mutations associated to MVC resistance were analyzed, as well as the association of the subtype with clinical, epidemiological, virological, and immunological variables. The subtypes detected using the C2V3 region were CRF20, 23, 24_BG (35 patients, 30.4%); Subtype B (33 patients, 28.7%); CRF19_cpx (30 patients, 26.1%); CRF18_cpx (10 patients, 8.7%); and others (7 patients, 6.1%). Overall, 60 per cent of the viruses exhibited R5 phenotype, 14.8 per cent were R5X4 and 25.2 per cent were X4. Interestingly, CRF19_cpx virus was associated with having phenotype X4 [46.7%, $P = 0.0047$, odds ratio (OR): 3.96, 95% confidence interval (95% CI): 1.59–9.84], with infection in young individuals (39.1%, $P = 0.025$, OR: 3,548; 95% CI: 1,136–11,077) and with higher values of viral load ($P \leq 0.05$). The comparison of the amino acid sequences of the V3 loop showed differences between the B and non-B subtypes ($P = 0.0001$). Mutations reported to be associated with MVC resistance, were detected in 75.7 per cent of the samples, in positions 11 (6.1%), 13 (49.6%), 25 (6.1%), 316 (7.0%), 323 (11.3%), and 319 (3.5%) of Gp120, particularly in the recombinant forms CRF19_cpx and CRF_BGs. HIV variants that use the CXCR4 co-receptor were associated with more than 10 years of diagnosis, with older individuals, in the AIDS stage, with low CD4 counts and higher viral load levels ($P < 0.05$). The results support the hypothesis previously stated that CRF19_cpx viruses could be more pathogenic and would have limitations for the use of MVC. The high rate of mutations associated to MVC among non-B Cuban subtypes should be further studied.

A8 Epidemiological study of transmission clusters in a local HIV-1 cohort

Carmen M. González Domenech,[1] Isabel Viciana-Ramos,[2,3] Gabriel Sena-Corrales,[2] Laura Mora Navas,[2] Guillermo Ojeda,[2] Enrique Nuño,[2] Encarnación Clavijo,[2] Rosario Palacios,[2,3] and Jesus Santos[2,3]

[1]Department of Microbiology, University of Granada, Granada, Spain, [2]Infectious Diseases and Clinical Microbiology Unit, Virgen de la Victoria University Hospital, Malaga, Spain and [3]Institute of Biomedical Investigation of Malaga (IBIMA), Malaga, Spain

Integration of molecular, clinical, and demographic data represents a powerful tool to understand the dynamics of local HIV-1 transmission chains (TCs). The aim of our study was the phylogenetic analysis of the TCs within a HIV-1 cohort and the description of the relevant patient data within a TC. We performed a phylogenetic analysis of 757 sequences from newly HIV-1 diagnosed patients in Málaga (Southern Spain) during the period 2004–15. We used partial *pol* gene sequences in a preliminary phylogenetic reconstruction using the Neighbour Joining method (MEGA v6.06 program). After eliminating branches with bootstrap values <80 per cent, we constructed a new phylogeny by Maximum likelihood method (FastTree program). We considered as TC any cluster with bootstrap values ≥ 90 per cent. Patient sequences within and outside TCs were compared. Resistance mutations in the protease (PR) and reverse transcriptase (RT) sequences were analyzed using the Stanford algorithm. Four hundred and fifty-one out of 757 patients (59.6%) were grouped into fifty-three TCs, seventeen of them with five or more subjects. The largest number of patients associated within a TC was ninety. Patients younger than 40 years [odds ratio (OR) 1.75, 95% confidence interval (95% CI) 1.2–2.4, $P = 0.002$], men who have sex with men (MSM) (OR 2.14, 95% CI 1.3–3.2, $P < 0.0001$), non-Spanish (OR 1.48, 95% CI 1.0–2.1, $P = 0.038$), with a non-B subtype HIV-1 (OR 3.12, 95% CI 2.0–4.8, $P < 0.0001$), and presenting primary resistance mutations (OR 14.1, 95% CI 3.1–62.6, $P = 0.001$) were more likely to be associated within a cluster. Ninety-four out of 118 patients (79.6%) with transmission resistance mutations were included in some TC. The most frequent mutations associated with clusters were T69D/N, L210W, and K219E/Q, for NRTIs, K103N, and G190A/S for NNRTIs, and the I54L/M and L90M mutations for PIs. The prevalence for resistance to NNRTIs in TCs was 13.7 per cent. There were two TCs of rarer non-B subtypes: CRF19_cpx, with twenty-one individuals, sixteen of them (76.2%) with mutation G190A; and CRF51_01B with thirty-nine patients, twenty of them with the K103N mutation. Approximately 60 per cent of newly HIV-1 diagnosed patients were included in a TC. Younger patients, MSM, non-Spanish, with non-B subtype HIV-1 and primary resistance mutations were more likely to belong to a cluster. NNRTI mutations were the most frequent ones among patients in TCs. We observed two TCs represented by infrequent non-B subtypes in our area—CRF19_cpx and CRF51_01B—both of which were associated to the transmission of primary resistance.

A9 A method to obtain full-length HIV proviral sequences and their sites of integration

Sean C. Patro,[1] Xiaolin Wu,[2] Shuang Guo,[2] Michael J. Bale,[1] Ann Wiegand,[1] Jonathan Spindler,[1] Christine M. Fennessey,[3] Elias K. Halvas,[4] Brandon F. Keele,[3] Stephen H. Hughes,[1] John W. Mellors,[4] John M. Coffin,[5] Jason W. Rausch,[6] and Mary F. Kearney[1]

[1]HIV Dynamics and Replication Program, National Cancer Institute, Frederick, MD 21702, USA, [2]Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702, USA, [3]AIDS and Cancer Virus Program, Leidos Biomedical Research, Inc., Frederick National Laboratory, Frederick, MD 21702, USA, [4]Department of Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA, [5]Department of Molecular Biology and Microbiology, Tufts University, Boston, MA 02111, USA and [6]Basic Research Laboratory, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702, USA

Accurate definition of the HIV-1 reservoir on antiretroviral therapy (ART) is of paramount importance to the development of curative strategies. Much of this reservoir is derived from clonal expansion of latently infected CD4+ T cells. Methods used to characterize the reservoir include near full-length single-genome sequencing (NFL-SGS) and integration site analysis (ISA). However, current technologies do not link the intact proviruses detected by NFL-SGS to their sites of integration. Therefore, we developed a method to obtain both near full-length single-proviral sequences and their sites of integration. We call our method full-length integrated proviral single-genome sequencing (FLIP-SGS). Genomic DNA from ACH2 and CEM cells mixed at 1:1,000, or patient samples were diluted to a single proviral endpoint. An in-house, optimized whole genome amplification (WGA) method was performed on wells at the endpoint, generating multiple copies of all DNA molecules within each well. The number of proviral copies after WGA was determined by droplet digital PCR targeting the long terminal region (LTR). Forty per cent of each WGA reaction was used to obtain the provirus–host integration sites with ISA (linker ligation, nested PCR, and Illumina sequencing). The remaining fraction was used to amplify the full-length proviruses in four overlapping fragments (LTR-pol, gag-int, int-env, and env-LTR) for Sanger sequencing. WGA performed on the endpoint-diluted ACH2:CEM DNA amplified single-copy HIV-1 proviral templates greater than 500-fold, making it possible to obtain unique integration sites from single proviruses in ACH2 cells, including one that was previously reported (in the NT5C3A gene on chromosome 7) and two that were not previously reported (in the EIF4ENIF1 gene of chromosome 22 and an unknown region of chromosome 6). Near full-length PCR amplification and Sanger sequencing was performed on proviruses integrated in the NT5C3A gene. FLIP-SGS was applied to peripheral blood mononuclear cells from one HIV-1 infected donor with viremia suppressed on ART and yielded integration sites of four genomes that appear to contain large internal deletions. We report a method for near full-length HIV-1 single-genome sequencing combined with host integration site detection that we call FLIP-SGS. This assay will further define clonal expansion of infected CD4+ T cells as a mechanism that maintains the HIV-1 reservoir and as the source of identical sequences observed during therapy and rebound, rather than from ongoing replication.

A10 Presence and frequency of M184V mutation in the MOBIDIP trial

A. Armero,[1,2] M. L. Chaix,[1,2] M. L. Nere,[1,2] E. Delaporte,[3,4] M. Peeters,[3,4] and C. Delaugerre[1,2]

[1]Virologie, Hôpital Saint-Louis, Assistance Publique Hôpitaux de Paris, Paris, France, [2]INSERM UMR 941, Université de Paris Diderot, Sorbonne Paris Cité, France, [3]Institut de Recherche pour le Développement UMI 233, Montpellier, France and [4]INSERM U1175, Université de Montpellier, Montpellier, France

The MOBIDIP trial evaluated the simplification by protease (PI/r) monotherapy for HIV infection versus dual therapy and boosted protease inhibitor plus lamivudine (PI/r + 3TC) in controlled patients under second-line regimens. MOBIDIP was interrupted because of a significant number of patients with virological failure (VF) at week 48 (W48) in PI/r (33/133, ~25%) versus in PI/r + 3TC (4/132, ~3%). At the time of first-line VF, 96 per cent of patients harbored the M184V mutation. The presence of the M184V mutation was related to a protective effect against VF in the

PI/r + 3TC arm. We developed a methodology that allows to determine the frequency of M184V/I mutations in the HIV reverse transcriptase (RT) gene in peripheral blood mononuclear cells (PBMC) obtained before MOBIDIP simplification. Paired-end sequences were obtained from 252 PBMC samples covering the first 855 bp of the RT gene (HXB2: 2485–3405) by MiSeq technology. These sequences were subjected to an in-house Bioinformatics pipeline. The results of our pipeline were compared to the output of PASeq (https://www.paseq.org), an open web-tool for the identification of drug resistance mutations. The M184V mutation was identified at a frequency greater than 1 per cent in 178 individuals (∼71%). The M184I mutation was observed in 34 patients (∼13%), always in the presence of stop codons, and is in agreement with expectations, as this mutation is a known APOBEC-targeted site. Sixty-seven patients (∼27%) had a frequency of the M184V mutation with values greater than 75 per cent. PASeq confirmed the presence of M184V mutation in 173 patients. The frequencies estimated by the PASeq tool and in-house pipeline were correlated up to 99.5 per cent. We found a significant loss of the M184V mutation archived in PBMC between the first-line regimen treatment failure and the beginning of the MOBIDIP trial. In patients under long-term antiretroviral therapy, as in our case, viral sub-populations could be lost, reducing the presence, and frequency of a mutation. In the next step, we will evaluate the association between the presence and frequency of M184V mutation and MOBIDIP results.

A11 Evaluation of phylogenetic inference methods to determine direction of HIV transmission

R. Rose,[1] A. D. Redd,[2] S. Lamers,[1] S. F. Porcella,[2] S. E. Hudelson,[3] E. Piwowar-Manning,[3] M. McCauley,[4] T. Gamble,[4] E. A. Wilson,[5] J. Kumwenda,[6] M. C. Hosseinipour,[7] J. G. Hakim,[8] N. Kumarasamy,[9] S. Chariyalertsak,[10] J. H. Pilotto,[11] B. Grinsztejn,[12] L. A. Mills,[13] J. Makhema,[14] B. R. Santos,[15] Y. Q. Chen,[5] T. C. Quinn,[2] M. S. Cohen,[7] S. H. Eshleman,[3] and O. Laeyendecker[2]

[1]BioInfoExperts, LLC, Thibodaux, LA, USA, [2]NIAID, NIH, USA, [3]Department of Medicience, Johns Hopkins University, Baltimore, MD, USA, [4]FHI-360, USA, [5]Vaccine and Infectious Disease Science Division, Fred Hutchinson Cancer Research Institute, Seattle, WA, USA, [6]College of Medicine-Johns Hopkins Project, Blantyre, Malawi, [7]Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, [8]University of Zimbabwe, Harare, Zimbabwe, [9]YRGCARE Medical Centre, Chennai, India, [10]Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand, [11]Hospital Geral de Nova Iguaçu and Laboratorio de AIDS e Imunologia Molecular, Rio de Janeiro, Brazil, [12]Instituto Nacional de Infectologia Evandro Chagas-INI-Fiocruz, Rio de Janeiro, Brazil, [13]CDC Division of HIV/AIDS Prevention/KEMRI-CDC Research and Public Health Collaboration HIV Research Branch, Kisumu, Kenya, [14]Botswana Harvard Aids Institute, Gabarone, Botswana and [15]Servico de Infectologia, Hospital Nossa Senhora da Conceicao/GHC, Porto Alegro, Brazil

It has been postulated that the direction of HIV transmission between two individuals can be determined by phylogenetic analysis of HIV sequences. This approach may be problematic, since HIV sequences from newly infected individuals are often more similar to index sequences from samples collected years before transmission, compared to those from samples collected at the time of transmission. We evaluated the accuracy of phylogenetic methods for determining the direction of HIV transmission by analyzing next-generation sequencing (NGS) data from index–partner pairs enrolled in the HIV Prevention Trials Network (HPTN) 052 trial. HIV-infected index and HIV-uninfected partner participants were enrolled as serodiscordant couples; samples were analyzed from couples with index-to-partner HIV transmission that was confirmed by genetic linkage studies. NGS for HIV gp41 (HXB2 coordinates: 7691–8374) was performed using plasma samples from thirty-nine index–partner pairs (seventy-eight samples collected within 3 months of partner seroconversion). Maximum likelihood trees were generated using the entire dataset using FastTree v.2. Topological patterns of HIV from each index–partner pair were analyzed. The analysis included 9,368 consensus sequences and 521,145 total sequence reads for the seventy-eight samples analyzed. In 10 per cent (four out of thirty-nine) of couples, the phylogeny was inconsistent with the known direction of transmission. In 26 per cent (ten out of thirty-nine) of couples, the phylogeny results could not discern directionality. In 64 per cent (twenty-five out of thirty-nine) of couples, the results correctly indicated index-to-partner transmission; in two of these twenty-five cases, only one index sequence was closest to the most recent common ancestor. Phylogenetic analysis of NGS data obtained from samples collected within 3 months of transmission correctly determined the direction of transmission in 64 per cent of the cases analyzed. In 36 per cent of the cases, the phylogenetic topology did not support the known direction of infection, and in one-third of these cases the observed topology was opposite to the known direction of transmission. This demonstrates that phylogenetic topology alone may not be sufficient to accurately determine the direction of HIV transmission.

A12 Modeling residual HIV replication and the emergence of drug resistance on ART

J. Michael Bale,[1] F. Valerie Boltz,[1] John W. Mellors,[2] John M. Coffin,[3] Stephen H. Hughes,[1] and Mary F. Kearney[1]

[1]HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, Frederick, MD, USA, [2]Department of Medicine, University of Pittsburgh, Pittsburgh, PA, USA and [3]Department of Molecular Biology and Microbiology, Tufts University, Boston, MA, USA

There are conflicting reports regarding the presence of low-level HIV replication during suppressive antiretroviral therapy (ART). We simulated varying levels of replication and estimated the number of generations needed to obtain linked, drug resistance mutations to explore the effects of replication during ART. HIV replication was simulated with varying population sizes (10 to 3,000,000). Each population size was modeled ten times. Each genome was given a Poisson-distributed number of mutations according to its length and the average replication error rate (3.4×10^{-5} sub/nt/cycle). Simulations were run a maximum of 20,000 generations with endpoints defined as detection of a variant with resistance mutations to at least two ARVs. In all simulations, variants that were resistant to all three ARVs emerged in less than 20,000 generations. The time to emergence ranged from 148–16,156 generations in the various simulations, depending on the replicating population size (4.8 months to 44.3 years if the generation time is 1 day). Clinically detectable virologic failure can result from linkage of two mutations conferring resistance to two ARVs in a regimen. In our simulations, two linked mutations emerged in from 9 to 6,429 generations (9 days to 17.6 years). Our simulations suggest that in patients continually suppressed on ART for at least 10 years, the replicating population size would have to be less than ten, or virologic failure would have occurred from emergence of two ARV-resistant variants. Because most patients on ART do not experience virologic failure, our simulations suggest that any residual replicating population on ART is very small and thus not likely to either sustain or significantly contribute to the HIV reservoir.

A13 Phylodynamic analysis of HIV in Florida

V. Richards, S. Rich, R. Cook, and M. Prosperi

Department of Epidemiology, University of Florida, Gainesville, Florida, USA

We are interested in using phylodynamics to better understand molecular clusters of HIV within the state of Florida, USA. To our knowledge, there is currently no study using such methodology to understand the epidemic in Florida. Viral sequences collected from 2007 to 2017 (about 50,000) will be linked with individual-level data (demographics—sex, race/ethnicity—and mode of transmission) accessed through the Patient Reporting Investigating Surveillance Manager (PRISM), and the Enhanced HIV/AIDS Reporting System (eHARS) via the Florida Department of Health (FDOH). Through the use of HIV-TRACE, which analyzes genetic distance matrices, we will create molecular transmission networks including data on mode of transmission, race/ethnicity, and sex. Furthermore, a maximum likelihood phylogenetic tree will be created using software (e.g. IQ-Tree, PhyML, and FastTree2) run on a high-performance cluster. Phylogenetic comparative analysis will be performed to assess the association between phylogenetic clades and demographics (including extended demographics like syphilis and other comorbidities queried from PRISM and eHARS).