(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

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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

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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

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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 nenatal 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