

children through contaminated catheters and needles, and from children to mothers through breast-feeding. Since then the infection was transferred both vertically and horizontally. The source of the outbreak was the husband of one of the infected mothers who was previously infected in Congo. Before the end of 1990s, all samples of HIV subtype G in Russia were considered to originate from that outbreak. Here, we study the phylogenetics of HIV subtype G in Russia. The dataset consisted of 1,169 HIV-1 subtype G sequences of protease and reverse transcriptase, including 12 Russian sequences of unknown origin and three Russian sequences from the Elista outbreak sequenced in the Central Research Institute of Epidemiology (CRIE), and 122 sequences of Russian origin from the Los Alamos National Laboratory (LANL) HIV database (lanl.hiv.gov). Sequences were aligned with Clustal Omega, and the alignment was then manually edited in AliView. A maximum likelihood tree was reconstructed using RAxML. In the resulting phylogeny, the 120 Russian LANL sequences and the three CRIE sequences from the Elista outbreak fall into a clade nested within the sequences from Democratic Republic of Congo as expected for the 1988 Elista outbreak. By contrast, the remaining thirteen other Russian sequences, including twelve CRIE sequences and one LANL sequence, formed a strongly supported clade nested within sequences from Portugal, next to 2003–2004 sequences from Denmark. This phylogenetic evidence suggests a mixed origin of the subtype G, with a previously unreported influx of subtype G in Russia in mid-2000s from Western Europe. About eight out of twelve subtype G sequences of presumably Western European origin were later fully sequenced. Recombination analysis performed with jpHMM has shown that all of them share the same recombination with subtype B (positions 790–1,160 based on HXB2 numbering). In contrast, analysis of full-genome subtype G sequences from the Elista outbreak has not shown any recombination events. These results also support the hypothesis of mixed origin of subtype G in Russia.

A12 Transmitted HLA pre-adapted polymorphisms in the GAG protein influences viral evolution in the new host

D. Mónaco,¹ D. Dileria,¹ A. Gartland,² K. Qin,³ K. Dennis,¹ J. Tang,³ J. Gilmour,⁴ A. Bansal,³ S. Allen,^{1,5} P. Goepfert,³ and E. Hunter¹

¹Emory University, Atlanta, GA, USA, ²Fred Hutchinson Cancer Research Center, Seattle, WA, USA, ³University of Alabama at Birmingham, Birmingham, AL, USA, ⁴International AIDS Vaccine Initiative (IAVI), London, UK and ⁵Zambia-Emory HIV Research Project, Lusaka, Zambia

HIV escapes adaptive cellular immunity by selecting mutations that are associated with the individual's HLA-I alleles. These mutations can be transmitted and have been shown to determine disease progression in the new host but their impact on subsequent viral evolution is poorly understood. In a group of seventy-six Zambian heterosexual transmission pairs, we obtained the gag sequence at zero (donor and recipient; median = 46 dpi) and at three, six, nine, twelve, eighteen, and twenty-four months after infection (recipient; median = 772 dpi). A neighbor joining phylogenetic reconstruction of these sequences (MEGA 6.06) confirmed that the donor clustered with the corresponding recipient sequences, and showed a star-like shape in most recipients. Using both 0 months sequences, we calculated the proportion of HLA-linked sites according to the recipient's HLA alleles that harbored a mutation associated with CTL escape. We found a median of 25 per cent of pre-adapted sites and confirmed that these polymorphisms impaired immune recognition, since individuals infected with highly pre-adapted virus (>50 vs. <20 per cent) had a lower number and proportion of CTL IFN- γ responses in ELISpot assays performed

using predicted peptides ($P=0.004$). Using the longitudinal sequences, we calculated both the rate of reversion of pre-adapted polymorphisms, which was significantly lower than that of all transmitted polymorphisms ($P<0.0001$), and the proportion of adapted sites after two years of infection, which was significantly increased to a median of 40 per cent ($P<0.0001$). Interestingly, the patients where the proportion of adapted sites was increased had one that was initially significantly lower ($P=0.0014$). We also looked for evidence of adaptive evolution using the Codon-based Z-test of Selection (MEGA 6.06). We found that 50 per cent of recipients showed significant positive selection during the first two years of infection. Interestingly, female recipients that showed evidence for positive selection, excluding those expressing the B*57 protective allele, had a lower initial proportion of pre-adapted sites than those that did not show evidence for positive selection (17.7 vs. 28.6 per cent; $P=0.06$). This study reveals that, even before an immune response is mounted in the new host, the degree of pre-adaptation of the transmitted variant determines the dynamics of viral evolution in the newly infected individual.

A13 Social affects phylogeny: Exploration of ongoing and active transmission chains

M. Siljic,¹ V. Cirkovic,¹ S. Lukovic,¹ D. Salemovic,² I. Pesic-Pavlovic,³ D. J. Jevtic,² and M. Stanojevic¹

¹Institute of Microbiology and Immunology, University of Belgrade School of Medicine, Belgrade, Serbia, ²IV/AIDS Unit, Infectious and Tropical Diseases University Hospital, Clinical Center Serbia, Belgrade, Serbia and ³Virology Laboratory, Microbiology Department, Clinical Center Serbia, Belgrade, Serbia

In this research, we combined phylogenetic and population based statistical approaches to assess HIV transmission dynamics and identify clusters that harbor strong potential for onward transmission. Over 300 full protease and partial RT HIV-1 subtype B sequences from Serbia were analyzed. A phylogenetic tree was constructed in MEGA with 1,000 bootstrap resamplings using both a neighbor joining and maximum likelihood approach under the GTR+G+I nucleotide substitution model. In addition, Bayesian estimation of phylogeny was performed using MrBayes. Identification of phylogenetic clusters was based on sets of criteria involving bootstrap support, genetic distance, Bayesian posterior probability value and Phylopart analyses. The largest transmission clusters detected were further subjected to logistic growth model (LGM) analyses implemented in the mathematical application Geogebra. The model assumed that in one year some subjects in an identified cluster were not yet diagnosed, and some had already been infected and diagnosed. Clusters were classified in three groups, as having low (<5 per cent), moderate (5–15 per cent), and high probability (>15 per cent) of hidden infection and potential for future HIV transmissions. Lastly, we examined the relationship between cluster size and socio-epidemiological profiles of cluster members. For this investigation, latent class analysis (LCA) was applied to different categorical covariates including: HIV risk factor, year of diagnosis, place of residence, education, age at diagnosis, other STIs, and clinical stage at the time of diagnosis. Phylogenetic analyses revealed the presence of fourteen transmission clusters. The majority of clustering sequences, were from male MSM patients living in Belgrade. LGM analysis of two largest transmission clusters detected, comprising thirteen and eight sequences, respectively, identified them as having very high (58 per cent) and moderate probability (10 per cent) of harboring undiagnosed infection. LCA identified four classes based on the lowest AIC that were associated with cluster size. Class 1 included high prevalence of young (20–30 years), MSM patients

(85 per cent), living in Belgrade, presenting in CDC stage A (84 per cent) with high potential to be part of a transmission cluster. Classes 2 and 3 included older patients (>40 years), heterosexual transmission category presenting in CDC stage C. Class 4 included a mix of MSM and heterosexual transmission category with high prevalence of patients presenting at CDC stage C.

A14 Ultra-wide and ultra-deep sequencing increases the detection rate of dual HIV-1 infections and recombinants

K. Van Laethem,^{1,2} A. Thielen,³ Y. Schrooten,² F. Ferreira,¹ L. Vinken,¹ and M. Daümer³

¹KU Leuven, Department Microbiology and Immunology, Rega Institute for Medical Research, Leuven, Belgium, ²AIDS Reference Laboratory, University Hospitals Leuven, Leuven, Belgium and ³Institute of Immunology and Genetics, Kaiserslautern, Germany

Since the 1980s, HIV-1 non-B subtype infections have been observed in Belgium and they have been mainly associated with sub-Saharan African migrants and heterosexual risk behavior. In the last decade, a rapid spread of subtype F1 was recognized among men having sex with men, previously predominantly characterized by subtype B infections. In this setting of co-circulating subtypes within a local risk group, we want to estimate the frequency of dual infections and the emergence of recombinant strains using next-generation sequencing. At least one plasma sample was selected from each HIV-1 patient who was diagnosed with a subtype F1 ($n=41$) or BF1 recombinant ($n=6$) infection. The subtype was defined based upon population-based PR-RT consensus Sanger sequences (Rega v3 subtyping tool). In addition, samples from ten HIV-1 patients whose PR-RT sequence displayed high similarity (>97.5 per cent) within the B fragment of several BF1 recombinants were included. Near full-length consensus HIV-1 sequences were obtained for eighty-five samples by multiplexing six overlapping amplicons. Samples were fragmented using Illumina's Nextera XT DNA Library Prep kit and sequenced on a MiSeq sequencing system (2×250 bp). Analysis was performed using the bioinformatics pipeline developed at the Institute of Immunology and Genetics. Consensus sequences were deduced from nucleotide frequencies (15 per cent threshold) and were submitted to the Rega v3 subtyping tool. A concordant subtype classification was obtained for thirty-one patients initially diagnosed with a subtype F1 infection (76 per cent), for nine subtype B (90 per cent) and six BF1 infections (100 per cent). Nine subtype F1 infections were reclassified as BF1 recombinants and two superinfections were identified (F1→BF1 and B→F1). When the threshold for consensus generation was reduced, it resulted into reclassifications for an additional ten patients. This study confirms that the diversity within a HIV-1 epidemic is underestimated when PR-RT Sanger sequencing is used for surveillance studies. However, the next-generation sequencing methodology used was developed for the reconstruction of the consensus sequence that summarizes the viral population within a patient, but could also be subject to inaccuracies due to in vitro and in silico selection biases and recombination events. Alternative strategies are required to perform a more in-depth analysis of dual infections and recombination.

A15 HIV-1 whole-genome NGS analysis to characterize virus evolution following dendritic cell immunotherapy and analytical treatment interruption

C. Lungu P. H. M. Boers, C. M. E. Schapendonk, S. L. Smits, D. A. M. C. van de Vijver and R. A. Gruters

Department of Viroscience, Erasmus MC, Rotterdam, The Netherlands

A dendritic cell (DC)-based therapeutic vaccination was evaluated in a phase I/II clinical immunotherapy trial in which seventeen HIV-1 infected patients on cART received autologous DCs electroporated with mRNA encoding HIV-1 Tat, Rev, and Nef (DC-TRN) before analytical treatment interruption. The trial provided a unique longitudinal set comprising samples from up to seven years prior administration of immunotherapy, at time of cART initiation and up to two-years post-vaccination. To determine the potential effects of DC vaccination on viral evolution and CD8+ T-cell epitope regions, pre- and post-vaccination sequences of whole genes within and outside tat, rev, and nef were obtained from plasma RNA before initiation of cART and after vaccination during viral load rebound following analytical treatment interruption. Control sequences for virus evolution in untreated HIV-1-infected individuals were obtained from the LANL HIV Sequence Database. The first set of data was obtained with Sanger sequencing, which revealed that viral sequence evolution in the tat, rev, and nef genes of vaccinated patients was similar to that of controls. Furthermore, the number of mutations observed inside and outside CD8+ T-cell epitopes was comparable for vaccine-targeted and non-targeted proteins, although occasional escape from CTL pressure was observed. Based on consensus sequence analysis, no evidence for a widespread impact of vaccine-induced or enhanced immune responses on the number of mutations inside or outside epitopes was found. Subsequently, whole-genome NGS on plasma samples from the same patients and time points as that for Sanger sequencing was performed. An in depth analysis of the NGS data to characterize virus evolution in tat, rev, and nef versus the rest of the genome is still lacking. Further analysis is required to search for minor variants that mutate under CTL pressure. These results may guide further research on plasma as well as PBMC samples that are still available.

A16 Primary drug resistance among children infected with HIV-1 in Mozambique: Impact of maternal and neonatal prophylaxis

Nália Ismael,¹ Dulce Bila,¹ Nedio Mabunda,¹ Martina Penazzato,² and Adolfo Vubil¹

¹National Institute of Health of Mozambique, Maputo, Mozambique and ²World Health Organization, Geneva, Switzerland

In Mozambique, HIV prevalence among children aged one to four years old was 1.4 per cent in 2009. The challenge of long-term adherence in adults as well as children during HIV treatment can be associated with the emergence of drug-resistant viruses resulting in treatment failure. Understanding the pattern of primary drug resistance and the impact of maternal and neonatal prophylaxis to define correct HIV treatment regimens is crucial. The main aims of this study are to describe the emergence of primary drug resistance among HIV infected children, the impact of maternal and neonatal prophylaxis associated with the emergence of drug resistance mutations, and the molecular epidemiology of HIV among these children. Retrospectively, dried blood spot (DBS) samples collected from HIV positive children used for routine HIV diagnosis using DNA PCR in four different laboratories in Mozambique were selected. DNA was extracted from DBS samples and used for genotyping. Drug resistance mutations and subtype distribution were analyzed by Stanford resistance algorithms. The relationship between drug resistance mutations to nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTI and NNRTI) and maternal/neonatal prophylaxis was performed by regression models. In total, 496 DBS were collected and 429 (86.5 per