A20 The search for replication-competent HIV during effective therapy

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Current antiretroviral therapies for HIV-1 are not curative because a small number of CD4+ T-cells remain infected with a latent, replication-competent provirus that contributes to viral rebound after the cessation of therapy. Several approaches to purge persistent HIV-1 reservoirs are in the beginning phases of clinical trials. To ensure future curative therapies target replication-competent HIV-1 proviruses for eradication, a thorough understanding of the distribution of replication-competent HIV-1 within T-cell subsets and how activation and proliferation of these cells contribute to the maintenance of the replication-competent HIV-1 reservoir is required. This study will employ a full-length single-proviral sequencing assay based on Next Generation Sequencing (NGS) techniques to sequence the entire HIV-1 genome of proviruses isolated from CD4+ T-cell subsets (central, transitional, and effector) sorted from peripheral blood and lymphoid tissue after 5–15 years of suppressive therapy from two groups of participants (1) three participants who initiated therapy during acute/early infection and (2) three participants who initiated therapy during chronic infection. Replication-competent proviruses will be identified by the absence of deletions and APOBEC3G induced hypermutation. The infection rates of replication-competent proviruses located in specific cell populations between participants will be compared along with the frequencies of replication-competent proviruses between different T-cell populations and within tissue-derived cells from these participants. This important study will allow us to determine whether specific cellular compartments harbour replication-competent HIV-1 and will provide valuable information for future curative HIV-1 clinical trials.

A21 HIV-1 sub-subtype F1 outbreak among MSM in Belgium

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HIV-1 non-B subtype infections have been observed in Belgium since the 1980s. However, subtype B predominates amongst men having sex with men (MSM), whereas other subtypes are mainly associated with sub-Saharan African migrants and heterosexual risk behavior. In the last decade, subtype F1 diagnoses have increased substantially in Belgium, representing 9% of newly diagnosed and therapy-naïve HIV-1 patients linked to care in 2014. In the present study, the Belgian subtype F1 epidemic has been characterized within a global context, where F1 is responsible for <1% of HIV-1 infections. The Belgian AIDS Reference Laboratories collected HIV-1 pol sequences from patients linked to care and sub-subtype F1 was verified using Rega v3 and COMET v1.0 subtyping tools. Concordant F1 sequences were retained from 293 patients, who were diagnosed with HIV-1 between 1988 and 2015. The number of F1 diagnoses increased from three in 2001–2 to 83 in 2013–4. Seventy-seven percent were men, with 52% homosexual, 15% bisexual, and 15% heterosexual contact as the probable transmission route (18% not registered). Belgium was the probable country of infection for 54% of the patients, whereas for 38% this information was not registered. A reference dataset from countries with a high burden of F1 infections or with a potential role in the global origin of sub-subtype F1 was collected from public and private databases and the phylogeny was reconstructed using RAxML and BEAST. These analyses indicate that 190 Belgian F1 sequences, 97% from men, and 72% with homosexual/bisexual risk behavior (17% not registered), belong to a monophyletic group with two sub-clades. Together with a Spanish clade, the Belgian clade is embedded in the Brazilian subtype F1 diversity and probably emerged after single or two migration events from South-America with one dead-end lineage (2 strains) and one actively spreading cluster (188 strains). This study reconstructed the structure of the local HIV-1 F1 epidemic and showed that onward transmission of subtype F1 occurs extensively among MSM. It illustrates the introduction and dissemination of strains in one geographically restricted risk group in which the subtype was previously absent.

A22 Increase in the numbers of HIV-1 non-B subtypes and potential recombinant forms circulating among Slovenian MSM in recent years

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In Slovenia, as in many Western countries, subtype B is still a predominant HIV-1 subtype and was historically correlated with the epidemic among men who have sex with men (MSM). In recent years, several reports demonstrating an increasing prevalence of non-B subtypes have been published. The majority of infections with non-B subtypes were linked to the heterosexual mode of transmission in previous Slovenian studies, thus the aim of this study was to investigate whether non-B subtypes are also becoming more prevalent among MSM in Slovenia. Between the years 2000 and 2014, a total of 520 people were diagnosed with HIV-1 infection in Slovenia. During the

study of HIV-1 transmitted drug resistance, filled questionnaires were obtained for 440 patients. Homosexual contact as the most probable mode of HIV acquisition reported 326/440 patients. Subsequently, partial *pol* sequences were obtained for 252 MSM patients who were included in the present study. Sequences were analyzed using the following automatic subtyping tools: REGA 2.0, REGA 3.0, COMET HIV-1 1.0, jpHMM, and SCUEAL. Sequences that gave divergent subtyping results were considered to be potential recombinant forms. Temporal trend of the proportion of non-B and potential recombinant sequences (both combined termed as "non-pure subtype B" sequences) was evaluated with Fisher exact test used for the assessment of statistical significance. All five subtyping tools gave concordant subtyping results in 230/252 (91.3%) of sequences. Pure subtype B was assigned to 226/252 (89.7%) sequences and subtype A, subtype C, subtype F and CRF01_AE were determined in one patient each (0.4%). The remaining 22/252 (8.7%) sequences yielded divergent results with at least one of the subtyping tools, an indication to a possible recombination event in the past. An increase in the proportion of "non-pure subtype B" HIV-1 variants was noted over the years with 0% (95% confidence interval (CI): 0–16%), 5% (95% CI: 1–15%), 4% (95% CI: 0–13%), 11% (95% CI: 4–21%), and 25% (95% CI: 15–39%) determined in the years 2000–2, 2003–5, 2006–8, 2009–11, and 2012–4, respectively. The marked increase was on account of an increasing number of potential recombinant sequences with subtype B as one of the founder subtypes, since this subtype was identified in 21/22 of divergent sequences. The remaining divergent sequence was a complex recombinant containing subtypes D and G. Additionally, all 4 pure non-B subtyped sequences were determined in patients diagnosed in the last two years (2013–4). The results obtained indicate that subtypes other than B are entering the HIV-1 MSM epidemic in Slovenia, making recombination events among different subtypes more plausible. A marked increase in the numbers of non-B subtypes and potential recombinant forms was observed among MSM in Slovenia in recent years.

A23 Identification of HIV drug resistance mutation patterns using illumina MiSeq next generation sequencing in patients failing second-line boosted protease inhibitor therapy in Nigeria

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There is limited information of patterns of protease inhibitor (PI) resistance in adults failing second-line therapy. Next generation sequencing (NGS) detects drug resistance mutations as low as 1%. However, the clinical implications of these minority variants to treatment outcomes are still in debate. Approximately 5% of antiretroviral treatment (ART) exposed patients in our treatment program are on second-line boosted protease inhibitor (PI). Population-based sequencing conducted on some of these patients revealed no major HIV drug resistance mutations (DRMs) to PIs. We compared population-based sequencing and NGS results with the view of identifying patterns of drug resistance mutations and minority variants. Forty-eight plasma samples from 40 patients on second-line NRTI/NNRTI and boosted PI regimens with evidence of virologic failures (VL ≥1,000 copies/ml) were used in this study. Of these, eight were obtained at the time of first-line failure while the remaining 40 at the time of second-line failure. Ultra-deep sequencing sample preparation was achieved using Illumina Nextera XT protocol. This required that target amplicon be subjected to fragmentation, tagging, indexing, size exclusion bead purification, normalization, and pooling. MiSeq data analysis was performed using the Geneious software by applying 1% cut-off at major drug resistance sites. Electropherogram data were generated using ABI 3130 genetic analyzer and analysis performed using Stanford Genotyping Resistance Interpretation Algorithm available at http://sierra2.stanford.edu/sierra/servlet/JSierra and IAS-USA 2015 Drug Resistance Interpretation list. MiSeq sequencing showed that 53% ($n=23$) of the patients developed PI resistance, 93% ($n=40$) had NRTI resistance, and 70% ($n=30$) had NNRTI resistance. Of the DRMs detected in protease, L90M mutation was the most common mutation (28%, $n=12$) followed by L76V (21%, $n=9$), then I47V (7%, $n=3$) and I84V (7%, $n=3$). Among the NRTI associated mutations L74V was the predominant mutation (77%, $n=33$) followed by M184V/I (60%, $n=26$) then TAMS (51%, $n=22$). Of these, 33% of patients ($n=14$) showed NRTI + NNRTI mutations, 39% ($n=17$) showed NRTI + NNRTI + PI mutations, 7% ($n=3$) showed NRTI + PI mutations, whereas 21% ($n=9$) and 2.3% ($n=1$) exclusively showed NRTI and NNRTIs mutations respectively. Twenty-eight samples that had both MiSeq and Sanger sequencing data were available for a comparison of mutational patterns in the PI region. Miseq sequencing revealed minority PI mutations in 10 samples that were wild type by Sanger sequencing and one sample showed mutations in both Sanger and NGS. The ten samples revealing mutations based on MiSeq data comprised of minority variants including L90M (50%, $n=5$), L76V (20%, $n=2$), I50V (10%, $n=1$), and N88S (20%, $n=2$). Our data suggest that even in the absence of PI mutations based on Sanger data, those minority variants can be present. NGS revealed the presence of PI resistance mutations in patients who had wild-type using population-based sequencing. Given that patient regimen revealed that minority variants were unlikely selected by ART pressure, our results suggest poor adherence as the likely contributor to second-line failure due to the high genetic barrier of PIs. Since ART adherence in these patients was monitored using clinico-immunological parameters and virological tests only when treatment failure was suspected, our results suggest the need for routine virological monitoring. This should provide early opportunity for adherence intervention and thereby avoiding the need for switch to salvage or third-line treatment options, which is more expensive and not readily available in our setting.

A24 Application of large-scale sequencing and data analysis to research on emerging infectious diseases

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Many human diseases are caused by emerging pathogens, such as the SARS and MERS coronaviruses. Timely understanding of the behaviors of these pathogens plays an important role in helping doctors and scientists in searching for treatment methods and designing vaccines. The development of next-generation sequencing (NGS) has led to significant breakthroughs in the production of large amount of unbiased DNA sequence data from field and human clinical samples, providing the capacity