PI/r + 3TC arm. We developed a methodology that allows to determine the frequency of M184V/I mutations in the HIV reverse transcriptase (RT) gene in peripheral blood mononuclear cells (PBMC) obtained before MOBIDIP simplification. Paired-end sequences were obtained from 252 PBMC samples covering the first 855 bp of the RT gene (HXB2: 2485–3405) by MiSeq technology. These sequences were subjected to an in-house Bioinformatics pipeline. The results of our pipeline were compared to the output of PASeq (https://www.paseq.org), an open web-tool for the identification of drug resistance mutations. The M184V mutation was identified at a frequency greater than 1 per cent in 178 individuals (∼71%). The M184I mutation was observed in 34 patients (∼13%), always in the presence of stop codons, and is in agreement with expectations, as this mutation is a known APOBEC-targeted site. Sixty-seven patients (∼27%) had a frequency of the M184V mutation with values greater than 75 per cent. PASeq confirmed the presence of M184V mutation in 173 patients. The frequencies estimated by the PASeq tool and in-house pipeline were correlated up to 99.5 per cent. We found a significant loss of the M184V mutation archived in PBMC between the first-line regimen treatment failure and the beginning of the MOBIDIP trial. In patients under long-term antiretroviral therapy, as in our case, viral sub-populations could be lost, reducing the presence, and frequency of a mutation. In the next step, we will evaluate the association between the presence and frequency of M184V mutation and MOBIDIP results.

A11 Evaluation of phylogenetic inference methods to determine direction of HIV transmission

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It has been postulated that the direction of HIV transmission between two individuals can be determined by phylogenetic analysis of HIV sequences. This approach may be problematic, since HIV sequences from newly infected individuals are often more similar to index sequences from samples collected years before transmission, compared to those from samples collected at the time of transmission. We evaluated the accuracy of phylogenetic methods for determining the direction of HIV transmission by analyzing next-generation sequencing (NGS) data from index–partner pairs enrolled in the HIV Prevention Trials Network (HPTN) 052 trial. HIV-infected index and HIV-uninfected partner participants were enrolled as serodiscordant couples; samples were analyzed from couples with index-to-partner HIV transmission that was confirmed by genetic linkage studies. NGS for HIV gp41 (HXB2 coordinates: 7691–8374) was performed using plasma samples from thirty-nine index–partner pairs (seventy-eight samples collected within 3 months of partner seroconversion). Maximum likelihood trees were generated using the entire dataset using FastTree v.2. Topological patterns of HIV from each index–partner pair were analyzed. The analysis included 9,368 consensus sequences and 521,145 total sequence reads for the seventy-eight samples analyzed. In 10 per cent (four out of thirty-nine) of couples, the phylogeny was inconsistent with the known direction of transmission. In 26 per cent (ten out of thirty-nine) of couples, the phylogeny results could not discern directionality. In 64 per cent (twenty-five out of thirty-nine) of couples, the results correctly indicated index-to-partner transmission; in two of these twenty-five cases, only one index sequence was closest to the most recent common ancestor. Phylogenetic analysis of NGS data obtained from samples collected within 3 months of transmission correctly determined the direction of transmission in 64 per cent of the cases analyzed. In 36 per cent of the cases, the phylogenetic topology did not support the known direction of infection, and in one-third of these cases the observed topology was opposite to the known direction of transmission. This demonstrates that phylogenetic topology alone may not be sufficient to accurately determine the direction of HIV transmission.

A12 Modeling residual HIV replication and the emergence of drug resistance on ART

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There are conflicting reports regarding the presence of low-level HIV replication during suppressive antiretroviral therapy (ART). We simulated varying levels of replication and estimated the number of generations needed to obtain linked, drug resistance mutations to explore the effects of replication during ART. HIV replication was simulated with varying population sizes (10 to 3,000,000). Each population size was modeled ten times. Each genome was given a Poisson-distributed number of mutations according to its length and the average replication error rate (3.4×10^{-5} sub/nt/cycle). Simulations were run a maximum of 20,000 generations with endpoints defined as detection of a variant with resistance mutations to at least two ARVs. In all simulations, variants that were resistant to all three ARVs emerged in less than 20,000 generations. The time to emergence ranged from 148–16,156 generations in the various simulations, depending on the replicating population size (4.8 months to 44.3 years if the generation time is 1 day). Clinically detectable virologic failure can result from linkage of two mutations conferring resistance to two ARVs in a regimen. In our simulations, two linked mutations emerged in from 9 to 6,429 generations (9 days to 17.6 years). Our simulations suggest that in patients continually suppressed on ART for at least 10 years, the replicating population size would have to be less than ten, or virologic failure would have occurred from emergence of two ARV-resistant variants. Because most patients on ART do not experience virologic failure, our simulations suggest that any residual replicating population on ART is very small and thus not likely to either sustain or significantly contribute to the HIV reservoir.

A13 Phylodynamic analysis of HIV in Florida

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We are interested in using phylodynamics to better understand molecular clusters of HIV within the state of Florida, USA. To our knowledge, there is currently no study using such methodology to understand the epidemic in Florida. Viral sequences collected from 2007 to 2017 (about 50,000) will be linked with individual-level data (demographics—sex, race/ethnicity—and mode of transmission) accessed through the Patient Reporting Investigating Surveillance Manager (PRISM), and the Enhanced HIV/AIDS Reporting System (eHARS) via the Florida Department of Health (FDOH). Through the use of HIV-TRACE, which analyzes genetic distance matrices, we will create molecular transmission networks including data on mode of transmission, race/ethnicity, and sex. Furthermore, a maximum likelihood phylogenetic tree will be created using software (e.g. IQ-Tree, PhyML, and FastTree2) run on a high-performance cluster. Phylogenetic comparative analysis will be performed to assess the association between phylogenetic clades and demographics (including extended demographics like syphilis and other comorbidities queried from PRISM and eHARS).