Background and Abstract

Antiretroviral therapy (ART) can reduce plasma HIV-1 levels below the limit of detection of clinical assays. However, incomplete suppression of virus can lead to rapid emergence of significant drug resistance mutations (DRM) that render certain drugs ineffective. The integration of drug resistant viruses into the host DNA and persistence in CD4+ cells in a latent state poses a major obstacle to effective treatment strategies and challenges new universal treatment paradigms for the eradication of HIV infection. The latent cellular reservoir functions as a historical archive of viral genotypes that have previously circulated in the host including provirus harboring DRM. However, little is known about the duration, persistence, and decay of DRM in the proviral reservoir over time.

To address these questions, we identified subjects with known dates of virologic failure of Efavirenz-based regimens with emergence of drug resistant viremia (with the K103N mutation). These subjects were previously enrolled in ACTG trials but were subsequently treated with an effective second-line, boosted protease inhibitor-based regimen. CD4 count and viral load were measured and cryopreserved PBMC were collected every 6 months for up to 11 years. Following a failed first-line regimen, we hypothesize that consensus resistance mutations rapidly decay to levels below detection (<20%) in proviral DNA but persist in the latent reservoir with gradual decay over months to years.

To test this hypothesis, our proposed study will
1) Examine the frequency of DRM in proviral DNA following virologic failure and the detection of DRM in plasma RNA
2) Define the factors that affect fixation, retention, and decay over time. PBMC proviral DNA was genotyped to determine the kinetics of retention and decay of K103N and other DRMs.

The information generated will have important implications for developing future treatment options for ART strategies to reduce clinical failure and mitigate the transmission of drug resistance.

Methods

Subjects under second-line suppression
We assessed proviral DNA for the presence of drug resistance mutations in cells obtained 6-12 months after viral suppression to <50 copies/ml in 29 subjects from ACTG364 and ACTGS095 with Efavirenz failure and documented K103N in plasma viral RNA.

Population sequencing
Genomic DNA was collected from whole peripheral blood mononuclear cells (PBMCs) but enriched CD4+ Whole HIV-1 pol was amplified from proviral DNA and sequenced. Editing, amino acid alignment, and phylogenetic analysis were performed using Geneious 6.2. For most samples, genotypes were obtained at 3 time points (first suppression (t1), most recent suppression (t2), and an intermediate (t3)).

Next-generation sequencing
Regions of the RT gene harboring DRMs were amplified to generate size-appropriate amplicons to barcode for multiplex: 2x250bp paired-end sequencing using Illumina MiSeq v2 (600 cycle). For comparison, whole HIV-1 pol was sequenced on PacBio RS II using P4-C2 chemistry.

Statistical Analyses
Repeated measures logistic regression (generalized estimating equations) was used to identify factors associated with detectable K103N in proviral DNA. All of the analyses were performed on SAS 9.2.

Results

<table>
<thead>
<tr>
<th>Subject</th>
<th>Mutation</th>
<th>Time point</th>
<th>K103N</th>
<th>K103K</th>
<th>M41I</th>
<th>M184V</th>
<th>E138A</th>
<th>Y181C</th>
<th>Y181F</th>
<th>Y188L</th>
<th>T215C</th>
<th>T215Y</th>
<th>T215SF</th>
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