
**Anthony Kebira Nyamache**

*Dr. Anthony Kebira Nyamache is currently a lecturer in the Department of Microbiology, Kenyatta University.*

## ISID Small Grant Program Report

# Evaluation of HIV Evolution and Its Role in Antiretroviral Drug Resistance, in Nairobi Cohort

*Anthony Kebira, Dip, Bsc, MSc, PhD, Kenya*

## Introduction

HIV-1 is partly characterized by its extensive genetic diversity, caused by errors introduced during the synthesis of cDNA from RNA. HIV-1 is thus subdivided into four groups (M, N, P and O), group M being itself subdivided into nine subtypes (A, B, C, D, F, G, H, J and K).[1,2] Genetic recombination events among different genetic subtypes of HIV-1 group M have been identified. Some of these mosaic HIV-1 genomes are unique, but others play a major role in the AIDS pandemic[3] and to date 48 circulating recombinant forms (CRF).[2] Genetic variability is a central feature of HIV-1. The high frequency of mutations during HIV-1 replication leads to the development of viral quasispecies in vivo and contributes to genetic heterogeneity among HIV-1 isolates.[4] There is a growing appreciation that HIV-1 genetic diversity, including the existence of distinct genetic subtypes and the evolution of drug-resistant genotypes, can greatly affect the diagnosis and treatment of HIV-1 infection.[4,5]

Combination therapy with reverse transcriptase and protease inhibitors is the most common current treatment of HIV-1 infection.[6,7] Despite the success of this therapy, namely reducing morbidity and mortality of HIV-1 infected patients,[8–11] it has adverse effects and drug resistant HIV-1 strains emerge.[12–14]. The continued development of novel antiretroviral agents for the treatment of HIV-1 has most recently culminated in the introduction of a new therapeutic classes the strand transfer integrase inhibitors (InSTI)[15] CCR5 antagonists, Fusion inhibitors; despite the challenges of drug resistance in patient management, disease control and surveillance especially in resource-poor countries.

## Methods

We conducted a crossectional prospective study Nairobi having a cohort of 188 study subjects. The study subjects were HIV positive comprising 103 male and 85 female with total 112 of them drug naive and 76 on first line antiretroviral therapy. We took demographic data, clinical history and collected blood samples at a month interval of all participants and peripheral mononuclear cells (PBMCs) and plasma separated. Plasma samples were evaluated for viral load, blood tested for CD4 counts. Viral RNA was extracted from plasma, translated and cDNA amplified using nested polymerase chain reaction on targeted genes; *pol*-RT, Protease, *env C2V3*, *env* gp41 and Integrase regions. The amplicons were cloned and others directly sequenced. Generated sequences were aligned and phylogenetically analysed using known reference subtypes sequences and drug resistance mutations interpreted and substitutions determined.

## Results

Phylogenetic analysis based on env C2V3 region revealed A1 (59.6%), C (18.1%), D (10.6%), B (2.1%), G (2.1%), CRF02_AG (3.2%) and the rest of 6.9% were CRFs. In HIV-1 co-receptor switch showed R5 tropism (69.6%) while X4 (30.4%). In addition, 2.4% T97A that is associated with reduced susceptibility to Raltegravir and 26.2% had secondary mutations associated with resistance to intergrase inhibitors. In fusion inhibitors, the following mutations were detected; A316T/I323V (2.6%) combination, A316T (63%), I323V (1.1%) for Maraviroc, (10%) K305R, (3.2%) G321E, (35.1%) R315Q, (4.5%) K305R/R315Q, (62.8%) T320R for Vicriviroc and (1.6%) A316T+ K305R+ R315Q, (12.7%) A316T+R315Q, (3.2%) R3I5Q+A316T+I323V, (0.5%) R315+A316T+G321E

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for Maraviroc and Vicriviroc combinations. In addition, 4.2% intermediate resistance associated to Enfuvirtide was detected. The point mutations at; N42S was detected in 16.7% of all the samples, while N42D was detected in 4.2%, S138L /T 3.1%, L44M 2.1% and 1% each for in the following mutations; N43I and L45V drug resistance mutations. In evolutionary rate; 12.5% had *d*N*d*S ration 1>, 88.5% *d*N*d*S ration < 1 and in those with *d*N*d*S ration ≠1.

## Discussion

The results indicate that HIV-1 subtypes in Nairobi cohort like the rest of the country, is predominated by HIV-1 subtype A1, though there could be possibility of an increase proportion of HIV-1 subtype C prevalences. Existence of diverse HIV-1 recombinants indicated viral mixing among the population, possibly as a result of dual infections. Evolutionary rate of the virus showed natural selection with high proportions of R5 strains suggestive of feasibility of use of maraviroc (CCR5 antagonists) in Kenya. However, multiple drug resistance mutations observed in the newly classes of drug-prior to their introduction, there is a need for constant monitoring of HIV-1 genetic diversity and drug resistance. Drug resistance seen to common drugs was minimal (2%), indicating that we need to continue to prescribe the current used drugs. In addition, the new classes of entry and fusion inhibitors are feasible as first line and intergrase inhibitors as third line drugs. In addition, it was realised that it is not necessary to carry out resistance testing at baseline unless there is strong evidence of virological failure.

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