Drug Resistance Mutations in Naïve HIV-1 South African Patients, and Construction of Molecular Clones to Phenotype Putative Resistance Mutations

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By

Mavhandu Lufuno Grace

To the Department of Microbiology
School of Mathematics and Natural Sciences
University of Venda, South Africa

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Supervisor: Dr Pascal Obong Bessong
Co-supervisors: Dr David Rekosh
                     Dr Marie-Louise Hammarskjold

Summary

In countries such as South Africa where access to therapy is progressing data is required on patterns of resistance and evolution of resistance. Thirty protease (PR) and 31 reverse transcriptase (RT) amino acid sequences of HIV primary isolates from drug naïve patients from rural settings in South Africa were examined for resistance mutations. Samples were collected between May and August 2007. Phylogenetic analysis showed that all the sequences were HIV-1 subtype C in both the protease and reverse transcriptase genes. The mean genetic distances among the sequences were 0.0170-0.0786 for the protease, and 0.0045-0.0890 for the reverse transcriptase. However, it was noted that 3 pairs of samples 07VGNF5ZA and 07VGNF6ZA, 07VGNF7ZA and 07VGNF8ZA, 07VGNF10ZA and 07VGNF13ZA did not show any genetic variability among their protease sequences. No major resistance mutation was observed among the protease sequences. However, the following minor resistance mutations were noted: L10I/V (3/30), A71T (1/30), and T74S (2/30). Examination of the reverse transcriptase gene for resistance mutations reveal the presence of V118I (1/30), V179D (1/30), K103N (2/30). Most of the RT sequences were wild-type, although V118I (3.3%) and k103N (6.7%) associated with resistance to lamivudine and nevirapine, respectively, were observed. In summary, this study has shown that most of the viruses in Limpopo Province, representing the northeastern part of South Africa are HIV-1 subtype C, and that the prevalence of resistant mutations among the drug naïve patients is still low.

Although combination antiretroviral therapy has resulted in a considerable improvement in the treatment of human immunodeficiency virus type 1 (HIV-1) infection, the emergence of resistant virus is a significant obstacle to the effective management of HIV infection and AIDS. Systems to be used in the testing of phenotypic drug resistance and susceptibility are being developed. These may intimately be used in guiding therapy to improve long term suppression of HIV replication. Two proviral chimeric clones making use of pMJ4 and pNL4-3, and two vector plasmids which deletions of sequences encoding HIV-1 protease or reverse transcriptase were constructed for cloning of HIV-1 PCR products. Growth of constructs was monitored by p24 antigen production. Susceptibility to protease and reverse transcriptase inhibitors was measured by using resistance test vectors that contain a Luciferase indicator gene. Cells were co-transfected with packaging plasmids, pLuc, and pEnv, resulting in the production of virus particles that were

used to infect target cells. Luciferase activity was measured following a single round of replication. The chimeric constructs MJ4 carrying the NL4-3 ApaI-HpaI cassette (MJ4/NL4-3) and NL4-3 carrying the MJ4 ApaI-HpaI cassette (NL4-3/MJ4) were successfully developed as shown by restriction digestion analysis. Considering growth of the constructed chimeras NL4-3/MJ4 was better than MJ4/NL4-3 although not robustly. Good p24 production was obtained from all four *gap-pol* plasmids. MJ4/NL4-3 worked better in delivering luciferase to the target cells while NL4-3/ML4 appeared totally devoid of any infectivity. The vectors pCMVGagPol(MJ4)-RREr and pCMVGagPol(NL4.3)-RREr were created and both expressed the viral *gag-pol* protein. Viral inhibition test showed that the vectors can be inhibited by NRTI, NNRTI and PI. Inhibition was seen in all drugs in different concentrations indicating that the system works. The results showed that vector systems constructed can be used to evaluate putative drug resistant mutations, coding for resistance to protease and reverse transcriptase inhibitors, detected in patient viruses. In addition, the system can also be used to evaluate candidate drugs and assist in the development of new drugs that are active against resistant HIV-1 virus.