VIRUS EVOLUTION

Volume 3 Supplement 1 2017

# 21st International BioInformatics Workshop on Virus Evolution and Molecular Epidemiology

## A1 Signature pattern and phylogenetic analysis of full-length env genes in 20 hemophiliacs infected with Korean subclade of HIV-1 subtype B

Young-Keol Cho,[1] Jung-Eun Kim,[1] Brian T. Foley,[2]

[1]Department of Microbiology, University of Ulsan College of Medicine, Seoul, South Korea; and HIV Databases and [2]Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, New Mexico, USA

Twenty patients with hemophilia (HP) were diagnosed with HIV-1, one to two years after exposure to domestic clotting factor IX (DCF), in 1989–90. Previous analysis of pol and vif genes confirmed that HIV-1 transmission to 20 HPs occurred through infusion of DCF. In this study, we determined full-length env gene sequences (about 2,550 bp) in 21 HPs, three plasma donors whose plasma were used in DCF production, and 45 local controls. The env gene from frozen stored sera obtained 1–3 years after diagnosis as well as from samples collected years after infection, was amplified by RT-PCR, and subjected to direct sequencing. Phylogenetic analysis revealed that all sequences were subtype B, with 109 sequences from 64 patients (20 HPs, 3 serum donors, 41 local controls, LCs) belonging to the Korean subclade (KSB) and 13 sequences from 5 patients (1 HP infected outside Korea, 4 LCs) not in the KSB. Sequences of the env gene from donors O and P plus the 20 HPs comprised two subclusters within KSB. In addition, signature pattern analysis revealed signature nucleotides at 45 positions between the HPs and LCs ($P < 0.05$). Surprisingly, specific signature nucleotides positions were conserved 100% in clusters O and P only (at 4 and 1 positions, respectively) with none in LCs. Within both clusters, sequence identity was high. These results are consistent with our previous conclusion that these 20 HPs were infected with viruses in the DCF used for treatment. In addition, we found that there are 25 signature pattern residues in amino acids in env gene of KSB distinct from subtype B.

## A2 HIV transmission networks among injection drug users in Pakistan

Francois Cholette,[1] John Ho,[1] Hillary McCoubrey,[1] Kiana Kadivar,[1] Laura Thompson,[2] James Blanchard,[2] Faran Emmanuel,[2] James Brooks,[1] John Kim,[1] Paul Sandstrom,[1]

[1]National HIV and Retrovirology Laboratories, JC Wilt Infectious Disease Research Centre, Public Health Agency of Canada, Winnipeg, MB, Canada and [2]Community Health Sciences, Centre for Global Public Health, University of Manitoba, Winnipeg, MB, Canada

Pakistan is currently facing a concentrated HIV epidemic among injection drug users (IDUs). Well-defined transmission networks, based on molecular epidemiology, have the potential to assist in the development of targeted screening and prevention strategies as well as identify hidden epidemic drivers. Here we present molecular transmission networks re-constructed from HIV-1 pol sequences collected from injection drug users located in major urban centres in Pakistan. Dried blood spots (DBS) were collected on Whatman 903 cards from IDUs in Karachi ($n = 300$), Hyderabad ($n = 300$), and Peshawar ($n = 257$). Nucleic acids were extracted using an easyMAG instrument. Protease and part of the reverse transcriptase genes were amplified and sequenced using an in-house HIV genotyping assay. Transmission networks were re-constructed using publicly available bioinformatics software. Most of the HIV pol sequences (75%) from 96 individuals formed connected nodes arranged in 7 clusters, ranging in size from 3 to 33 individuals. Clusters were mainly homogeneous, in which 87 inferred transmission events (90.6%) occurred within a particular city. A small number (9.4%) of transmission events occurred between cities. The highest number of inter-city transmission events (7.3%) occurred between Karachi and Hyderabad while only 2 (2.1%) occurred between Karachi and Peshawar. Transmission events were not observed between Hyderabad and Peshawar. The majority of sequences analyzed formed localized transmission networks partitioned according to city of collection, although some inter-city linkages are apparent. Reconstruction of transmission networks based on sequencing-based approaches has the potential to expand our understanding of the HIV epidemic currently taking place in Pakistan especially if used in combination with epidemiological data. Particular attention maybe warranted for Karachi since transmission linkages were observed with all the other cities included in this study.

## A3 Genetic analysis and natural polymorphisms in HIV-1 gp41 isolates from Maputo, Mozambique

Nália Ismael,[1] Dulce Bila,[1,2] Diana Mariani,[2] Adolfo Vubil,[1] Nedio Mabunda,[1] Celina Abreu,[2] Ilesh Jani,[1] Amilcar Tanuri,[2]

[1]National Institute of Health, Maputo, Mozambique and [2]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Enfuvirtide is an HIV fusion inhibitor that prevents fusogenic conformation and inhibits viral entry into host cells. Resistance to Enfuvirtide is conferred by mutations occurring in the HR1 region of HIV gp41, involving residues 36–45. Mozambique, a sub-Saharan country with an HIV prevalence of 11.5%, provides

first line and second line HAART based treatment. In resource-poor settings such as Mozambique the lack of adequate infrastructure, high costs of viral load tests and the availability of salvage treatment has hindered the intended objective of monitoring HIV treatment, suggesting an important concern regarding the development of drug resistance. The general aim of this study was to evaluate natural occurring polymorphisms and resistance-associated mutations in the gp41 region of HIV-1 isolates from Mozambique. The study included 78 patients naive to ARV treatment and 28 patients failing first line regimen, recruited from the Alto Mae Health Centre in Maputo. The gp41 gene from 103 patients was sequenced and resistance associated mutations for Enfuvirtide were screened. Subtype analysis revealed that 93% sequences were classified as subtype C, 2% as subtype G, 1% as subtype A1, and the other 4% as mosaic recombinant forms. No Enfuvirtide resistance associated mutations in HR1 of gp41 were detected. The major polymorphisms in the HR1 were: N42S, L54M, A67T, and V72I. This study suggests that this new class of antiviral drug may be effective as a salvage therapy in patients failing first line regimens in Mozambique. However further phenotypic studies are required to determine the clinical relevance of these polymorphisms detected in this study.

### A4 The transmission dynamics over ten years of human immunodeficiency virus type 1 in Vietnam

V.P. Thao, [1,*] V.M. Quang,[2] N.V. Vinh Chau,[2] J. Day,[1] G. Thwaites,[2] T. Le,[1,3]

[1]Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit, HCMC, Viet Nam, [2]Hospital for Tropical Diseases, Ho Chi Minh City, Viet Nam and [3]Hawaii Centre for AIDS, University of Hawaii at Manoa, Honolulu, USA

The HIV epidemic in Vietnam is evolving from a concentrated epidemic in men who inject drugs and female sex workers to sub-epidemics of men who have sex with men and to the general population. Understanding the infection dynamics and the temporal and geographical trends in transmission events in the population are crucial for national HIV control and prevention strategies. In collaboration with two largest primary and referral hospitals for HIV treatment in Ho Chi Minh City and Ha Noi, we have conducted cohort studies of over 1,000 patients on antiretroviral therapy (ART) and have access to approximately 800 HIV polymerase sequences from ART-naïve patients collected over a 10-year period from 2004 to 2014. The first-line ART cohorts includes 220 patients initiating ART in Ho Chi Minh City from 2005 to 2007 and 650 patients initiating ART in Hanoi from 2011 to 2015. The median age was 36 years and 70% were male. Median CD4 cell count was 90 cells/mm$^3$ and the median HIV RNA level was 5.2 log10 copies/mL. The second-line ART cohort enrolled 330 patients who failed first-line ART and were switched to second-line ART from 2006 to 2011. We propose to perform phylogenetic analyses including molecular clock calculations to investigate HIV evolution, HIV transmission dynamics, and trends over 10 years in Vietnam. HIV transmission dynamics includes the pattern of transmission, transmission of drug resistant strains, drug-resistance mutational pathways, and rapidity of viral spread.

### A5 Peripheral blood cells contribute to HIV-1 viremia induced by romidepsin

A. Winckelmann,[1,2,*], K. Barton,[2] B. Hiener,[2] W. Shao,[3,4] L. Østergaard,[1] T. Rasmussen,[1] O. Søgaard,[1] M. Tolstrup,[1] S. Palmer,[2]

[1]Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark, [2]The Westmead Institute for Medical Research, University of Sydney, Westmead, Australia, [3]Leidos Biomedical Research, Inc., Advanced Biomedical Computing Center, Reston, VA, USA and [4]Frederick National Laboratory for Cancer Research, Frederick, MD, USA

Anti-retroviral therapy (ART) suppresses viral replication and restores immune function in HIV patients. However, cessation of treatment results in viral rebound from persistent proviruses in latently infected cells. A recent clinical trial investigated the ability of the latency-reversing agent romidepsin to increase HIV-1 transcription as part of an approach to clear persistent proviruses. The administration of romidepsin once weekly for three consecutive weeks to individuals on suppressive ART revealed quantifiable increases of intracellular and plasma HIV-1 RNA in 5 of 6 participants which coincided with the romidepsin infusions. However, the origin of the romidepsin-induced plasma HIV-1 RNA is unknown. To address this, we compared intracellular HIV-1 DNA and RNA sequences from peripheral blood CD4+ T cells to HIV-1 RNA sequences obtained from the plasma during romidepsin treatment. CD4+ T-cells were obtained at baseline, following the second and third romidepsin infusion, and 10 weeks after the final romidepsin treatment. Plasma was collected 24 and 72 h following each romidepsin infusion. Single-genome sequencing of the env region was used to genetically characterize the virus from intracellular proviral DNA, the transcribed intracellular HIV-1 RNA as well as the plasma RNA pool and MEGA 6.0 was used to perform phylogenetic analysis. The intracellular HIV-1 DNA and RNA sequences obtained during romidepsin therapy contained a mean of 13.5% and 36% defective sequences, respectively. However, 8% defective sequences were found in plasma-derived HIV-1 RNA. Plasma-derived RNA and intracellular HIV-1 DNA and RNA sequences intermingled throughout the phylogenetic tree. In one participant, we identified one plasma-derived HIV-1 RNA sequence identical to, and another highly similar (>99.7%) to, intracellular HIV-1 DNA sequences. Another participant had 16 plasma-derived HIV-1 RNA sequences that were >99.7% similar to intracellular HIV-1 RNA or DNA sequences. One of these plasma sequences was identical to both intracellular RNA and DNA sequences. The plasma-derived HIV-1 RNA sequences in this participant also contained three large clonal populations. Our findings demonstrate that romidepsin induced transcription from proviruses in peripheral blood cells, which contributed to viremia in patients on suppressive ART. Intermingling of plasma-derived HIV-1 RNA sequences with intracellular HIV-1 RNA and DNA sequences indicates activation of multiple infected cells. In one participant the clonal plasma HIV-1 RNA sequences indicated that a subset of transcriptionally activated proviruses contributed to the majority of viremia. Therefore, HIV-infected cells in the blood are important reservoirs of HIV-1 during effective therapy and harbor proviruses capable of contributing to viremia during romidepsin therapy.

### A6 Persistence and transmission of H7N9 influenza virus in Guangdong, China 2013–2015: implications for live poultry market intervention

Changwen Ke,[1,2] Jing Lu,[2] Runyu Yuan,[1,2] Lirong Zou,[1,2] Jie Wu,[1,2]

[1]Key Laboratory for Repository and Application of Pathogenic Microbiology, Research Center for Pathogens Detection Technology of Emerging Infectious Diseases, Guangdong Provincial Center for Disease Control and Prevention, Guangzhou 511430, PR China and [2]WHO Collaborating Centre for Surveillance, Research and Training of Emerging Infectious Diseases

The increasing spread of H7N9 influenza virus and its potential for human-to-human transmission pose a heavy burden to global public health. Since the novel avian influenza A H7N9