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Nigeria has been reported to have the highest number of AIDS-related deaths in the world. In this study, we aimed to use molecular epidemiology to investigate the HIV-1 diversity and phylogenetics in Nigeria. We analyzed 1,442 HIV-1 *pol* sequences collected from 1999 to 2014 from seven geopolitical zones in Nigeria. The main circulating strains, CRF02\_AG (44.1% of the analyzed sequences), Subtype G (8.3%), and CRF43\_02G (16.4%), were introduced to Nigeria in the 1960s, 1970s, and 1980s, respectively. The number of effective infections decreased in Nigeria after the introduction of free antiretroviral treatment in 2006. We also found a significant number of unique recombinant forms (22.7%), the majority of which were recombinants between the two or three of the main circulating strains described above. In addition, phylogeographic analysis indicates multiple occasions of HIV-1 transmission between Lagos and Abuja (two of the main cities in Nigeria). Our results may be relevant for HIV-1 intervention and contribute in making informed decisions in strategies aiming at reducing further spread of HIV-1 in Nigeria.

#### **A4 An amplicon-based approach for universal amplification, sequencing, and assembly of full-length HIV-1 samples from the DRC**

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Phylogenetic studies have contributed to our understanding of the early epidemic onset of HIV-1 in the Democratic Republic of Congo (DRC); however, the factors driving its early emergence and establishment in human populations still remain unresolved. In order to determine the key aspects of its successful epidemic spread, complete genome data are required from samples representative of the viral diversity in the DRC. In this study, we have established a universal PCR-assay that uses seven different panels of primers to produce overlapping amplicons covering the complete HIV genome. To circumvent the limitations of purifying these fragments and sequencing them with traditional approaches, we have developed a massive parallel sequencing method and a protocol for efficiently assembling HIV-1 genomes. A total of thirty-six samples, collected between 1997 and 2001 from different locations across the DRC, have been obtained, and, at this stage, we are focusing on complementing our dataset with more archival samples that can be used as HIV 'molecular fossils'. By generating complete genome phylogeographic data from the DRC, we aim to create a genomic window into the past evolutionary and epidemiological dynamics of HIV-1 in Central Africa and understand the natural history of this devastating pandemic.

#### **A5 Near full-length HIV-1 genome sequencing in newly diagnosed individuals in Sweden**

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The Swedish HIV-1 epidemic is characterized by a high diversity in HIV subtypes and recombinants as a result of migration. To study the time from infection through viral diversification, transmission patterns, and drug resistance in minor quasiespecies, a robust protocol for pan-genotypic near full-length HIV-1 genome (HIV-NFLG) next-generation sequencing (NGS) is key. Our group has established two protocols for HIV-NFLG on the Illumina platform that we aim to compare and, if necessary, modify to find a method with optimized coverage, depth, and subtype inclusivity. Zanini et al. (<https://doi.org/10.7554/eLife.11282.001>) have developed a method with one-step RT-PCR with six overlapping primer sets, followed by NGS and quality filtering and assembly with in-house methods. Aralaguppe et al. (<https://doi.org/10.1016/j.jviromet.2016>

07.010) have designed amplification in two fragments, followed by multiplexed NGS and quality control and assembly with Iterative Virus Assembler and VICUNA. Both methods have high coverage per nucleotide and low error rates in amplification and sequencing and can reliably identify SNPs at 1 per cent of the viral population with linkage within the quasiespecies. Subtype inclusivity remains a challenge even though both methods show success in amplifying and sequencing subtypes B, C, and the common recombinants 01\_AE and 02\_AG. Therefore, we aim to evaluate and optimize our NFLG NGS methods on a panel of patient samples that more completely reflects HIV-1 diversity in Sweden. Patient samples from fifty treatment-naïve viremic individuals representing the genotypic HIV-1 panorama in Sweden, including CRFs, are being amplified and sequenced by both protocols. Coverage of the genome, error rate, and possible depth of quasiespecies analysis is being evaluated. We will compare number of reads, coverage across the HIV genome, and representation of minor single nucleotide variants as well as subtype inclusivity and impact of plasma RNA levels. To do this we will use an in-house bioinformatic pipeline. The NFLG sequences will also be analyzed with phylogenetic tools for determination of subtypes including CRFs and URFs.

#### **A6 Does treatment cause virulence changes in HIV-1?**

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Antiretroviral treatment (ART) has provided substantial benefits for HIV-1-infected patients and has reduced incidence in areas with high uptake since its introduction in the late 1980s. As ART has led to shifts in the worldwide epidemiology of HIV-1, it may also have the potential to cause concomitant selective pressure on the virus population. Evidence for changes in HIV-1 virulence since the introduction of ART appears to be inconsistent. As well as reviewing both empirical and theoretical studies on the likely impact of ART on HIV-1 virulence, we developed a mathematical framework to evaluate the likely impact of ART on virulence selection under the widespread treatment programs and the future impact of recent test-and-treat recommendations. By quantifying both the relationship between virulence changes with the transmissibility through disease progression and the speed of diagnosis and treatment, we reconcile observational studies on virulence changes with the mathematical model predictions. On adoption of new test-and-treat programs—synonymous with early detection and immediate treatment—it is likely that increased virulence will be observed. Our findings highlight the potential public health consequences of mass treatment and the ensuing requirement for greater access and adherence to nullify the public health effect of these virulence changes.

#### **A7 Co-receptor tropism determined by genotypic assay in HIV-1 non-B subtypes circulating in Cuba: Implications for pathogenesis and Maraviroc resistance**

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The V3 loop of the HIV-1 envelope (*env*) gene is involved in binding to the chemokine receptors CCR5 and CXCR4, thus determining viral tropism. With the aim of genetically characterizing the C2V3 *env* region of HIV-1 samples from Cuban patients, naïve to Maraviroc (MVC) therapy, 115 plasma samples were taken in the period of 2014–6 and analyzed by sequencing of the C2V3 region. HIV-1 subtyping was performed using COMET V.2 and Rega subtyping toolV.3 software. Subtypes were confirmed by phylogenetic analyses using Mega-6. Prediction of co-receptor tropism was performed using the geno2pheno algorithm. The viral

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