks of transmission from HIV sequence data collected through routine drug resistance genotyping. A diverse number of nonparametric clustering methods have been applied to this purpose, but we have observed that these methods may be biased to detect variation in rates of sampling instead of transmission. We describe a new model-based approach to extract clusters from virus phylogenies based on a Markov-modulated Poisson process (MMPP).

We used the MMPP to model variation in branching rates as a continuous-time Markov chain with a finite number of rate classes. The likelihood of a tree under this model was calculated by Felsenstein and Pupko’s algorithms with censored terminal branches. Maximum likelihood parameters were estimated by a covariance matrix adaptation evolution strategy implemented in C. To evaluate the MMPP method alongside current nonparametric clustering methods, we used MASTER to simulate trees under a compartmental epidemic model with varying rates of transmission and/or sampling among subpopulations. Sequence evolution was simulated along these trees using INDELIBLE, and phylogenies were reconstructed by neighbor-joining or maximum likelihood.

The nonparametric methods were generally ineffective at detecting variation in transmission rates – for example, patristic distance clustering averaged ~50% sensitivity at 80% specificity. On the same data, our method clustered transmission rates with a mean sensitivity and specificity of 82% and 90%, respectively. However, it was uninformative about variation in sampling rates. Our program required about 35 seconds to process a tree with 1000 tips.

There is rapidly growing interest in using genetic clustering to inform public health decisions in HIV prevention. We describe a significant and pervasive deficiency in nonparametric clustering methods that may be overcome by a model-based approach. Source code for this program is available at http://github.com/rmcclosk/netabc.
DETAILED ANALYSIS OF HIV TRANSMISSION CHAINS: INPUT OF ULTRA-DEEP SEQUENCING

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Background. The net value of Ultra Deep Sequencing (UDS) over Sanger sequencing (SS) for phylogenetic HIV transmission studies remains to be established, particularly in the context of transmitted drug resistance (TDR). We explored the epidemiological linkage of a cohort of HIV positive men who have sex with men (MSM) using both approaches, and evaluated their impact on transmission and TDR detection.

Methods. Reverse transcriptase, protease and integrase sequences were obtained by SS and UDS from 70 HIV-1 infected, treatment-naïve MSM diagnosed between January 2012 and July 2013 in Paris. Pairwise genetic distances and maximum likelihood phylogenies were computed from both datasets. Transmission events were identified as clades with branch support ≥70% and intra-clade genetic difference <2.5%. TDR mutations were recognised from the consensus list of TDR surveillance. Transmission directionality, directness and inoculum size were inferred from the tree topologies.

Results. SS and UDS data concurred in the identification of 7 transmission pairs and 1 cluster of 3 patients. With UDS, direct linkage and direction of transmission was unambiguous inferred in 3/7 and 2/7 pairs, respectively. The polyphyletic nature of the sequences from 3/7 suggested multiple founder viruses and no unobserved intermediary links. By SS, the prevalence of TDR mutations in the linked patients was 5.7% and 13% with SS and UDS, respectively. No minority resistant variants were transmitted.

Conclusion. While SS and UDS identified the same transmission chains, UDS allowed a better resolution of transmission events despite a weak phylogenetic signal. These results highlight the benefits of UDS data in the phylogenetic identification of transmission chains, allowing the inference of direct linkage and multiplicity of founder viruses in the recipients, and potentially of direction of transmission.
MOLECULAR EPIDEMIOLOGY OF HIV 1 SUBTYPE A IN FORMER SOVIET UNION COUNTRIES

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BACKGROUND: While in other parts of the world it is on decline, incidence of HIV infection continues to rise in the former Soviet Union (FSU) countries. HIV epidemic in this region was initially driven by people who inject drugs (PWID), and then spread into heterosexual population, where now the prevalence has surpassed that in PWID in most countries.

OBJECTIVE: The present study was conducted to investigate the patterns and modes of HIV transmission in the FSU countries.

METHODS: To characterize the regional HIV epidemiology we analyzed 2093 publicly available HIV-1 subtype A pol sequences (HXB2 nucleotides: 2622-3252) from seven FSU countries, namely Armenia, Azerbaijan, Georgia, Kazakhstan, Kyrgyzstan, Russia, and Uzbekistan. The phylogenetic trees were constructed using MEGA 6.0, and modified using Figtree v1.4.2.

RESULTS: Our phylogenetic analysis showed that the clusters from FSU countries were intermixed, indicating a possible role of cross-border migration in HIV transmission. Further analysis of phylogeny in the context of high risk behavior showed that injection drug use was the most frequent mode of transmission. Furthermore, the clusters from PWID and heterosexual transmission were intermixed, indicating bridging of HIV infection across populations.

CONCLUSION: Major HIV transmission routes in FSU countries appear to be through cross-border migration, injection drug use and heterosexual behavior. To control the expanding HIV epidemic in this region, harm reduction strategies should be focused on high risk groups associated with these three modes of transmission.
PHYLOGEOGRAPHY OF THE HIV-1 SUBTYPE G IBERIAN VARIANT: ANCESTRY IN CAMEROON AND SPREAD FROM THE IBERIAN PENINSULA INTO CAPE VERDE AND WESTERN AND EASTERN EUROPE


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An HIV-1 subtype G variant widely circulates in Portugal, and, with lower prevalence, in Spain. Previously, we determined that the Iberian subtype G variant (GIb) originated in Cameroon and migrated from Portugal to Spain. Subsequently, new subtype G sequences from different countries have become available, notably from Cape Verde and Europe. Using data base sequences, other authors (de Pina-Araujo et al. 2015, PLoS ONE 10: e0127384) recently proposed that the subtype G variant circulating in Portugal migrated from Central Africa, suggesting an Angolan ancestry, to Cape Verde, and from there to Portugal. Here we perform new analyses, using all subtype G protease-reverse transcriptase sequences from Africa and Europe deposited in data bases and four newly obtained from Moscow, Russia. Maximum likelihood phylogenetic analyses were performed with PhyML, assessing node support with the aLRT. Ancestry was analyzed using the Bayesian method implemented in BEAST, estimating migration pathways with the Bayesian stochastic search variable selection approach. The GIb clade included sequences from Portugal, Spain, France, United Kingdom, Belgium, Luxemburg, Poland, Denmark, Romania, Russia, Cyprus, Cabo Verde and Tunisia, with sequences from Denmark (n=7), Romania (n=11), and Russia (n=5) grouping in respective monophyletic clusters, and was nested in a clade that included sequences from 7 sub-Saharan African countries. GIb origin was estimated in Portugal (PP=0.84), around 1985, and its ancestry in Cameroon (PP=0.83). tMRCA of Danish, Russian and Romanian clusters were estimated around 1994, 1996 and 2004, respectively. Well supported (BF>6) migration pathways were from Cameroon to Portugal; from Portugal to Spain, Cape Verde, and Denmark; from Spain to Cape Verde and Romania; and from Denmark to Russia. In conclusion, using newly available sequences, we confirm the ancestry of HIV-1 GIb variant in Cameroon and its origin in Portugal, with further diffusion from the Iberian Peninsula into Cape Verde, Romania, Denmark, and Russia.
NONDISCLOSED MSM LINK TOGETHER IN HIV TRANSMISSION NETWORKS

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Phylogenetic analysis has revealed the existence of self-reported heterosexual (HET) men within networks dominated by men who have sex with men (MSM). We characterised this group. HIV pol sequences were obtained from the UK HIV Drug Resistance Database and from public databases. Clusters with a maximum genetic distance of 4.5% were selected in maximum likelihood phylogenies then time-resolved in BEAST. Networks were created by linking nodes if sequences shared a phylogenetic ancestor ≤5 years. Self-reported heterosexual men who clustered only with men were classed as potential nondisclosed MSM (pnMSM). We compared the centrality (a measure of connectedness) of pnMSM and MSM and calculated assortativity (the propensity for nodes sharing attributes to link) by self-reported risk group. Finally, we evaluated whether pnMSM linked MSM and heterosexuals.

Network analysis allows for multiple subtypes to be analysed concomitantly and >50,000 subtype A1, B and C pol sequences were analysed here. Of these, 14,405 linked within 5 years and were represented in the network, including 8,452 MSM, 1743 female HET and 1341 male HET. We identified 223 network clusters comprising only men: 955 MSM and 249 pnMSM. pnMSM represented 18.6% of linked self-reported heterosexual men, more than twice the proportion of women clustering with MSM (131/1743; 7.5%). Betweenness centrality was lower for pnMSM than for MSM (2.37 vs 4.11, p<0.005), underlining their peripheral position in MSM clusters. Assortativity by risk group was higher than expected (-0.124 vs -0.196, p=0.05) indicating that pnMSM linked to each other. Self-reported male heterosexuals were much more likely than female heterosexuals to link MSM and heterosexuals (Fisher’s exact test; p<0.0005; OR 2.24).

pnMSM appear to have fewer partners and to partner preferentially with other pnMSM. They are at higher risk for HIV than heterosexuals and may put female partners at risk by linking the MSM and heterosexual epidemics.
IDENTIFYING THE SOURCES OF RECENT HIV INFECTION IN A UK COHORT

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Background Between 13-23% of HIV infections are undiagnosed in the UK, however they account for up to 82% of transmissions. A previous phylogenetic analysis found 74% of recent HIV infections (RHI) within Brighton, UK, had no likely transmitter, suggesting an undiagnosed source. We aim to identify sources of RHI more accurately within this cohort, to determine whether real time phylogenetic reconstruction is a feasible component of intervention in this population.

Methods Subtype B sequences were retrieved from the Brighton population (predominantly MSM), diagnosed 1981-2015 (n=1,840) and the most similar UK and global sequences obtained. A maximum likelihood tree was built in RAxML (GTR+Γ), with dated phylogenies reconstructed in BEAST. Demographic and clinical data was collected for Brighton patients, including available CD4 and viral loads, STIs, AIDS diagnoses and antiretroviral history. RHI were identified using testing history and serological markers. Likely transmitters to RHIs were identified according to an algorithm considering phylogenetic and clinical data at transmission. Chronic infections linked to RHI, undiagnosed during transmission with appropriate parameters were considered potential transmitters.

Results 389 RHI were identified, for which a likely transmitter was identified for 189(49%). 178(94%) transmitters were male, 170(90%) were white. 174(92%) transmissions were between MSM. 111(59%) infections were acquired from the local population, 77(41%) from elsewhere in the UK; 31 from the nearest major city (London), and one from America. 26(14%) were diagnosed shortly after the transmission period midpoint. 139 were linked to a potential transmitter, 108 being undiagnosed at the time of transmission. 61 RHIs had no potential source.

Conclusions A significant proportion of HIV transmissions are acquired from outside the local population, suggesting a previous overestimation of the burden of undiagnosed transmitters in this cohort. Nevertheless, 50-58% have acquired HIV from an unknown, possibly undiagnosed source and this remains an important focus for public health interventions.
HIV TYPE 1 GENETIC DIVERSITY, TRANSMISSION NETWORKS AND PHYLOGEOGRAPHIC ANALYSIS OF VIRAL SPREAD IN FISHING COMMUNITIES OF LAKE VICTORIA AND THE NEIGHBOURING GENERAL POPULATION IN UGANDA.


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Fishing Communities (FCs) in Uganda are disproportionately affected by HIV-1 with prevalence and incidence rates 4-5 times and 5-10 times higher than the national average respectively. However the patterns of HIV-1 transmission in the FCs are not well enough understood to implement targeted interventions. A cross-sectional community-based survey was implemented in 8 FCs of Lake Victoria and 2 general population (GP) cohorts in Uganda.

HIV-1 partial pol sequences from 606 individuals were analysed by phylogenetic methods and complemented by participants’ socio-demographic data. Sequences were classified into viral subtypes and maximum likelihood trees generated by RAxML were analysed using Cluster Picker to identify networks. Time-calibrated phylogenies in BEAST were used to estimate HIV transmission times. A phylogeographic analysis was visualized in SPREAD to dissect the viral diffusion.

In the FCs and GP, HIV-1 Subtype A1 (46% and 50%) was the major viral strain followed by subtype D (33% and 34%). Initial network analysis at £4.5% genetic distance (GD) and ³90% bootstrap support (BS) showed that 13% (81/606) of sequences grouped into 35 transmission pairs and 3 clusters. At £1.5% GD and ³90% BS, 4%(26/606) of sequences grouped into 13 distinct pairs. Half (54.5%, 6/11) of the transmission pairs represented recent transmissions with a ‘pair depth’ of 5 months corresponding to the estimated time-to-infection. Phylogeography revealed a localized viral diffusion pattern (posterior probabilities >0.9) with strong support (Bayes Factor³100) for viral migration between FCs in Mpigi and Kampala.

Among FCs of Lake Victoria, Uganda, HIV-1 transmission occurred largely within communities with limited inter-community viral dispersal. HIV-1 subtype A1 predominates in the FCs and GP respectively followed by subtype D and other inter-subtype recombinants. Consequently the design and implementation of interventions in FCs should prioritize high-risk groups in networks associated with recent HIV-1 transmission and target transmission hotspots with recurrent viral migration.
A common approach when investigating HIV transmission using viral sequence data has been to define clusters based on a genetic distance threshold between consensus sequences derived from each infected individual. This approach produces cluster graphs which are dense, having many more edges than the plausible number of infection events, and cannot suggest the direction in which the virus moved between hosts, or give a confidence level for the inferred associations between those hosts.

The increased resolution granted by next-generation sequencing (NGS) techniques have allowed us to refine the approach to this problem. When multiple sequences are available from infected individuals, the topology of a phylogeny built from all those sequences can indicate the direction of ancestries amongst the pathogens infecting them. This in turn indicates the direction of links in the transmission chain between the individuals themselves, with or without unsampled intermediate individuals in that chain. When combined with a genetic distance threshold we can then identify groups of patients infected with closely-related viruses and reconstruct the direction of transmission between them.

This approach is implemented in our tool phyloscanner, which analyses short-read NGS data by sliding a window across the genome and building separate phylogenies for the reads overlapping each window. Subsequently, a partial transmission tree is reconstructed from each phylogeny using a maximum-parsimony approach. Variation in the reconstructions across windows allows us to assess the strength of inferred cluster membership and directionality in the face of phylogenetic uncertainty.

We use HIV genomic data from the BEEHIVE study as a demonstration. We establish that, where phyloscanner suggests a direction of transmission, this is usually in concordance with the order of dates of sampling and first infection of the relevant individuals. We then present the results of an exploration of the clusters identified from a dataset of around 2600 patients.
DEVELOPMENT OF SIMILAR BNAB SPECIFICITIES IN TWO SUBJECTS INFECTED WITH CLOSELY RELATED STRAINS OF HIV


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The rational design of HIV immunogens that trigger the development of broadly neutralizing antibodies (bNabs) requires understanding the viral determinants and evolutionary pathways that influence this process. We sought to identify subjects infected with similar strains of HIV who develop bNAb specificities to characterize the evolutionary pathway(s) giving rise to this response.

HIV env was amplified from patient plasma and sequenced utilizing both next generation sequencing and single genome amplification (SGA). For subjects AC049 and AC053, pseudoviruses expressing Env genes isolated both prior to and following the development of bNAb activity, were used to assess sensitivity to common bNAb specificities including CD4bs, V1/V2, glycan-V3 and MPER.

Phylogenetic analysis of env sequences from our Boston Acute cohort identified 10/141 subjects comprising a putative transmission cluster, including subjects AC049 and AC053 who were previously shown to exhibit bNAb responses (Mikell et al, 2011). SGA sequences confirmed their infection by a highly similar strain, but also revealed dual infection in subject AC049. Early Env pseudoviruses from AC049 and AC053 were sensitive to neutralization by V1/V2-specific PG9/PG16 antibodies and both developed >2-log resistance to these antibodies at later times. While the prototypic N160 PG9 contact site remained intact in both subjects, mutations in and around other known PG9/PG16 contact sites arose through shared and unique pathways. Additionally, while both AC049 and AC053 envelopes display similar sequence evolution in the MPER epitopes, AC053 developed >1-log sensitivity to MPER antibodies 4E10 and 10E8, while AC049 develops resistance to the same monoclonal antibodies. Further efforts to define the env mutations governing development and escape from these responses are underway.

Identification of two individuals infected with a highly similar HIV strain, both of which appear to develop similar bNAb responses, provides remarkable insight into how shared and unique bNAb responses can evolve from a similar Env antigen.
SHIV CH505 REPLICATION IN INDIAN Rhesus Macaques AND CONVERGENT EARLY ESCAPE FROM AUTOLOGOUS NEUTRALIZING ANTIBODIES

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SHIVs designed from transmitted/founder (T/F) HIV-1 Envs, which in humans elicited bNAbTs or bound to germline unmutated common ancestors (UCAs) of bNAbTs, can be used as challenge strains to study desirable bNAbs epitopes. Here we describe sequence evolution in rhesus macaques experimentally infected with a SHIV construct clade-C TF Env from the human donor CH505. We compare Env evolution away from the TF Envs in the human and macaque infections. We substituted complete tat-rev-vpu-env (gpl60) or vpu/env (gpl40) T/F HIV-1 cassettes into the SIVmac766 T/F SIV backbone. Substitution of one naturally occurring amino acid at position 375 in the Phe43 binding pocket increased rhCD4 affinity without altering antigenicity or neutralization sensitivity. Infection of RMs with SHIV CH505 recapitulated early viral kinetics, Nab development, and early escape in the human infected by an identical TF envelope. Monospecific NAb responses toward loop D N276 led to early virus escape mutations in two macaques. In a third, a different NAb response was directed at V1 and V5. We found remarkable convergence of mutational events between the human CH505 and three macaques infected with CH505-TF-Env SHIV. Convergence included regions under antibody-driven positive selective pressure in CH505. Specific amino acid changes and precise indels occurred in both. Selection for CTL escape in CH505 was not recapitulated in the RMs, a counterpoint to antibody-related regions. For vaccine design, a particular Env may induce similar early immune responses across individuals. Vaccine-induced antibody responses across hosts may be partially predictable. SHIVs that parallel immunopathogenesis of corresponding HIV-1 Envs in natural human infection can facilitate future work in HIV vaccine development.
IMPUTING HUMAN GENOTYPES FROM VIRAL SEQUENCES

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The HLA-mediated immune response results in the predictable selection of adaptive mutations in HIV. Given large enough datasets and appropriate statistical models, the specific amino acids that are under selection from specific HLAs can be inferred. For example, in one study of chronically subtype B infected, antiviral-naïve subjects, we identified 1,923 HLA-amino acid associations, that covered 725 unique sites across all proteins in HIV. Importantly, different HLA subtypes tend to select for distinct amino acids, even when presenting the same epitope. Thus, in the context of chronic infection, substantial information about host HLA types appears to be imprinted upon the HIV genome.

We recently developed a probabilistic model that estimates the sampling distribution of HIV sequences as a function of host HLA haplotype, denoted p(HIV | HLA). Here we explore whether the posterior distribution, p(HLA|HIV) ~ p(HIV | HLA)p(HLA) can be used to impute host HLA alleles from viral sequence alone. We use as our prior distribution published, ethnicity-specific, estimated haplotype frequencies (and marginalize over ethnicity when unknown).

On holdout data, the posterior probabilities were well calibrated and ROC curves demonstrated average per-allele AUC values of 85%; 15 alleles achieved AUC>95%. When applied to longitudinal sequences from linked transmission pairs, recipient HLA types could not be imputed until 6 months post-infection. Moreover, HLA imprints from the donor’s HLA alleles dominated the recipient’s sequences: even at 24 months post-infection, the donor’s alleles were easier to impute than the recipient’s (donor AUC=0.81, recipient AUC=0.67, p<2x10-5). When applied to sequences in the Los Alamos database, 49% of clade B and 38% of clade C sequences yielded at least one high confidence (>90%) 4 digit HLA imputation call. Notable examples included HXB2 (B0702-C0702, 95%) and MJ4 (A74, 82%).

The ability to impute host genetic data from viral sequences alone has some interesting implications. First, it highlights the extent to which host genetic information is imprinted on the viral genome. Second, in scenarios in which HLA types are unavailable, this approach allows fuzzy HLA assignment to individuals and sub-populations, which may inform population-level parameter estimates. Third, the ability to impute donor HLA alleles suggests an approach to inferring the direction of transmission, and possibly whether the donor was chronically infected at the time of transmission. Finally, imputation of human genetics from viral sequences has ethical implications in scenarios where the latter are publicly released but the former are withheld out of concerns for patient privacy.
USING MOLECULAR DYNAMICS TO ILLUSTRATE THE CHANGES IN THE GLYCAN SHIELDS OF TWO HIV-1 ENVELOPE TRIMERS AFTER THE LOSS OF A GLYCAN.

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The glycan shield of the HIV-1 envelope trimer forms a barrier between the virus and humoral immune response, thereby protecting the virus from neutralisation. As the glycoprotein mutates to escape further immune attack, the composition of the glycan shield can vary substantially between isolates and over time. The loss of a single glycan on the viral surface, or the shift of a glycan by only a few residues, has been shown to have a significant effect on the neutralisation sensitivity and antibody breadth developed. Here, we analyse the changes in the glycan shields of two HIV-1 envelope glycoproteins when a single glycan-site mutation (N301A) is introduced. The antibody neutralisation assays for these two viruses indicated that the loss of the N301 glycan resulted in a shift from resistant to sensitive for only one of the isolates, which suggests that further structural features and glycan interactions influence the conformation of the glycan shield. Trimeric homology models were generated using these subtype C pseudovirus sequences (~90% identity) and their N301A mutants. All four models were computationally glycosylated with high-mannose glycans (Man-9) and molecular dynamics simulations were carried out for 500ns using Amber 14.

Our results show a significant difference between the dynamics of glycans on the original and N301A mutant models. We observe a difference in the range of movement for some glycans, as well as a change in their interaction with adjacent glycans (whether within or between monomers), which forms one explanation for the variation seen in the experimental neutralisation assays.

This study illustrates the complexity of the glycan shield, where the impact of a loss of a glycan depends on the potential of the remainder of the glycan shield to compensate for this loss through structural rearrangement. Such rearrangements, however, can have a substantial effect on the virus' neutralisation profile.
Neutralizing antibodies provide protection against many viral diseases, and are desirable for an HIV vaccine, but the immunological processes favoring their generation amid competing antigen-specific B cells have not been elucidated. Here, we investigated the nexus of the transmitted-founder (T/F) envelope (Env), early Env diversification, monoclonal antibodies (mAbs) specific for the T/F Env, and autologous and heterologous neutralization in 21 participants identified during recent infection at two African sites. Sequence analysis revealed that a lower ratio of NXS to NXT-encoded glycan motifs in the T/F Envs correlated with neutralization breadth. Further analysis comparing substitutions, insertions/deletions, and glycan motif alterations between the T/F and autologous early Env variants revealed that diversification in the V2, V4, and V5 regions of gp120, accompanied by contemporaneous viral escape, favored the development of breadth. These results suggest that more efficient glycosylation of subtype A and C T/F Envs is more likely to elicit antibodies that can transition from autologous to heterologous neutralizing activity following exposure to gp120 diversification. To investigate this further, mAbs specific for the T/F gp120 (n=149) were recovered 7 months after infection from two subjects with disparate levels of plasma neutralization capacity. Striking genetic and functional differences in the antigen-specific antibody landscapes of the two individuals were observed. Robust CD4 binding site neutralizing antibodies arose in one individual amongst balanced and diverse heavy and light chain germline gene usage and pairing, low affinity antigen binding, and weak clonal competition. In contrast, neutralizing antibodies did not develop in the other individual where the environment exhibited high affinity, clonally expanded antibodies that vigorously competed for binding to an immunodominant epitope. These results illustrate how the interplay between Env immunogenicity and the ensuing B cell microenvironment during early HIV-1 infection can promote immunodominance or B cell diversity, which can influence the development of neutralizing antibodies.
HIV POPULATION-LEVEL ADAPTATION CAN RAPIDLY DIMINISH THE IMPACT OF A PARTIALLY EFFECTIVE VACCINE


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Development of an HIV vaccine is essential to ending the HIV/AIDS pandemic. However, vaccines can result in the emergence and spread of vaccine-resistant strains. Indeed, analyses of breakthrough infections in the HIV vaccine trial RV144 identified HIV genotypes with differential rates of transmission in vaccine and placebo recipients. We hypothesized that, for HIV vaccination programs based on partially effective vaccines similar to RV144, HIV adaptation will diminish the expected vaccine impact. Using two HIV epidemic models, we simulated large-scale vaccination programs and, critically, included HIV strain diversity with respect to the vaccine response. We show here that rapid population-level viral adaptation can lead to decreased overall vaccine efficacy and substantially fewer infections averted by vaccination, when comparing scenarios with and without viral evolution (depending on vaccination coverage, vaccine efficacy against the sensitive allele, and the initial resistant allele frequency). Translating this to the epidemic in South Africa, a scenario with 70% vaccination coverage may result in 250,000 new infections within 10 years of vaccine rollout that are due solely to HIV adaptation, all else being equal. These findings suggest that approaches to HIV vaccine development, program implementation, and epidemic modeling may require attention to viral evolutionary responses to vaccination.
NEUTRALIZING ANTIBODY SIGNATURES IN HIV-1 ENV AND APPLICATIONS FOR VACCINE DESIGN AND PREDICTING ANTIBODY SENSITIVITY PROFILES


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We systematically resolved HIV Envelope (Env) genetic signatures of ~50 broadly neutralizing antibodies (bNAb), identifying recurrent genetic signatures across bNAb with shared specificities. This provided an overview of the imprint of neutralizing antibody escape on HIV evolution both within and outside of the direct contact regions. We defined signatures for four antibody classes: those that target the CD4 binding site, the V2 apex, the V3-glycan region, or the MPER. Signature sites were identified using a phylogenetically corrected method to resolve specific amino acids and glycosylation sites in particular positions in Env associated with bNAb sensitivity/resistance. Env hypervariable region characteristics and subtype associations with neutralizing antibody sensitivity were also explored. These signatures both recapitulate prior findings and present many novel associations.

We next used these bNAb signatures in two clinically relevant applications. First, we used them to provide a focused framework to inform machine learning predictions of neutralization activity for specific bNAb/Env combinations based on Env sequence data. Depending on the bNAb, some predictions were quite accurate and so may be useful for screening or enabling interpretation of clinical studies that use bNAb as a therapeutic agent. Second, the V2 glycan and V3 glycan bNAb signatures were used as a roadmap for introducing modifications into Env for vaccine design. The signature-based vaccine immunogens gave enhanced breadth and potency against difficult to neutralize tier 2 viruses when experimentally tested in guinea pigs and compared to a wildtype immunogen.
CLUSTER GROWTH DYNAMICS SUGGEST STRATEGY FOR TARGETED INTERVENTION IN NEW YORK CITY PUBLIC HEALTH HIV-1 SURVEILLANCE REGISTRY

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All persons newly diagnosed with HIV in New York City are offered linkage to care and notification, testing, and referrals of named partners, and, more recently, “partners” with highly similar pol sequences who are viremic or out of care. The ability to use routinely reported genotype data to target rapidly growing clusters of genetically linked persons has the potential to improve outcomes in hyperendemic populations. It has been suggested that genetic clusters may be a sampling artifact and not reflect underlying growth dynamics. If true, there would be little or no benefit from targeting services to clusters. To address this question, we tested whether past cluster growth predicts future cluster growth. We compared the ability of various targeting strategies to predict cluster growth between 2008 and 2015 in NYC: network-informed strategies (i.e., total and proportional cluster growth and machine learning incorporating demographic variables) versus two network-naive approaches (random selection and selection based on the presence of recent cases).

We inferred an HIV-1 transmission network using baseline genotypes from 63,942 individuals in the NYC HIV Surveillance Registry using HIV-TRACE, which computes genetic distance between sequences and identifies those that are sufficiently close to imply direct or indirect epidemiologic linkage. This network contained 3415 clusters of two or more persons and represented 21.3% of records (13,633/63,942). Network-informed targeting strategies predicted an order of magnitude more new cases than network-naive approaches: clusters with the greatest growth proportional to the square root of cluster size grew, on average, by 25.9 new diagnoses per 100 cases in the following year, compared with random cluster selection (2.1 new diagnoses) or selection of only clusters with recent diagnoses (8.5 new diagnoses). Machine learning approaches performed comparably to growth-based schemes, suggesting that—after accounting for growth—demography does not substantially improve predictions.

These findings suggest that network-based prediction of cluster growth may be a useful tool for directing services aimed at reducing HIV transmission.
UNDIAGNOSED HIV-1-CASES IN SWEDEN CLOSE TO 10% UNAIDS TARGET BASED ON A MULTIPLE BIOMARKER ESTIMATE OF INFECTION TIMES


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Background: Accurate estimation of date of infection (DI) for HIV-1-infected patients is vital for understanding disease epidemiology, including proportion of undiagnosed cases, and for informed public health decisions.

Methods: We developed a model that combines three biomarkers: CD4 counts, the BED-enzyme immunoassay and ambiguous nucleotides in HIV-1 pol sequences. The biomarkers growth was described in a Bayesian non-linear mixed model that was fitted on longitudinal data from 31 treatment-naive patients with well-defined DI. The model was used to estimate DI on data from 1,357 Swedish patients diagnosed between 2003 and 2010. As a majority of HIV-1 cases in Sweden are immigrants, the model also included the estimated time- distribution between arrival in Sweden and diagnosis. Based on these data we estimated the HIV-1-incidence in Sweden during 2003-2015 and quantified the number of undiagnosed cases.

Results: The combined biomarkers model had a better predictive ability to estimate DI than single biomarker models (RMSE nearly halved), especially within the first year from infection. The incidence estimation revealed no major changes in Sweden over the last years. Around 42% (21-85%) of all individuals were estimated to have been diagnosed within the first year of infection. One quarter of individuals was infected in Sweden and delayed diagnosis was especially common among heterosexuals. Delayed diagnosis was common also among individuals infected abroad, but around 74% of these individuals were diagnosed within the first year after entering Sweden.

Conclusions: Our multiple-biomarker model produces more accurate estimates of DI for HIV-1 patients than any single biomarker, which allows more precise incidence estimation. Estimation of incidence and proportion of undiagnosed cases is further improved by explicitly modelling the contribution from people infected prior to entering the country. The estimated number of undiagnosed cases in 2015 was 749(711-796). We are further developing the method by incorporating a NGS-based viral diversity biomarker.
ASSESSING THE DANGER OF SELF-SUSTAINED HIV EPIDEMICS FROM PHYLOGENETIC CLUSTER ANALYSIS


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Assessing the danger of transition of HIV transmission from a concentrated to a generalized epidemic is of major importance for public health. Although HIV transmission in Swiss heterosexuals has never led to a self-sustained epidemic, the unknown potential of imported infections either from abroad or from other transmission groups in Switzerland remains a large concern. We investigated how far from a self-sustained epidemic HIV transmission within Swiss heterosexuals is, and assessed its time trend and determinants. From a phylogenetic tree containing Swiss and background HIV sequences, we identified 3100 Swiss heterosexual transmission clusters (estimated infection dates between September 1980 and July 2014). The demographic characteristics of the individuals forming these clusters were extracted from the highly-representative Swiss HIV Cohort Study. To capture the incomplete sampling of HIV sequences, the delayed introduction of the imported infections to the Swiss heterosexuals network, and potential factors associated with higher basic reproductive number R0, we extended the basic branching process model to infer transmission parameters. Overall, the R0 of HIV in Swiss heterosexuals was estimated to be 0.44 (95%-confidence interval 0.42-0.46). The model also showed that subtype B had lower R0 (0.35) compared to non-B subtypes with the highest R0 occurring for CRF02_AG (0.62), highlighting the heterogeneity between subtypes. When assessing the time trend, we found that R0 was decreasing by 11% per 10 years (4%-17%). The multivariate model revealed that non-B subtype, reported sex with occasional partners and longer time to diagnosis were significantly associated with larger R0, while age and the earliest CD4 cell count did not exhibit significant effect. These findings indicate that there is no imminent danger of a self-sustained epidemic among Swiss heterosexuals, but rather diminishing HIV transmission far below the epidemic threshold. Generally, our approach allows to assess the danger of a self-sustained epidemic from HIV sequence data.
IMPLEMENTING A REAL-TIME HIV SURVEILLANCE PLATFORM


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Background: Real-time surveillance of pathogen sequences is an area of increasing interest, especially for epidemics of acute viruses. HIV does not stand out as a good candidate for such analysis, as it is endemic in many parts of the world, and diagnosis often occurs long after infection. However, recent outbreaks of HIV among black men who have sex with men (MSM) in Jackson, Mississippi and among people who inject drugs in Scott County, Indiana demonstrate the potential for dramatic, rapid spread of HIV in some settings. A system to screen HIV sequence data as they are generated has already demonstrated potential in British Columbia. We sought to develop a similar system that could be distributed under an open-source license.

Methods: We developed a computational platform for automated analysis and reporting of HIV pol sequence data and associated metadata. Submitted sequences are aligned against HXB2; resistance interpretations generated; clustering and subtyping performed using a reference dataset of over 118,000 sequences from the Los Alamos database; phylogenetic analyses performed for each subtype; and a dynamic, interactive report is generated.

Results: To simulate surveillance in real time, we applied our platform to retrospective data from the Vanderbilt Comprehensive Care Clinic, comprising 4,728 sequences from 2916 individuals sampled between 1998 and 2015 from middle Tennessee, US. Our analysis identified active transmission of HIV, particularly among young MSM, as well as clusters of non-B subtypes consistent with local transmission.

Conclusions: We have developed a self-contained system for automated molecular epidemiological analysis, which can be ‘containerized’ to run identically on different computer platforms. Our analyses have the potential to inform public health strategies for reducing transmission. We are working on how best to identify ‘unusual’ sequences that might warrant further attention, as well as how to present results to a public health audience effectively.
PHYLODYNAMIC ANALYSIS TO GUIDE ALLOCATION OF PRE-EXPOSURE PROPHYLAXIS IN THE UNITED KINGDOM

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Background: Pre-exposure prophylaxis (PrEP) promises to reduce acquisition of HIV infection in the United Kingdom. PrEP effectiveness depends on infections averted by directly protecting vulnerable individuals as well as infections averted indirectly by preventing transmission by those who would have been infected if not receiving PrEP. Therefore the impact of PrEP can be maximised if limited resources are concentrated on protecting individuals who are at greater risk of transmission as well as greater risk of HIV-acquisition. Analysis of HIV phylogenies reveals risk factors for transmission, which we examine as potential criteria for allocating PrEP.

Methods: We analysed 5,572 HIV-1 partial pol sequences collected from men who have sex with men (MSM) in the United Kingdom combined with global reference sequences. Dated phylogenies for subtypes A, B, C and CRF02_AG were estimated by maximum likelihood and least-squares dating. Coalescent models were fitted to phylogenies which adjust for stage of infection, global migration of HIV lineages, and changing incidence through time. Multiple patient-level covariates were examined to ascertain if they conferred enhanced transmission risk.

Results: Young age (<10% quantile) was found to confer higher odds of genetic distance clustering (OR=1.54) implying that transmission may be higher in that group. Structured coalescent models including young age showed smaller and statistically insignificant effects. We compared simulated interventions where PrEP is targeted on young London MSM or randomly allocated. We predict that over a five year horizon, 2.5% of new infections can be averted if providing 1% of vulnerable individuals with PrEP. Discussion: Concentrating limited PrEP doses on individuals with greater transmission risk can avert more infections than random allocation. PrEP allocation could be further optimised by identifying more patient-level covariates that enhance transmission risk.
VALIDATING THE DSPS-HIV, A HIGHLY-CUSTOMIZABLE AGENT-BASED HIV EPIDEMIC SIMULATOR, AGAINST THE UK HIV EPIDEMIC

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The Discrete Spatial Phylo Simulator HIV (DSPS-HIV) is an individual-based stochastic model that simulates HIV transmission within populations composed of diverse individuals, and produces realistic epidemiological statistics, viral phylogenies, and viral sequences. The DSPS-HIV was used in the PANGEA_HIV initiative to evaluate the ability of phylogenetic techniques to detect changes in HIV epidemics from sequence data, and to investigate “missing men” in the UK heterosexual HIV epidemic. But in order for the DSPS-HIV to realise its full potential as an epidemic modelling tool it must be validated against real epidemic data.

We replicated the approach taken by Phillips et al. (HIV Medicine, 8: 536–546; 2007) and aimed to match epidemic parameters from the UK HIV epidemic from 1980 to 2006. However, unlike the Phillips model, the DSPS-HIV simulates contact networks including both infected and uninfected individuals in the population, which greatly increases the computational load of the simulation.

Through extensive optimization, we have enabled the DSPS-HIV to simulate HIV epidemics in populations comprising hundreds of thousands of individuals across multiple risk groups. For each sexual risk group modelled (MSM, heterosexual female, heterosexual male) we compared yearly number of infections, number of deaths, number with AIDS, and number on treatment against the observed data. We also compared time-to-AIDS and time-to-death before and after the introduction of HAART. Finally, unlike the original Phillips model, we were also able to compare the simulated sequence diversity to the diversity observed in the real UK sequences.

Here we present the results of our validation and conclude that DSPS-HIV can be used to realistically and reliably reproduce the UK HIV epidemic.

CHARACTERIZING HIV TRANSMISSION CLUSTERS WITHIN A MIDDLE TENNESSEE CLINICAL COHORT

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Background: The Southeastern US is the epicenter of the US HIV epidemic, accounting for the greatest number of new diagnoses. Targeting ongoing transmission clusters provides new opportunities to intensify interventions, but the success relies on timely cluster detection. However, little is known about transmission clustering in middle Tennessee. We assessed cluster characteristics, growth, and associations with clustering.

Methods: HIV-1 partial pol sequences collected from 2001-2015 among persons in care at the Vanderbilt Comprehensive Care Clinic. Transmission clusters were identified using maximum likelihood methods and patristic distance differences. Demographic, risk behavior, and clinical factors were assessed with logistic regression.

Results: Among 2915 patients, with 4721 HIV-1 sequences, 77% were male, 44% black, and 57% were men who have sex with men (MSM). Most (96%) sequences were subtype B. Using first available sequence, 963 (33%) were identified in 292 clusters (<1.5% distance, range 2-39 members, 61% patients in clusters ≥3 members). During 2011-2015, most clusters (181/292; 62%) were “active”, either being newly identified (n=80) or had growth (expansion on existing cluster, n=101), and involved 690 patients. Among 1,027 patients with first sequence obtained 2011-2015, 39% were identified in these active clusters. Factors independently associated with these clusters included MSM risk (OR 1.86, 95%CI: 1.27-2.84) and age ≤30 years (OR 2.63, 95%CI: 1.96-3.52) but not black race (OR 0.72, 95%CI: 0.54-0.97). Active clusters were more likely to be composed of MSM (60% vs. 41% inactive clusters had ≥50% MSM; P=0.002). Highly related clusters (<0.5% distance) involved 348 persons, of whom 90% were men.

Conclusions: We characterized “active” clusters, identifying a high proportion of closely related sequences. Young MSM were most likely to be identified in active clusters highlighting importance of interventions among this group. Prospective cluster analysis would be feasible in this cohort and allow timely detection of new or expanding clusters.
ACCOUNTING FOR DONOR VIRAL DIVERSITY GIVES HIGH ESTIMATES OF THE NUMBER OF HIV FOUNDER VIRIONS IN RECIPIENTS

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After observations that most sexually transmitted HIV infections are initiated by single strains, it was hoped that signatures of transmission would be identified and used as targets for vaccine development. Selection occurring at the transmission bottleneck can solve the paradox of a very small transmission probability per contact but multiple transmitted/founder (T/F) strains when successful transmission does occur in 20-40 percent of cases. However, genotypic and phenotypic signatures of transmission have been difficult to find. Using a probabilistic modeling approach, we show that selection need not be invoked to explain this apparent paradox. If transmission is only possible for a minority of contacts and changing viral diversity in donors throughout their courses of infection is accounted for, it is possible to resolve the low transmission probability and new infections being founded by multiple strains 20-40 percent of the time. We apply our modeling framework to published whole-genome deep sequencing data, and infer the distributions of the number of virions and the number of distinct strains establishing infections in a population. We find that the numbers of T/F virions and strains are not identical: there is not necessarily even a positive correlation depending on the assumptions made about contact rates in the population. This is important, since different studies have suggested that either the initiating volume of virus or number of T/F strains are predictors of set point viral load. Decoupling these is therefore important for making accurate predictions of quantities characterizing infection. Furthermore, we show that it is possible for most individuals to be infected by single strains despite the complex quasispecies observed in data from individuals later in infection, without requiring selection at transmission. This is due to changing strain diversity in donors throughout their courses of infection combined with stochasticity in the strains transferred at each transmission event.
IN VIVO MUTATION RATES AND THE LANDSCAPE OF FITNESS COSTS OF HIV-1 IN CHRONIC INFECTION

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Absolute values of mutation rates and fitness costs are difficult to measure in vivo. Using deep longitudinal whole genome sequence data by Zanini et al, we estimate the mutation rates and distribution of fitness costs from the dynamics of variation. At approximately neutral sites, mutations accumulate with rates similar to those measured in vitro. Diversity at other sites saturates and we can estimate the fitness costs at those sites from the time to saturation and the level at which diversity saturates. Using these methods, we quantify the distribution of fitness effects in different proteins, at different positions within codons, and investigate their dependence on protein properties such as disorder or solvent accessibility. Fitness costs estimated from in-vivo diversity explain up to 50% of the sequence diversity within subtypes, but sites associated with HLA types tend to be globally diverse even if mutations at these sites decrease fitness.
SEX, DRUGS, AND PHYLOGENY: THE ABCS OF A SCIENTIFIC SOAP OPERA


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Using a modified Approximate Bayesian Computation (ABC) method we present an analysis of a transmission case involving 3 parties and multiple accusations of HIV transmission. Parties MP1 and MP2 are married IDUs who divorce in April. Six months later MP2 is diagnosed with HIV. MP2 accuses MP1 of infecting him and the case goes to trial. As part of the evidence, 20 clones are sequenced from each party. Later it is determined that the current partner of MP2, MP3, is also infected. 588 days after sequencing MP2, 19 clones are sequenced from MP3. The joint phylogeny shows a complex mixture of monophyletic, paraphyletic, polyphyletic relationships among the tip labels suggesting transmission of at least 7 lineages among MP2-MP3. The question we will address is, can we quantify the evidence for who infected whom when (WIW) and can we differentiate different modes of transmission (instantaneous versus ongoing)?

Our tools are a set of 6 time-variable coalescent models that represent alternative hypotheses as to what occurred among this triad. For inference we use an ABC method based on 9 statistics measured on the joint phylogeny that we have shown to be related to WIW in previous studies. We directly incorporate both the density and correlation of the tree statistics into our method by considering not just a single ML tree, but a large sample of trees from a posterior distribution. We find clear support for the instantaneous transmission model and, ironically, MP3 as the donor contradicting what a naive reading of the phylogeny would suggest. We discuss our results in terms of using multi-sample sequences to quantify epidemiologic relationships.
META-ANALYSIS OF PHYLOGENETIC RECONSTRUCTION OF HIV-1 TRANSMISSION IN DOCUMENTED DONOR-RECIPIENT CASES

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Because HIV-1 evolves during its spread from host to host, there is a connection between who infected whom and the resulting virus phylogeny sampled from the infected hosts. While the evolution within each host facilitates this connection, it is not a trivial one-to-one correspondence. The connection between transmission tree and the resulting virus phylogeny can be understood via the so called pretransmission interval, which explains why phylogeny-based transmission times become backwards biased in time and who-infected-whom inference may become jumbled. However, accounting for within-host virus evolution makes it possible to infer meaningful HIV-1 phylogenetic transmission patterns. To systematically evaluate HIV-1 phylogenetic transmission patterns we reanalyzed 204 previously documented transmission chains with data in the LANL HIV database. We included cases with multiple sequence clones from both donor and recipient(s) to facilitate detection of transmission direction and potential unsampled links. Many donor-recipient cases consisted of >2 individuals and many had data from >1 genomic region, resulting in 1508 sequence data sets with 2 epidemiologically linked individuals. Furthermore, the data contained different risk groups, subtypes, and in many cases we had some information about time of transmission. Using a Bayesian framework, we analyze common and distinguishing features in cases with direct transmission, common source transmission, and transmission with intermediary links. We find that the donor’s HIV-1 population often, but not always, defines the root, and that transmission of multiple lineages is not unusual.
DUAL HIV-1 INFECTION IN SEROCONVERTERS: PREVALENCE, DETERMINANTS AND BIOLOGICAL EFFECT

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INTRODUCTION Dual infection is the infection of an individual by two phylogenetically distinct HIV viruses. We report on dual infection in a cohort of 2,525 treatment naïve seroconverters from Europe.

METHODS Phyloscanner was used to infer phylogenies of within and between host viral diversity from whole-genome deep-sequencing data. Putative dual infections were detected as phylogenetically distinct subtrees formed from viruses from one patient. To reduce the impact of false positives, results were aggregated across multiple windows spanning the whole genome. A mathematical model was developed to simultaneously estimate the proportion truly dually infected, the false positive detection rate, the biological effect of dual infection on viral load, and the effect of viral load on our method's ability to detect dual infections.

RESULTS Because dual infection is rare, true dual infections are easily outnumbered by false positives, so aggregation is important to reduce the false positive rate. We estimated that our method produced a true discovery rate of 73%. Thus, of 158 (6.3%) patients that we found empirically to be dually infected, we estimate that 115 (4.7%) are truly dually infected. We estimated that the biological effect of dual infection is an increase of 0.29 log10 units of viral load per ml (p<0.001). We found no association of dual infection with country of sampling, with gender, with mode of transmission, or with the dominant viral subtype. We found no association with time from seroconversion to sampling. Dual infection rates were found to decrease over time (OR=0.73 per decade elapsed, p=0.04).

CONCLUSIONS Dual infections are common in all HIV infected populations in Europe. Dual infection prevalence does not depend on patient gender, location, mode of transmission, or viral subtype, but decreased over time in this sample. Dual infection occurs early in infection and is associated with an increase in set-point viral load.
RE-EVALUATING THE ROLE OF LATENCY IN HIV EVOLUTION AT THE WITHIN-HOST AND EPIDEMIOLOGICAL LEVELS

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HIV-1 evolves approximately an order of magnitude more slowly at the epidemiological level than at the within-host level. The Store and Retrieve hypothesis posits that this discrepancy results from preferential transmission of latent virus due to an intrinsic transmission or establishment advantage. However, the putative reduction in the between-host evolutionary rate through this mechanism depends on the dynamics of the latent reservoir, and the loss of transmissibility during the course of infection, neither of which are well characterized. We develop a model of within-host HIV dynamics and evolution, and re-evaluate this hypothesis in light of available data on the dynamics of the latent reservoir and transmission on the epidemiological level. Our model, which incorporates a detailed mutation process including substitution and recombination, tracks the divergence of the virus population through the course of infection, accounting explicitly for the loss of transmissibility due to mutation of the viral envelope. We inform latent reservoir dynamics and turn-over based on the within-host evolutionary rate, and the relative size of the reservoir in acute and chronic infection, and reconcile the decay in transmissibility with empirically observed probabilities of transmission during different stages of disease. We find that preferential transmission of latent virus can result in a significant evolutionary rate discrepancy only if 1) transmissibility decays rapidly during acute infection, 2) the activation rate is orders of magnitude higher during untreated than treated infection, and 3) the latent reservoir consists primarily of clonally expanded virus deposited into the reservoir in the first few days of infection. However, the latter condition is not consistent with the high prevalence of CTL escapes in the latent reservoirs of patients treated during chronic infection, suggesting that the Store and Retrieve hypothesis cannot explain the evolutionary rate discrepancy under realistic parameter settings.
DISSECTING HIV VIRULENCE: HERITABILITY OF SETPOINT VIRAL LOAD, CD4+ T CELL DECLINE AND PER-PARASITE PATHOGENICITY

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Pathogen strains may differ in virulence because they cause different pathogen load, or because they induce disease-causing mechanisms independent of load. In evolutionary ecology, the latter is referred to as “per-parasite pathogenicity”. Strains with low per-parasite pathogenicity cause benign infections despite high pathogen load. Using viral load and CD4+ T cell measures from 2014 individuals enrolled in the Swiss HIV Cohort Study, we investigated if virulence and per-parasite pathogenicity are heritable from donor to recipient. This would imply that these traits are affected by viral genetic factors. We focused on individuals infected with HIV subtype B. We measured virulence as the decline of the CD4+ T cells over time, and per-parasite pathogenicity as the residual of a population-wide regression of CD4+ T cell decline versus set-point viral load. We determined heritability of virulence and per-parasite pathogenicity by simple donor-recipient regressions applied to 196 previously identified transmission pairs, and methods based on a phylogenetic tree constructed from viral sequences obtained from the 2014 individuals. The phylogenetic methods assumed either neutral evolution or stabilizing selection of the traits. Applying the donor-recipient regressions to the CD4+ T cell decline and per-parasite pathogenicity did not yield heritability estimates significantly different from zero. With the phylogenetic mixed model approach, we find that the heritability of the decline of CD4+ T cells is 25% (95% CI: 9%–40%) assuming neutral evolution of this traits, or 17% (95% CI: 6%–29%) assuming stabilizing selection. The heritability of per-parasite pathogenicity is estimated with phylogenetic mixed models as 22% (95% CI: 5%–39%) assuming it evolves neutrally, and 17% (95% CI: 4%–29%) for stabilizing selection. Further, we confirm previous studies that established the heritability of the set-point viral load. In summary, we present the first comprehensive study of the heritability of HIV virulence including its virus-load-dependent and -independent components. We find evidence for the heritability both of these virulence components. Our results suggest that viral genetic factors affect virulence in two ways: indirectly through influencing the set-point viral load, and directly by modulating the per-parasite pathogenicity of the virus.
HEPATOTOXICITY AND ANAEMIA CO-MORBIDITY IN TREATED HIV PATIENTS IN FUNDONG SUBDIVISION IN THE NORTHWEST REGION OF CAMEROON

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Hepatotoxicity and anaemia are relevant adverse effects of ART and can cause interruption of therapy and death. However, there is dearth of information on hepatotoxicity and anaemia co-morbidity especially in rural areas. The aim of the study was to identify the prevalence of Hepatotoxicity and Anaemia co-morbidity among HIV patients treated with first line ART.

In total, 150 patient age between 15 and 74 years visiting the day hospital in Fundong District Hospital between January-March 2015 and have been followed up for 18 months were recruited into the study. Baseline and 18 months levels of CD4 counts, alanine transaminase (ALT), and aspartate transaminase (AST) and Haemoglobin concentration (Hb) were determined.

HIV was diagnosed using Alere determine HIV rapid test kit and Oral Quick test kit used for confirmation. CD4 counts were determined using the Pima™ CD4 machine. Hb, ALT and AST counts were determined by colometric enzymatic reaction and classified based on age and sex.

The majority of patients were female 115(76.7%) and belonged to the <30 years age range 48(32%). The prevalence of anaemia decreased from 86(57.3%) to 69(45.6%) at the end of the study period. In all, 46(30.7%) patients had hepatotoxicity and anaemia co-morbidity which was higher in the age group <30years 30(41.7%) and in female 37(32.2%). A total of 1(0.7%) and 10(6.7%) patients developed severe hepatotoxicity using ALT and AST respectively. The prevalence of hepatotoxicity was higher in male (31.4% and 62.9%) and in the age group 30-39years (29.5% and 68.2%) for ALT and AST, respectively. The prevalence of anaemia and elevated AST and ALT were higher in persons with CD4 <200cells/μl. There was a significant correlation (P=0.00) between CD4 and Hb (r= 0.193), CD4 and ALT(r=-0.149) and CD4 and AST(r=-0.193).

Hepatotoxicity especially Grades 1-2 and not anaemia is a significant adverse effect of ART upon time.
EPIDEMIOLOGY OF HIV AMONG YOUNG PEOPLE IN SOUTH KIVU PROVINCE: IMPLICATIONS FOR PREVENTION.

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Background: South Kivu province (Democratic Republic of Congo) is still one of the high TB burden province. The burden of TB in non-Congolese patients can be obtained from TB screening that is conducted in migrant in 2010, 1,284,920 migrants were screened, 5400 diagnosed as having tuberculosis (420 per 100,000) comparing to Congolese 198/100,000. Moreover, there are high default rates in new smear-positive cases for TB treatment among non-Congolese TB patients.

Methods: This project aims to study on TB care and treatment program for improving access to TB care among non-Congolese migrants in five hospitals located in South Kivu province. This involves in three holistic aspects including epidemiology, operational research and intervention views. The researcher conducted an opinion survey with 136 health personnel using structured questionnaires, performed a prospective study with 420 migrant TB patients, and provided health education for improving patients' compliance for 100 undocumented migrant TB cases.

Results: The key concerning issues for health providers about migrant TB cases are related to cleared policy on health and migrants, quality of care, DOTS strategy, security issue, humanity, and MDR-TB. The important influential factors associated with treatment success rate are having good knowledge about TB, good behaviors, good DOT observers, and understanding the diagnosis and treatment processes of TB service in health care settings. Moreover, for health education intervention, this could be improved a good appointment rate and patients' compliance among migrants up to 80%.

Conclusions: The results from this study will be benefited for TB program to develop appropriate interventions and services to control TB among migrants in particular those providers caring for them should have a high concerning about access to care to early case detection & treatment for stop spreading TB to our Congolese & Non-Congolese residents.
DIVERGENT VARIANTS IN PURE AND RECOMBINANT CONTEMPORARY HIV-1 LINEAGES FROM THE DEMOCRATIC REPUBLIC OF CONGO REVEAL AN OLDER ORIGIN AND A HIGHER DIVERSITY DURING EARLY EPIDEMIC HISTORY OF SUBTYPE C


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We identified two divergent subtype C lineages during previous HIV-1 drug resistance surveillance surveys in the Democratic Republic of Congo (DRC). Here, we characterize and infer the evolutionary story of these novel genetic forms. We obtained near full-length genome sequences from eight treatment-naive infected patients sampled in 2008 and 2012 in Kinshasa (n=1), Mbuyi-Mayi (n=6) and Goma (n=1). Subtype/CRF designation was done using bootscanning and likelihood-based phylogenetic analyses. We inferred the time-scaled evolutionary history following a Bayesian approach and a partitioned analysis (pol, gag, env) or the partial Reverse Transcriptase (RT)-gene. Four strains were more closely related to subtype C over the entire genome while one harbored also two small A-like regions. Three other strains shared the same mosaic pattern with alternating fragments close to CRF02_AG, CRF26_AU, subtype A or C. The pure C-like sequences were always basal to the corresponding subtype C references and the TMRCA of the whole group was around the mid 1930s for any genomic region. Subtype C and CRF02_AG fragments from the mosaic strains were accordingly basal to the subtype C clade (including those described above) in pol and gag regions or to the CRF02_AG clade in the env region. Inclusion of this latter divergent subtype C fragment shifted the TMRCA of subtype C to around the mid 1920s. The transition to a faster phase of exponential growth was around the late 1950s and cladogenesis within novel groups occurs thereafter. These divergent subtype C forms represent 4.3% of all documented subtype C strains from DRC. We evidenced the current circulation of divergent subtype C variants in pure and mosaic HIV-1 strains in the DRC. Our findings suggest an older origin that previously thought and a higher diversity during early epidemic history of subtype C.
Viral sequencing as part of patient care is now routine for HIV patients in the U.K, and consequently a large fraction of the infected population is sampled prior to therapy. However, this is not the situation in low and middle-income countries (LMICs), and it is possible that people in some risk groups or geographic areas are under represented or only sampled at a late stage of infection. Phylogeographic studies of the spread of infection using sequence data have the potential to uncover the transmission network in the population and allow a quantification of transmission between risk groups and locations. But it has long been noted that biased sampling can lead to biased results in phylogeographic studies, and this seems to be a fundamental feature of the discrete trait phylogenetic model employed. We test the idea that fast phylogenetic tree inference with approximate time scales on repeated subsamples plus discrete trait model inference can give a reasonable approximation to the correct transmission pattern assuming a structured population. Also we show that this procedure is comparable to using the full bayesian phylogeographic models but with the advantage of improving model robustness by reducing over-representation, and reducing run-time for large data sets. Next, transmission matrix predictors incorporating host density, distance and known movement or connectivity patterns are fitted to the approximate phylogenies, using model selection and generalized linear modelling frameworks. Finally, we explore the how transmission via a missing population can be inferred with the generic transmission matrix predictors for the sampled and unsampled populations using HIV and Avian Influenza example data sets.
ANALYZING AND MODELING THE EFFECT OF IL7 INJECTIONS ON THE VIRAL RESERVOIR AND IMMUNE SYSTEM OF HIV INFECTED PATIENTS.

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In HIV infected patients, antiretroviral therapy efficiently blocks the viral replication and this is followed in most patients by a restoration of the CD4+ T cells pool. As IL-7 is a cytokine involved in T cell homeostasis, repeated injections of exogenous IL-7 have been tried for patients who fail to restore their CD4 pool. The INSPIRE 2 and 3 trials evaluate a first cycle of IL7 injections followed by a new cycle each times the patient is under 550 CD4 cells/μL, with a visit every 3 months. There are repeated measures of the total number of CD4, the proliferation marker Ki67+ and the HIV DNA for on 137 patients. Efficacy of this IL7 therapy on CD4 counts has been demonstrated but its impact on the HIV reservoir has still to be explored. We first propose a mixed-effect model for the evolution of infected CD4 cells after an injection. This first approach showed that, although the number of infected CD4 increases after an IL7 injection, their proportion remains constant. It may be explained by assuming that the proliferation rate of the infected and uninfected CD4 cells increases of the same amount after an IL7 injection. To better understand the mechanism of action of the IL7 injection on the viral reservoir, we use models based on ordinary differential equations with four compartments representing quiescent and proliferating infected as well as quiescent and proliferating uninfected CD4 cells. The model included random effects to take the inter-individual variability into account. We estimated the parameters of this model using the data of the INSPIRE studies. The interpretation of the parameters will allow us to understand how the injections of IL7 affect the dynamics of the CD4 and the viral reservoir. The first results tend to confirm the conclusions of the first approach.
6 NEAR WHOLE GENOME SEQUENCING OF NOVEL HIV-1 RECOMBINANTS FROM CAMEROON.


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Introduction: The broad diversity of circulating HIV-1 strains in Cameroon has contributed to the predominance of recombinant forms in this region. Circulating Recombinant Form 02_AG (CRF02_AG) and a plethora of Unique Recombinant forms (URFs) constitute more than 80% of prevalent strains. In recent years, we monitored a steadily increase in URFs, especially new URFs comprised of subtypes 02_AG and F2. There is dearth of identifying and characterizing these newly emerging URFs that pose monumental challenges to diagnosis, treatment, and HIV-1 vaccine approaches.

Method: In order to generate near whole genome sequences (NWGS) of the emerging URFs, we performed rational primer design and optimized PCR conditions with special regards to the prevalent 02_AG and F2 sequences. We established protocols to obtain NWGS either by one whole genome amplicon or amplifications of 2, 3 or 4 overlapping parts. NWGS are analyzed for recombination breakpoints, unique sequence signatures, and recurrent sequence patterns, relevant for drug and neutralizing antibody resistance.

Results: We identified 16 new URFs in previous screenings of our HIV+ Cameroonian cohort, whereof 12 NWGS have been completed comprising all functional genes. Four near whole genome sequences were obtained using the whole genome PCR amplification protocol with our optimized 5’ and 3’UTR primers while the other URFs needed amplification of smaller overlapping parts to cover their near whole genomes. We detected novel URFs with contributing subtypes 02_AG, F2, F1 and A1 that exhibit previously unidentified recombination patterns with novel arrangements of antibody epitopes.

Conclusion: Whole genome analysis of emerging URFs in Cameroon helps to monitor the steadily changing and increasing diversity of HIV strains. The identification of evolving drug and antibody resistance patterns within the new circulating URFs will assists future efforts for improved treatment and a globally successful HIV-1 vaccine.
Ebola virus (EBOV) causes a severe, often fatal Ebola virus disease (EVD), for which no approved antivirals exist. Recently, some promising anti-EBOV drugs, which are experimentally potent in animal models, have been developed. However, because the quantitative dynamics of EBOV replication in humans is uncertain, it remains unclear how much antiviral suppression of viral replication affects EVD outcome in patients. Here, we developed a novel mathematical model to quantitatively analyse human viral load data obtained during the 2000/01 Uganda EBOV outbreak and evaluated the effects of different antivirals. We found that nucleoside analogue- and siRNA-based therapies are effective if a therapy with a >50% inhibition rate is initiated within a few days post-symptom-onset. In contrast, antibody-based therapy requires not only a higher inhibition rate but also an earlier administration, especially for otherwise fatal cases. Our results demonstrate that an appropriate choice of EBOV-specific drugs is required for effective EVD treatment.
8 QUANTIFYING THE FITNESS COST OF DRUG RESISTANCE MUTATIONS IN THE SWISS HIV COHORT STUDY

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The emergence and subsequent spread of drug resistant HIV is a major threat to the long-term efficacy of treatment to prevent AIDS and new infections. Studies showing that anti-retroviral treatment can prevent onward transmission of the virus have led to recommendations that treatment should begin earlier in an infection. HIV sequence data is increasingly available, as pol sequences are routinely collected from infected individuals to test for drug resistance. These sequences can be used to reconstruct the phylogenetic relationship among viral lineages, which is an approximation of the transmission tree.

By employing a two-type birth-death model as the process generating the viral phylogeny, we are able to estimate the rates of transmission, the rates of de novo resistance evolution and reversion to a drug-sensitive virus. We analysed data from 4077 HIV infected individuals enrolled in the Swiss HIV Cohort Study, sampled between 1989 and 2015.

Our results suggest that the effective reproductive number, R, of drug sensitive viruses was above 2 before 1994, below the epidemic threshold of one between 1994 and 2001 and around 1 after 2001. We quantify fitness cost as the ratio at which transmission rates of drug resistant strains differs from transmission rates of drug sensitive strains. Among the considered resistance mutations, only the 90M mutation in the protease gene was found to have significantly higher fitness than the drug sensitive strains. The following mutations associated with resistance to reverse transcriptase inhibitors were found to be less fit than the sensitive strains: 67N, 70R, 184V, 219Q. The highest posterior density intervals of the transmission ratios for the remaining resistance mutations included in this study (103N, 138A, 41L, 210W, 215D, 215S) all included 1, suggesting that these mutations do not have a significant effect on viral transmissibility within the Swiss HIV cohort. These patterns are consistent with alternative measures of the fitness cost of resistance mutations such as site-directed mutagenesis or reversion rates.

Overall, we have developed and validated a novel phylodynamic approach to estimate the fitness cost of drug resistance mutations.
RECONSTRUCTING WITHIN-HOST HIV DYNAMICS FROM SEQUENCE VARIATION WITH A SIMULATION-BASED PHYLODYNAMIC METHOD

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Complex population dynamics of HIV within a host are shaped by virus growth, selection, and migration among cellular compartments. Hypotheses about HIV dynamics such as compartmentalization or latency are often evaluated by the visual inspection or summary statistics of the shapes of within-host phylogenies. Here, we develop a simulation-based framework for phylodynamic analysis of within-host dynamics.

We simulated coalescent trees from an ODE system representing a simple within-host dynamic model from Rong and Perelson. State transitions were reconstructed along branches by modified rejection sampling. To model the relative evolutionary stasis of cell-free virus and latent integrated virus, we collapsed the corresponding intervals from branch lengths. We simulated trees under a joint prior distribution centred at estimates from the literature. To quantify the similarity of tree shapes, we developed a kernel function that penalizes subset trees by their discordance in branch lengths and tip labels. We assumed latent and active infected cells could not be differentiated (same label).

Using cross-validation with support vector regression, most model parameters were identifiable through tree shape, especially the fraction of infections becoming latent, death rate of actively infected cells (δ), and activation rate of latent cells (mean R2=88%, 92% and 70% respectively). Next, we compared over 2000 simulated trees to a phylogeny reconstructed by RAxML from published longitudinal HIV RNA and DNA env sequences (MACS patient 2) and rooted by root-to-tip regression. We obtained posterior estimates of approximately δ=0.17/cell/day and T cell growth rate (Λ)=1000 cells/mL/day, although these rates were confounded.

Our results provide encouraging evidence that tree shapes are a rich and under-utilized source of information about within-host dynamics. Further work will focus on data-driven model criticism and generating accurate simulations from more realistic models.
A deeper understanding of HIV-1 transmission and drug resistance mechanisms can lead to improvement in current treatment policies. However, the rates at which HIV-1 drug resistance mutations (DRMs) are acquired and at which transmitted DRMs persist are multifactorial and vary considerably between different mutations.

We develop a probabilistic model of drug resistance acquisition and transmission and use it to estimate the time duration of different events (DRM emergence under drug selective pressure, DRM loss in the absence of ART), and the transmission rates for different types of patients (treatment-experienced or naive, infected with drug-sensitive or drug-resistant viruses). We estimate the rates separately for different mutations. This model refines the method we described in Mourad et al AIDS 2015.

Our model is Markov chain-based and describes both the state evolution along the tree branches, and the transmission process. The states describe a patient-virus pair. Given the model parameters (transmission and state transition rates), we provide an analytical solution for calculation of the likelihood of a transmission tree with known tip states. We show that our model has several important, non-standard properties. Most importantly, the characteristics of the transmission tree depend on the evolution of the states along the branches. For example, as under a successful treatment the viral load becomes undetectable, when the state is “treatment-experienced drug-sensitive” the number of transmissions becomes very low. Moreover, the model is non “ergodic”: The “vertical” stationary distribution of states along a virus lineage is different from the “horizontal” state distribution of a large infected population at a given time point.

Using simulated transmission trees, we show that maximum-likelihood optimisation allows for accurate estimation of rate parameters. We then apply our model to transmission trees reconstructed on the real data obtained from the UK HIV drug resistance database to make predictions for known DRMs.
OBSERVING EVOLUTION IN HIV-1 INFECTION: PHYLOGENETICS AND MUTANT SELECTION WINDOWS (PHYLOMSW) TO INFER THE INFLUENCE OF THE NATURAL ANTIBODY RESPONSE ON THE VIRAL QUASISPECIES

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During infection with HIV-1, the viral population constantly evolves alongside the adapting antibody response. To understand and in the future manipulate this co-evolutionary trajectory, we need to reconstruct the evolutionary paths of the antibody response and the virus population. When an antibody is newly generated, it is only abundant in low concentrations. This low selective pressure makes it possible for a resistant viral variant to evolve. Therefore, we need to determine under which antibody concentrations an antibody escape strain can out-compete its viral ancestor, referred to as mutant selection window (MSW). Here, we present a framework combining experiments with mathematical theory to determine the mutant selection windows. We first applied this framework to artificial ancestor/escape pairs of laboratory derived HIV-1 strains and then to longitudinally sampled viral strains from one HIV-infected individual (CAP256). We used the laboratory-derived pairs to demonstrate which forms of MSW can be observed before we applied this framework to in vivo derived longitudinal viral strains and the autologous broadly neutralizing antibodies (bnAbs) sampled of patient CAP256. With a phylogeny, we identified potential ancestor/escape virus pairs. The mutant selection windows of these pairs in respect to the autologous bnAbs inform us about (i) which strain will be out-competed (ii) which strain could be a possible ancestor of an escape variant and (iii) whether escape most likely happens via free virus or cell-cell transmission.

Our method has direct application for antibody based treatment strategies: It is important to find the right antibody dosage to suppress escape mutants from evolving and, in addition, a vaccine scheme should make use of the knowledge of which viral strains led to the evolution to a broadly neutralizing antibody response. The presented method informs us about both application of broadly neutralizing antibodies as anti-HIV therapies.
MULTISCALE, MECHANISTIC PIPELINE TO ASSESS THE PROPHYLACTIC EFFICACY OF HIV COMPOUNDS

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While HIV-1 cannot be cured to date, pharmaco-intervention strategies have been proposed to end the epidemic. Two strategies are prevalent: (i) Treatment-as-prevention (TasP) decreases the patients’ virus load and thereby markedly reduces the infectivity of the potential transmitter. However, onwards transmission may preferentially occur early after infection, when the transmitter is unaware of his infection, arguing that TasP may only prevent a small fraction of transmission events in reality. (ii) The second intervention is called pre-exposure prophylaxis (PrEP). Here, an uninfected, exposed individual takes anti-viral drugs to prevent infection upon viral exposure.

Our objective was to build a predictive mechanistic model for the drug-class and drug-specific efficacy of antiviral compounds in the context of PrEP, 'PrEP on demand' and post-exposure prophylaxis (PEP). By coupling our model with drug-specific pharmacokinetics, we assess the mechanisms of prevention, the pharmacological limitations and opportunities for various prophylactic schedules using approved and neglected drugs.

We derived analytical solutions for the probability of virus clearance, depending on a constant, drug-class specific viral inhibition and fixed inoculum size. The latter was used to deduce a polyhedron in state space in which infection is reversible. We then develop an extrande algorithm, sampling the stochastic dynamics within the eradication polyhedron, considering the time- and dosing dependent efficacy of anti-retrovirals (ARVs).

Our framework enables to integrate virus loads in the transmitter, mode of exposure, timing of viral challenge and dosing schedules to estimate the infection probability for unprotected sex for any particular ARV.

We observed drug-class specific prophylactic efficacies, with protease inhibitors exhibiting switch-like response profiles. Our simulations indicated that, unlike approved nucleoside reverse transcriptase inhibitors, currently neglected non-nucleoside reverse transcriptase inhibitors may be highly efficient, even when used in 'PreP on demand' or PEP. The latter warrants further clinical assessment, particularly since these compounds are extremely cost-efficient and thus suitable for large-scale roll-out in resource-constrained settings.
A HIGHER FRACTION OF DRUG RESISTANT PROVIRUSES EXPRESS UNSPLICED HIV RNA THAN THEIR WILD-TYPE PREDECESSORS


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Introduction: The fraction of proviruses persisting during ART that are latent vs. transcriptionally active has not been determined. To address this question, we investigated the expression of unspliced HIV RNA in vivo in single cells carrying either wild-type (WT) proviruses or those with drug resistance (DR) mutations.

Methods: PBMC were analyzed from Patient #1 in Maldarelli, et al., 2014. The fraction of the proviruses expressing HIV RNA was determined by sequencing of P6-PR-RT cell-associated HIV RNA and DNA from single cells. Proviruses capable of infectious virus production were identified using viral outgrowth assays (VOA). The levels of viral RNA present in infected cells were determined for the archival drug sensitive population, the recently infected DR population (resistant to the current drug regimen), and for clones carrying known intact and defective proviruses.

Results: We analyzed a total of 77 million PBMC, of which 10,450 contained HIV pro-pol sequences: 7137 were WT, 1714 were DR, and 1599 had obvious defects (contained stop codons). The mean fraction of proviruses that expressed RNA in cells with DR virus was 18%, whereas in cells with WT proviruses, it was 8% (p=2x10-11). Levels of expression in single cells with DR proviruses were also higher than in cells with WT proviruses (p=0.002). The median fraction of cells in apparent expanded clonal populations (determined by identification of multiple identical WT sequences) was 4%.

Conclusion: A small fraction of the proviruses in HIV infected cells expressed HIV RNA. The fraction and levels of proviral expression were significantly higher in more recently infected cells than in those that persisted during long-term ART. These findings show that ART can select both for cells infected before ART initiation that either do not express HIV RNA or express at low levels and for cells infected recently with drug-resistant viruses that express higher levels of HIV RNA.
EMERGENCE OF MUTATIONS IN HIV-1 CRF02_AG ASSOCIATED TO RESISTANCE TO FIRST LINE ANTIRETROVIRAL THERAPY OF REVERSE TRANSCRIPTASE INHIBITORS IN PRE AND POST HAART ERA IN CAMEROON


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Background: HIV-1 CRF02_AG drives the AIDS epidemic in Cameroon. Antiretroviral therapy was initiated in 2004 and currently, combination therapy of reverse transcriptase inhibitors (RTI) is used as first line regimens. The most frequently used combination being tenofovir (TDF), lamivudine (3TC) and efavirenz (EFV). A comparison is made of rates of resistance associated mutations (RAMs) in the pre- and post-HAART era in Cameroon (1996 to 2017).

Methods: HIV-1 CRF02_AG RT sequences and reference sequences of Group M subtypes and circulating recombinant forms from Cameroon were downloaded from the Los Alamos HIV Sequence Database. Overall, 306 and 673 sequences were assigned to the period before HAART was used in Cameroon (1996 to 2003) and during HAART (from 2004 to January 2017), respectively. Sequences from both groups were aligned using the MAFFT Program, neighbor-joining phylogenetic trees were generated using Tree Maker and Fig Tree to assess the evolution/comparability of both study groups, the WebLogo used for amino acid sequence analysis, and the Analyze Align Tool to calculate the frequency of each RAM.

Results: Some HIV-1 CRF02_AG variants of the post-HAART era, formed 2 distinct clusters in the phylogenetic tree. Twenty one major RAMs that can modulate susceptibility of tenofovir, lamivudine or efavirenz were identified with increased frequency at post-HAART era. M184V, which increases TDF susceptibility was reported at 29.59% in the post-HAART era compared to 3.59% in the pre-HAART. The T69Ins multidrug resistance (MDR) was reported at 0.3% in the pre-HAART compared to 2.5% in the post-HAART. An uncommon multi-nucleoside reverse transcriptase inhibitor complex Q151M, which confers intermediate resistance to TDF and 3TC, was absent in the pre-HAART era but present at 1.09% in the post-HAART.

Conclusion: The rate of RAMs increased in the viral population with increasing genetic diversity of HIV-1 CRF02_AG.
Deep sequencing of viral populations using next generation sequencing (NGS) offers opportunities to understand and investigate evolution, transmission dynamics, and population genetics. Currently, the standard practice for processing NGS data is to summarize all the observed sequences from a sample as a single consensus sequence, thus discarding valuable information about the intra-patient viral molecular epidemiology. Furthermore, existing analytical pipelines may only analyze genomic regions involved in drug resistance, thus are not suited for full viral genome analysis. Here we present HAPHPIPE, a HAploTtype and PHylodynamics PIPEline for genome-wide assembly of viral consensus sequences and haplotypes. HAPHPIPE includes modules for quality trimming, error correction, de novo assembly, alignment, and haplotype reconstruction. The resulting consensus sequences, haplotypes, and alignments can be further analyzed using a variety of phylogenetic and population genetic software. HAPHPIPE is designed to provide users with a single tool to rapidly analyze viral sequences generated from NGS platforms and provide informative phenotypic analysis to produce an overall report for a single sample and a group of samples.
High variation among viral strains of HIV are a result of high mutation rates, large population sizes, and short generation times, with the possibility of recombination between viral strains. Selection pressures, such as HAART, can influence the prevalence of mutations occurring in the HIV genome. While high variation is hallmark of HIV genetic diversity, it is hypothesized that a few viral strains may dominate the intra-patient genetic diversity, with a variety of lower frequency strains comprising the rest of the population. Analysis of intra-patient diversity using NGS data has proven to be a difficult task. Some existing software packages are unable to keep up with the bulk of data generated by current NGS platforms, while other packages may produce results that do not agree with expectations based on HIV-1 biology. We hypothesize that the failure of existing approaches is due to poor understanding of intra-patient diversity of HIV-1 and lack of a population genetic framework to inform haplotype reconstruction. Here, we test two probabilistic haplotype reconstruction methods using real patient data and simulated populations based on the coalescent. In the patient data, we found that neither method was able to reconstruct haplotypes that agree with expectations based on HIV-1 biology. Furthermore, neither approach could reconstruct haplotypes generated under simple coalescent models. We conclude that closer collaboration is needed among molecular biologists, computational biologists, and computer scientists in order to design realistic haplotype reconstruction algorithms.
HIV-1M SUBTYPES DISPLAY STRIKING DIFFERENCES IN THE LOCATIONS OF GENOMIC SITES THAT THEY CONTRIBUTE TO RECOMBINANTS

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It remains unclear whether inter-subtype HIV-1M recombinants tend to randomly inherit particular genome regions from their parental genomes, or whether some genomic regions from particular subtypes tend to occur more frequently in recombinants than what would be expected under random recombination in the absence of selection.

To differentiate between these two possibilities, we analysed the distribution within 79 CRFs and 88 URFs of genome fragments derived from subtype A, B, C, D, F, G and CRF01_AE viruses. We used a permutation-based test to identify genome regions that tended to be more frequently inherited from particular subtypes than could be accounted for by chance under random recombination.

While genome fragments derived from each of the subtypes were scattered throughout the analysed recombinant genomes, there were some notable differences between the subtypes with respect to the genome sites that they tended to most or least frequently contribute to these recombinants. Specifically, subtype A derived fragments tended to fall within env, subtype B derived sequences in pol, subtype C sequences in tat and vpu, subtype F sequences in gag and pol subtype G in gag and CRF_01AE sequences in gag and vpu. Our analysis also revealed the evidence of statistically significant hot-spots of subtypes A and 01_AE derived sequences in gp120, in RT for subtypes B and D, in vif for subtype C, in p24 for subtype F and in prot for subtype G. Conversely, statistically significant cold-spots were identified in vpu for subtype A derived sequences, in p24 and vif for subtype B, in RT for subtype C, in Nef for subtype D, in p17 and vpu for subtype F, in RT and p15 for subtype G and in RT for CRF01_AE.

The apparent non-randomness in the frequencies which different subtypes have contributed particular genome regions to known HIV-1M recombinants suggests that selection may either favour or disfavour the survival of inter-subtype recombinants that inherit particular genome regions from parental viruses.
THE PHYLOSCANNER METHOD: PHYLOGENETICS BETWEEN AND WITHIN HOSTS, ALL ALONG THE GENOME, SHOWS TRANSMISSION, DUAL INFECTION, RECOMBINATION AND CONTAMINATION

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Several projects are currently generating thousands of whole viral genomes through next-generation sequencing. Methods such as shiver, previously presented here, now facilitate reconstruction of consensus sequences from reads (short sequence fragments). However the reads contain a wealth of additional data on the minor variant mutations and their linkage. To fully exploit this, our tool phyloscanner constructs and analyses phylogenies using all reads from all patients in the study, in sliding windows along the genome. Each such phylogeny provides a model of both within-host and between-host evolution. A maximum parsimony reconstruction of the transmission process is used to (1) remove contaminant reads, (2) detect dually infected patients, and (3) infer viral ancestry. (1) is necessary to avoid distorting other conclusions. (2) Dual infections are important clinically and because they may generate new recombinant strains, accelerating viral adaptation. We detect points of recombination by the appearance of intermediate reads between distinct phylogenetic clusters. (3) When the inferred phylogeny reflects the true evolutionary history, ancestry -one quasispecies having evolved from another - implies transmission with known direction. This transmission could be direct or through unsampled intermediate patients. Identifying transmitters allows better determination of transmission risk factors and more targeted public health interventions. By combining and summarising phylogenies from many windows covering the whole genome, a consistent picture emerges out of the more limited phylogenetic resolution available at any one part of the genome. Using many small windows also bypasses the need to exclude all recombinant sequences before phylogenetic analysis. This greatly enhances the reach of phylogenetics in high-burden endemic areas where there have been more opportunities for recombinants to arise and circulate, and where targeted interventions are most needed. phyloscanner underlies the results analysed in three other submitted talks; here I propose to explain in detail the method itself, with examples from subtypes A-G.
Phylogenetic methods are used to reconstruct the evolutionary history of HIV amongst other pathogens and have provided key insights into its origin and spread. However, phylogenetic methods largely ignore the presence of selection forces shaping evolutionary trajectories. Since the utilized substitution matrices assume independent selection at distinct sites, epistatic interactions, which determine viral fitness and evolutionary pathways, are largely neglected. Recently, maximum entropy methods have been proposed to infer direct couplings between residues within genes. The couplings are typically estimated from multiple alignments which, depending on the dataset, may have emerged from different selection pressures and may be confounded by phylogenetic non-independencies.

We recently developed the Mutational Interference Mapping Experiment (MIME) which generates thousands of mutationally perturbed sequences in vitro and allows to estimate the biophysical effects of each perturbation on an investigated function. Favorably, the method does not create any phylogenetic bias and allows to determine the effects of fitness-decreasing and -increasing mutations alike, as well as epistatic interactions between mutations. Our goal was to first benchmark different computational methods for the correct inference of fitness and epistasis using an in silico experiment involving a library of mutationally modified RNAs that are selected with regard to protein binding. Subsequently, we use the methods to infer physically interacting sites from MIME data that shape the packaging domain in the 5’ UTR of the HIV-1 genome.

Our benchmark revealed that maximum entropy methods robustly predict fitness and epistasis, but they are highly dependent on an a priori choice of regularization. For the HIV-1 genomic RNA, we identified ≈150 positions to affect the Gag-RNA binding, quantifying ≈250 biophysical effects of individual mutations and predicted epistatic interactions mainly mapping to stem loop 1 (SL1) and 3 (SL3) in the viral 5’ UTR, which have been implicated in Gag-mediated viral genome packaging.
Sanger sequencing as a phylogenetic tool for investigating HIV networks cannot reveal directionality of transmission. Clonal analysis can reveal phyletic relationships between epidemiologically linked samples, but is laborious and low-throughput, and the specific region analysed may give inconclusive data. Here we show that whole-genome Next Generation Sequencing (NGS) can support the assignment of one or other direction of transmission between linked samples. Ten demographically diverse, epidemiologically linked pairs were identified, and sequence-capture NGS was performed on archive samples. Using in-house scripts and open-source software packages, complete HIV genomes were derived, together with ‘joint genomes’ from the combined FASTQ data from paired samples.

Method 1: Nucleotide frequency tables were obtained by mapping each individual FASTQ set to the joint genome, and processing the BAM files with an in-house script. The log frequencies of each sample’s majority base in the other sample were summated across all loci within the joint genome (and vice versa) to determine the relative likelihood of each having emerged from the other’s quasispecies.

Method 2: 204nt overlapping tiles were defined in HXB2, and corresponding regions within each sample’s genome determined. Haplotype sets comprising tile-delimited sequences from reads spanning entire tile regions were obtained from SAM files. Phyletic relationships between the 10 largest of the 88 paired haplotype sets were interrogated, allowing an overall directionality to be inferred.

In five pairs where directionality was known, four were strongly supported by both analyses. In the fifth, the methods were in disagreement, although a large temporal distance between samples may account for loss of signal. Of the five remaining pairs, both methods were in strong concordance in four, with samples in the equivocal fifth having eight years between diagnoses and sampling as a likely confounder.

Sequence-capture whole-genome NGS provides sufficient information to infer directionality with much greater confidence than existing methods.
RAPID AND RECENT TRANSMISSION OF HIV AMONG PEOPLE WHO INJECT DRUGS IN GLASGOW, SCOTLAND REVEALED THROUGH PHYLOGENETIC ANALYSIS

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Harm reduction has dramatically reduced HIV incidence among people who inject drugs (PWID). In Glasgow, Scotland, <10 infections/year have been diagnosed since the mid-90s. However, in 2015 a sharp rise in diagnoses was noted among PWID: all were subtype C with two identical drug resistant mutations and some displayed low avidity, suggesting the infections were linked and recent.

We collected Glasgow subtype C pol sequences and identified closely related sequences from the UK HIV Drug Resistance Database (UKRDB) and from Los Alamos National Laboratory (LANL). A phylogeny was reconstructed comprising 228 Glasgow sequences, 762 from UKRDB and 1144 from LANL. The outbreak cluster was extracted from the tree and time-resolved.

All 104 outbreak sequences originated from Scotland. Mean genetic distance was <1%, with short branches indicating rapid transmission. The outbreak subdivided into three subclusters, two of which displayed rapid and recent transmission events. The common ancestor of the outbreak dated back to 2004 and the oldest sequence represented a female PWID diagnosed in 2005. Five patients were diagnosed in 2008-2009 and all others between 2010 and 2016. We extracted node dates to estimate the timing of transmissions, demonstrating an acceleration of the transmission rate after 2008, culminating between 2013 and 2015 when 63 transmissions took place.

All patients reported injecting drugs, and the majority were men (59/94, 63%), suggesting that infections have been transmitted primarily through injection rather than sexually. While disclosure of needle sharing was variable, the majority (88/90, 98%) were co-infected with Hepatitis C.

The strain is limited to Scotland but transmission is ongoing. We are currently investigating associations between the outbreak and epidemiological parameters including homelessness.
**Source of HIV-1 Drug-Resistant Minority Variants in People Who Are Recently Infected**


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**Background:** Drug-resistant minority variants (DRMinVs) in patients who recently acquired HIV-1 can either be transmitted or generated de novo through replication errors. The former are likely to persist and result in treatment failure while the latter could arise stochastically. However, DRMinV transmission contradicts the current understanding that most HIV-1 infections arise from a single founder clone.

**Methods:** We performed ultra-deep sequencing on 655 men who have sex with men (MSM) ascertained to have recently acquired HIV between 2011 and 2014. Variant frequency thresholds for detection of DRMinVs and DR majority variants (DRMajVs) were 2-20% and >20%, respectively. Transmission cluster analysis was performed on DR-containing sequences and >100,000 HIV-1 pol sequences from the UK HIV DR Database (UKHDRD) generated as part of routine clinical care in the UK since 2000, using a bootstrap support of >90% and maximum genetic distances of 4.5% and 1.5% (the latter to detect the most recent transmissions).

**Results:** DRMajVs were detected in 53 (8.1%) and DRMinVs in 61 (9.3%) of recently infected MSMs. High levels of clustering to sequences in UKHDRD were observed for both DRMajV (n=39; 73.6%) and DRMinV (n=52; 85.2%) sequences. Of these, 34 (64.2%) with DRMajVs were in a transmission cluster with sequences that harboured the same DR mutation compared to only 2 (3.3%) of sequences with DRMinVs (p<0.001; \( \chi^2 \) test). Evidence of recent transmission of DRMajVs was observed for 15/53 (28.3%) whereas none was observed for the DRMinVs (p<0.001). Virological failure (VF) rate among those harbouring DRMinVs was 15% (6/40) vs 13% (55/416) among those with no DR (p=0.75). In contrast, VF rate was 29% (12/41) among those harbouring DRMajVs (p=0.006).

**Conclusions:** Using a densely sampled MSM population in the UK we show no evidence that DRMinVs were transmitted among recently infected MSM. Furthermore, the presence of DRMinVs had no significant impact upon VF rate.
A BIOINFORMATIC APPROACH TO DETERMINING HIV-1 DRUG RESISTANCE PROFILES IN NEXT-GENERATION SEQUENCING DATASETS

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Introduction: Next generation sequencing technologies are widely used to study the genetics of HIV-1 populations. Although software is available for processing large scale sequence data, no pipelines include an automated analysis of HIV-1 drug resistance mutations. Therefore, we developed a pipeline that constructs high quality consensus sequences from reads with identical primer IDs as reported previously (Jabara CB, et. al. PNAS, 108: 20166) and reports drug resistance mutation profiles for each consensus by interacting with the Stanford HIV database (https://hivdb.stanford.edu/hivdb/by-sequences/).

Methods: We developed a pipeline that, after binning sequence reads generated by paired-end Illumina Miseq technology according to their common primer IDs, applies a super-majority rule which requires ≥80% agreement at each nucleotide position in order to detect and eliminate PCR-based errors. Consensus sequences are subsequently generated from each group of primer IDs and are automatically processed in the Stanford HIV database. The pipeline is written in Perl.

Result: Our new bioinformatics pipeline produces high quality consensus sequences that have error rates equivalent to the gold-standard single-genome sequencing assay and generates an automated excel spreadsheet that reports each HIV-1 genome that contains drug-resistant mutations. The final report produced from the pipeline includes the frequencies of each HIV-1 drug resistance mutation in the total dataset, the frequencies of linked drug resistance mutations, and the characterization of the genetic backbones on which linked mutations occur.

Conclusions: Our new pipeline allows for the rapid profiling of drug resistance mutations in targeted, HIV-1 next-generation sequencing datasets and is a reliable and easy tool for highly sensitive HIV-1 genotyping.
ASSESSING THE ROBUSTNESS OF PHYLOGENETIC MODELS TO CHANGES IN SELECTION PRESSURES OVER TIME: A SIMULATION STUDY.

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Phylogenetic and population genetic analyses have both contributed immensely to the understanding of evolution. Phylogenetic models were originally developed to model evolution among species. Population genetic studies, on the other hand, focus on explaining evolutionary processes within a population. These fundamental differences ensure that similar concepts tend to be tackled with different techniques between the two fields of evolutionary biology. For example, natural selection is typically modelled in terms of the ratio of non-synonymous to synonymous substitutions (dN/dS) by phylogenetic analysts. In contrast, natural selection is often modelled explicitly in terms of a selection coefficient parameter (s) in the field of population genetics. In this study, we developed a flexible genetic sequence simulator by combining techniques from phylogenetics and population genetics. Consider the Halpern-Bruno mutation-selection model, where natural selection was modelled as a function of codon mutation and fixation probabilities. The authors used an approximation that assumed weak selection pressure. As a result, it had been shown that the model only accommodates negative selection. The simulator that is proposed here is such that natural selection is explicitly modelled without constraining s to small values. We show that dN/dS models approximate selection adequately when selection is weak and that they perform poorly when selection is strong. The adaptations to the mutation-selection models that are proposed in this work allow for simulations of sequences that realistically mimic HIV evolutionary pattern. They consequently provide for more rigorous studies about the dynamics that allow HIV to successfully evade treatments and its host immune system.
LACK OF HCV MOLECULAR CLOCK CAUTIONS AGAINST USING GENETIC DISTANCE AS A MARKER OF TIME

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Background: The linear increase of viral genetic distance (GD) over time in HIV and other infections provides information regarding length of infection and/or timing of transmission events. We tested this assumption for HCV by measuring viral GD in 90 chronically infected subjects in the Baltimore Before and After Acute Study of Hepatitis (BBAASH) cohort.

Methods: All subjects had at least two samples spaced at least one year apart. We used 744 E1 sequences (bp 943-1288 relative to H77) from 89 subjects and 136 5’-hemi sequences (bp 70-5296) from 20 subjects. We calculated intra- and inter-subject pairwise GD for each gene using the TN93 model. We compared intra-subject pairwise distances with the time between the two samples to determine correlation.

Results: The bulk of the distribution of intra-host E1 GD was <4%. Additional peaks were observed at 10%, 35%, 50%, and 65%, which corresponded to comparisons within genotype, between 1a and 1b, 1a/1b and 3a, and 1a/1b/3a and 2b, respectively, and indicate co- or re-infection of multiple viral strains within subjects. Hemi genetic distances followed a similar pattern with peaks at 10%, 25% and 40%. Correlation between the intra-subject GD vs. time was absent for both genes (R2<0.05), measured as both mean GD vs. length of infection as well as considering each individual pairwise GD vs. time. This result was unchanged by removing distances >4% to avoid biasing the analysis with known co/re-infection events, and by further including only subjects with samples spanning >5 years. A moderate (R2=0.2, p<0.001) correlation was observed between GD and time only when distances >1% were removed for the hemi region, but not for E1.

Conclusions: The lack of evidence for a consistent molecular clock warrants extreme caution when using measures of distance as a marker for time. Samples from different individuals with small GD do not indicate a recent transmission event.
HIV-1 SEQUENCES FROM EARLY INFECTION PREDICT THE AGE OF THE INFECTION


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3. AFRIMS, Thailand
4. WRP-Kericho, Kenya
5. MUWRP, Uganda

The date of HIV-1 infection is often unknown. The RV217 cohort allows a rare opportunity to compare precise estimates of the date of infection against phylogenetic dating methods. More than 2,300 seronegative individuals were enrolled for twice-weekly HIV-1 RNA tests, leading to 124 acute infections being identified. HIV-1 genomes from 36 individuals were sequenced at a median of 5, 32 and 170 days after diagnosis; the last negative test occurred 4 days before HIV-1 diagnosis. Within-host phylogenies were obtained using BEAST v1.8.2, under a Bayesian skyline model and uncorrelated log-normal relaxed clock. Most phylogenies showed no accumulation of mutations in the first month of infection, then many mutations were observed at six months. Twenty-six individuals had a single founding population. The beginning of the infection was estimated to have occurred a median of 12 days before the first HIV-1 positive test (range: -350, +7 days); it occurred in the window of transmission (starting 10 days before the last negative test) in 19 of 26 cases. Among the participants with less accurate estimates of the infection, there were 3 participants with subtype A1 viruses that showed limited diversity and who had very low set point viral load (2.7<Log10 <3.5).

Individuals infected with multiple founder variants had less accurate estimates of the date of infection (median: 200 days before diagnosis; IQR: 146-219), with a significantly larger interval (95% HPD) for the estimated infection date than for individuals with single founders (median: 367 vs. 29 days, p = 0.025). Splitting samples into founding events yielded better results, however the small number of sequences per subject (n=30) did not allow to resolve 4 of 10 cases.

Our results showed that Bayesian coalescence methods generally allow accurate dating of an HIV-1 infection event, providing estimates for use when the date of HIV-1 transmission is unknown.
ASSESSMENT OF NEIGHBOR-JOINING AND BAYESIAN METHODS FOR USE IN PHYLOGENETIC ANALYSES OF INTRA-PATIENT HIV-1 POPULATIONS


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Introduction: Different analytical methods can produce divergent phylogenetic trees and lead to conflicting conclusions. We compared tree topologies produced by Neighbor-Joining (NJ) and a Bayesian method (BEAST) using intra-patient HIV proviral single-genome sequences (SGS) to highlight differences in results and interpretation.

Methods: SGS of P6-PR-RT were obtained from HIV DNA in PBMC collected from 7 children in the CHER cohort at time points early after ART initiation (within one year of birth when diversity is limited) and after 7-8 years of suppressive ART. Phylogenetic trees were generated with the NJ algorithm and with BEAST using the GTR+I+F4 model assuming a strict molecular clock.

Results: NJ trees showed little to no genetic distance between variants in the early time point allowing identification of founder viruses. No sequence divergence was detected after 7-8 years on ART using NJ methods, consistent with a lack of detectable ongoing viral replication on ART. By contrast, the Bayesian-generated trees show evolutionary distances between identical sequences obtained at both time points, which could be interpreted as viral evolution from ongoing viral replication during ART. The evolutionary distances observed using BEAST were primarily driven by the input of sampling dates.

Conclusions: By inputting the times of the sample collections into BEAST, the sequences obtained from later dates are assumed to be from recently infected cells and are placed on branches distant from those obtained at earlier time points. NJ methods do not consider the dates of sample collections and thus are only influenced by the genetic distance of the sequences. Because HIV infected cells can persist and clonally expand over the course of infection without evolution, inputing the dates of sample collection into evolutionary analyses is misleading. NJ analysis of intra-patient longitudinal SGS is a more accurate way to assess HIV genetic divergence on ART.
DECONVOLUTING SEQUENCING ERROR FROM TRUE WITHIN-HOST VIRAL DIVERSITY THROUGH PHYLOGENETIC COMPARISON OF ILLUMINA AND NANOPORE SEQUENCE DATA OF HEPATITIS C.

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The MinION platform (Oxford Nanopore Technologies, ONT) offers potential for point-of-care virus genomics which could prove valuable for high burden pathogens such as HCV and HIV, particularly in resource-poor settings where routine disease surveillance, clinical genotyping and resistance testing is currently lacking. Through iterative optimizations to the chemistry, protein-pore design and base-calling algorithms accuracy and data yield has improved dramatically to a point where deep characterization of viral quasispecies is now possible. Furthermore, Nanopore can generate single reads long enough to span whole virus genomes, removing the ambiguity of haplotype construction from quasispecies sequence data.

We have previously described a method for sequencing RNA viruses that avoids virus-specific PCR and instead uses oligonucleotide baits to capture virus-derived nucleic acids for unbiased sequencing on the Illumina platform. Here, we adapt our approach to Nanopore and use it to sequence both HCV and Zika directly from plasma in a single sequencing run. Sequencing libraries were constructed using ONT compatible barcodes and sequenced as a multiplex on a MinION flowcell (R9 version). Sequencing both HCV and Zika both separately and simultaneously from artificial mixtures produced achieved complete genome coverage with at average read depths ranging from 30-417 bp. Using the phyloscanner pipeline, we constructed HCV phylogenies from aligned Illumina and Nanopore sequences and show that Nanopore sequencing error, while approximately 10%, is routed within an underlying population structure, determined by the less error-prone Illumina sequencing method, and thus does not impair our ability to distinguish sequences from separate infected individuals. Using a mathematical model of the Nanopore error process, and maximum-likelihood determination of its parameters from the “true” within-host diversity, we deconvolute the error component from the observed within-host diversity in order to make a priori estimates of HCV diversity using the Nanopore MinION platform.

Nanopore is emerging as a powerful tool for point-of-care studies of within-patient viral diversity for a range of pathogens.
EXPLORING MINION NANOPORE SEQUENCING TO INFER THE WITHIN-HOST VIRAL DYNAMICS FROM CLINICAL HIV-1 AND HCV SAMPLES

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The MinION nanopore sequencer is a small, portable single-molecule DNA sequencing device that produces long sequencing reads by measuring changes in ionic current when single-stranded DNA molecules translocate through protein nanopores. The use of such a platform has now provided new insights into real-time genomic surveillance of viral outbreaks such as Ebola and Zika. However, its application on understanding the complexity and diversity of genetically dynamic populations such as HIV-1 and HCV remains unknown.

HIV-1 full-length pYK-JRCSF and pNL4-3 plasmids were used to validate sequence quality on the MinION sequencing device. A range of clinical samples from acute HIV-1 and HCV infection were amplified for different viral segments (Full genome to smaller amplicons) and sequenced using a range of MinION chemistries (R7 – R9.4). Sequencing libraries were prepared according to Oxford Nanopore Technologies 2D library prep kit with barcodes used to generate libraries for up to 12 samples to permit sequential running on a single flow cell. FAST5 reads containing raw signal-level information were uploaded in real-time for base calling analysis using the cloud-based Miniorch 2D workflows. De novo assembly was performed using Canu v.1.3 with final polishing of the draft assembly using Nanopolish.

Our first attempts at MinION sequencing using an R7.3 FLO-MAP003 flowcell on HIV-1 and HCV revealed an average 2D read accuracy of 75.3% [IQR: 70.3% - 82.4%]. Nevertheless using our bioinformatics pipeline we generated a de novo consensus assembly genome that was 99.2% accurate when benchmarked against Illumina deep sequencing data. Subsequent improvements in basecalling and significant updates in MinION technology from R7 to R9 chemistry demonstrated a substantial improvement in both yield and accuracy. For instance, using a barcoded library of 12 HIV-1 samples on a MIN106-SpotON flowcell demonstrated an average read accuracy rate of 91.5% [IQR: 89.5% - 94.7%] and produced de novo consensus assembly genomes in excess of 99.7% similarity. Examination of intra-host diversity profiles demonstrated that high diversity variants were reliable detected with the MinION at concordant frequencies.

Taken together these results illustrate that the MinION Nanopore Sequencing can provide high-quality, long read viral sequences that accurately reconstruct the consensus genome and further can recapitulate diversity profiles to some degree of accuracy with those derived from clinical HIV-1 and HCV samples.
PRESERVING INTRA-PATIENT VARIANCE IMPROVES PHYLOGENETIC INference OF HIV TRANSMISSION NETWORKS

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Phylogenetic analyses of HIV sequences across patients are used to infer features of the transmission network, such as to identify transmission clusters. Relying on consensus sequences that discard intra-patient variation and using a single representative virus sequence per individual ignores potentially informative data that can be derived from Next Generation Sequencing (NGS) platforms. This is in part because standard phylogenetic methods require a single fully resolved virus sequence per patient. We predicted that preserving information about intra-patient HIV sequence variation could improve phylogenetic analyses across patients.

To test this prediction, we developed a new phylogenetic approach that uses the probabilistic aligner HMMER to generate per-site nucleotide frequency distributions from NGS data, generates a population of “synthetic” sequences that sample from this distribution, and then conducts a phylogenetic analysis that includes multiple synthetic sequences per patient to capture intra-patient sequence variation. On a dataset of adults diagnosed with HIV in 2013 in Rhode Island, USA, we compared (i) a phylogeny with one full genome consensus sequence/patient; and (ii) a phylogeny of NGS-derived full genome 10 synthetic sequences/patient. RAxML was used for maximum likelihood phylogenetic inference, leaf stability and tree certainty analyses.

In 63/67 patients with HIV-1 subtype B, five well-supported clusters (>90% bootstrap); and 4 less-supported clusters (50-80% bootstrap) were identified with the consensus method. The phylogeny produced with the new method was congruent with the consensus method, but with improved support, including resolution of sister group relationship within one cluster and collapsing two clusters into a well-supported one.

Our new phylogenetic method, which preserves the intra-patient variation available with longer and deeper NGS data, produced a more highly resolved virus phylogeny compared to commonly used methods that rely on consensus sequences. Enhanced transmission cluster detection has the potential to improve HIV transmission prevention.
A PROFOUND TRANSMISSION BOTTLENECK BETWEEN THE FEMALE GENITAL MUCOSA AND THE BLOOD LEADS TO A HOMOGENEOUS SYSTEMIC HIV-1 INFECTION


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Background: HIV-1 infection through heterosexual intercourse is characterized by a profound genetic bottleneck. In the majority of cases this process leads to the transmission of a single transmitted founder virus selected from a genetically diverse swarm present in the donor, while in rarer instances multiple transmitted viral clones are responsible for clinical infection. To get a better understanding of the selective events that occur during mucosal transmission we analyzed the viral envelope sequence diversity at early infection in the female reproductive tract and in blood.

Methods: Vaginal swab and plasma samples were collected from Ugandan and Zimbabwean women which were recruited during acute (0-3 months) and early (3-7 months) stages of HIV-1 infection. Using 454 deep sequencing we analyzed the genetic envelope diversity of HIV-1 isolated from the female genital tract and compared it to the diversity of HIV-1 isolated from blood. We included a total of 80 women and additionally were able to analyze 21 matched pairs of cervical swab and blood samples collected from the same patients at the same time points.

Results: Genetic analysis of the C2-V3 region of HIV-1 Env showed that HIV-1 isolates within blood comprise a more homogeneous genotype (d=0.005 s/nt), while HIV-1 clones in the female genital tract showed higher genetic diversity (d=0.011 s/nt). Interestingly viral diversity in cervical samples decreased from acute to early stage of infection while Env diversity in blood slightly increased. No differences in Env diversity were observed between HIV-1 subtypes A, C and D. We observed a greater CD4 T cell decline in individuals that displayed higher genetic diversity in blood during early infection.

Conclusion: We clearly demonstrate the presence of higher HIV-1 diversity in the vaginal tract but a more homogenous HIV-1 population in blood early in infection. Our work provides in vivo evidence for the presence of a genetic bottleneck in the mucosae that leads to the establishment of a systemic infection by only a few clones.
ARE HIV PHYLOGENETIC CLUSTERS ENRICHED WITH TRANSMITTING INDIVIDUALS?

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Introduction: Phylogenetic analyses have become commonplace in epidemiological studies of transmission, as patterns of phylogenetic clustering are assumed to reflect the process of viral transmission. However, the extent to which phylogenetic pairs or clusters reflect patterns of HIV transmission is not completely understood. In this study we explicitly tested assumptions related to the use of phylogenetic clusters as representative of ongoing transmission.

Methods: We used an HIV epidemic model to simulate a heterosexual HIV epidemic on a regional scale. Viral sequences were then evolved via simulation along the recorded transmission chains, followed by subset sampling of sequences, phylogeny reconstruction, and identification of phylogenetic pairs and clusters (with liberal and conservative thresholds). We then tested for associations between individual transmission histories, behavioral risk factors (as defined by the epidemic simulation), and clustering status, at increasing coverage levels.

Results: Unsurprisingly, the absolute number of transmission clusters and number of sequences in clusters increased as sample coverage increased. However, the proportion of sequences in clusters that were transmitters (defined by the simulation) remained constant at ~0.55 across coverage levels, and the relative risk (RR) of a transmitter being in a cluster was ~1.25. RRs of being a transmitter for medium and high-risk (defined by the simulation) individuals were ~6.22 and ~7.59, respectively, while the RRs of these individuals to be in a phylogenetic cluster ranged between 1.25 – 2.00 and 0.75 – 2.50, respectively.

Discussion: Our results indicate that phylogenetic clusters are not substantially enriched with transmitters and that increased sampling coverage does not automatically improve statistical inference of transmission risk factors. Additionally, the use of phylogenetic clusters as proxies for transmission is subject to misclassification bias that leads to decreased effect sizes for known transmission risk factors. We see these effects are best minimized with the use of conservative clustering thresholds.
LIFE CYCLE SYNCHRONIZATION IS A VIRAL DRUG RESISTANCE MECHANISM

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HIV can be effectively treated and prevented with antiretroviral therapy, but the evolution of drug resistance can cause treatment failure. Antiviral drugs typically target a specific phase of the virus's life cycle, and it is generally assumed that resistance arises from mutations that alter the virus's susceptibility to the direct action of the drug. Here we consider the alternative possibility that a virus population can evolve towards synchronizing its life cycle with the pattern of drug therapy. The periodicity of the drug treatment could then allow for a virus strain whose life cycle length is a multiple of the dosing interval to replicate only when the concentration of the drug is lowest. This process, referred to as "cryptic resistance", could allow the virus population to maximize its overall fitness without having to alter drug binding or complete its lifecycle in the drug's presence. We use mathematical models and stochastic simulations to show that life cycle synchronization can indeed be a mechanism of cryptic viral drug resistance. We show this effect is more likely to occur when the variability in both viral life cycle and drug dose timing are low. More generally, we find that in the presence of periodic drug levels, time-averaged calculations of viral fitness do not accurately predict drug levels needed to eradicate infection, even if there is no synchronization. We derive an analytical expression for viral fitness that is sufficient to explain the drug-pattern-dependent survival of strains with any life cycle length. We discuss the implications of these findings for clinically-relevant antiviral strategies for HIV as well as other viruses including hepatitis B and C and influenza.
Reduced bacterial species diversity, referred to as dysbiosis, has been observed in the gut or virginal microbiota during the onset of chronic infection of human immunodeficiency virus (HIV). Bioinformatic analysis of microbiome datasets obtained from sequencing of specific variable regions of 16S rRNA (16S rRNA amplicon sequencing) revealed enrichment of Enterobacteriaceae family as a major compositional change of a gut microbial community of HIV infected individuals. On the other hand, some of study exhibited enrichment of Prevotellaceae family as a major compositional change. These results imply that inherent individual heterogeneity may exist among HIV infected individuals, which gives rise difficulties in interpreting compositional change of microbiota as an indicator of HIV infection.

In this presentation, we report our recent progress on comparative study of inferring HIV associated metabolic changes in virginal and gut microbiota. Abioinformatics analysis pipeline is employed to calculate bacterial species composition from 16S amplicon sequencing data. To extract potential metabolic activity of a given microbiota, several existing databases for bacterial genome and metabolism were used to infer gene contents, followed by metabolic network inference. Based on these data-mining procedures, we investigate a set of metabolites which are invariant among different datasets. A plan of experimental validation will be discussed based on the inferred results.
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