

discovery are not effective enough. We have developed a bioinformatics pipeline to identify and classify all known viruses present in a metagenomic sample. Viral NGS reads are identified using a protein-based alignment method, DIAMOND, which is substantially faster than the standard BLAST method, and more reliable for viruses. These reads are automatically assembled into contigs using SPAdes, a *de novo* assembler. The contigs are then used to classify the virus at species level using a pan-viral typing tool based on all available taxonomic reference sequences from the International Committee on Taxonomy of Viruses (ICTV) database. This bioinformatics pipeline is Java-encoded and will include an easy-to-use web interface that is fit-for-purpose for researchers or clinicians. This tool can assemble viral contigs from paired-end reads generated by an Illumina MiSeq sequencer. So far 1865 viruses can be identified at species level resolution and 10 viruses (chikungunya virus, dengue virus, HBV, HCV, HHV8, HIV-1, HPV, HTLV-1, YFV, and Zika virus) at the genotype level. A web version of the pan-viral typing tool is already available and a web version with extended NGS functionality is currently being evaluated. Eliminating the need for virus-specific laboratory techniques, or targeted sequence capture, means a virome can be profiled in the context of its non-viral microbiome. Preliminary findings suggest our tool offers greater functionality than existing alternatives, with greater sensitivity to known viruses (including bacteriophages), automatic assembly and good quality phylogenetic analyses. A systematic comparison is underway.

#### **A35** Viral evolution and innate immune responses during acute HIV-1 infection and their association with disease pathogenesis

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The rate of HIV-1 disease progression varies widely between individuals. This has been attributed to a combination of virological and immunological events during acute HIV infection (AHI). However, the exact mechanisms explaining the relationship between HIV-1 diversity, evolutionary dynamics and host immune responses, and their effect on disease pathogenesis remain unclear. We aim to dissect HIV-1 viral diversity, evolutionary dynamics and select parts of the innate immune responses observed during AHI, and elucidate virus-host mechanisms involved in the regulation of HIV-1 disease pathogenesis during the acute and chronic stages of infection. A retrospective longitudinal study design from well-characterized AHI cohorts will be used. Archived samples from about 122 patients with AHI (defined as HIV-1 antibody negative and RNA or p24 antigen positive) from Europe (Sweden [ $n=32$ ]) and Africa (Kenya [ $n=32$ ], Rwanda [ $n=14$ ], Uganda [ $n=13$ ], Zambia [ $n=15$ ], and South Africa [ $n=16$ ]) will be included. Each patient will contribute plasma samples from four serial time points (<14, 30 [+/- 15], 90 [+/- 30] and 360 [+/- 180] days post estimated date of infection, EDI) collected prior to treatment initiation. HIV-1 ev

sequences determined by single genome sequencing (SGS), with 20 SGS clones from each time point, will be generated. In addition, a selected panel of innate immune markers will be profiled using the Meso Scale Discovery (MSD) electro-chemiluminescence-based platform and/or ELISAs. A multi-dimensional Bayesian framework of hierarchical phylogenetic models (HPM) will be applied, allowing for both fixed and random effects prior specifications to test for differences associated between and within patient group parameters, and where all measured virus-host parameters will be considered simultaneously. In addition, evolutionary parameters in different stages of the disease i.e. acute and chronic phases, will also be measured and accounted for in the HPM by addition of the epoch modeling approach to quantify the relationships between viral parameters, innate responses and their effect on disease pathogenesis. The proposed study is likely to constitute one of the largest virus-host dataset of longitudinally collected data of both virus sequences (covering a wide range of HIV-1 subtypes) and innate immune markers to date. The results of the proposed analyses will increase our understanding of HIV-1 pathogenesis and may have implications for therapeutic and prophylactic vaccine design.

#### **A36** Prevalence of HIV-1 subtypes in Slovenia with an emphasis on molecular and phylogenetic investigation of subtype A

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In Slovenia, a small country in Central Europe, less than 1 per 1,000 inhabitants are estimated to be infected with HIV-1. As in most of the Central and Western European countries, the majority of patients diagnosed with HIV-1 are infected with subtype B. However, due to migration, other subtypes can become more prevalent in the country. The aim of this study was to determine HIV-1 subtypes circulating in Slovenia and to further examine the molecular epidemiology of subtype A. A total of 367 Slovenian HIV-1 sequences were included in the study, representing 58% of all patients diagnosed in Slovenia until the end of the year 2013. Subtype was assigned by employing different HIV subtyping tools coupled with Maximum likelihood phylogenetic analysis. The latter was performed to examine the molecular epidemiology of subtype A as well. Identified clusters of Slovenian subtype A sequences were further analyzed for the determination of the time of the most recent common ancestor (tMRCA) by using Monte Carlo Markov chain (MCMC) method available in BEAST 2.1.3 software. We determined the prevalence of subtype B to be 85.3%, while subtype A was the most prevalent non-B subtype found in 18 patients (4.9%), followed by CRF02\_AG (2.4%), subtype C (1.1%), subtypes D, G and CRF01\_AE (0.8% each) and subtypes F1 and CRF22\_01A1 (0.3% each). Subtypes could not be assigned to 12 sequences (3.3%). The phylogenetic tree obtained by ML analysis of the subtype A and subtype A related recombinants revealed that Slovenian sequences were part of 6 major international clusters. Two clusters consisting only of Slovenian sequences were identified and thus additional MCMC analysis was employed. Results of a Slovenian cluster of 4 subtype A sequences showed a posterior probability value of 1 and a tMRCA between the years 1985 and 2008, with a mean in the year 2001. In conclusion, in a Central