

# Studies on HIV-1 Subtype C Drug Susceptibility: Development of a Phenotypic Resistance Assay and Evaluation of Plant-Derived Compounds

By

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## GENERAL ABSTRACT

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Access to antiretroviral therapy (ART) continues to expand with huge benefits to AIDS patients. However, a potential danger to the success and sustainability of ART is the development of resistance mutations. Phenotypic characterization of acquired resistance associated mutations and mutations of unknown significance are important for patient management. Currently available HIV phenotypic resistant testing assays can accommodate an incomplete fragment of the polymerase gene. However, it is important to have a system that comprises the complete group associated antigen (Gag) and the polymerase (Pol) genes so that changes in the Gag gene that influence mutations in the protease gene can be evaluated; and importantly too there is potential to determine the effectiveness of integrase inhibitors and future compounds targeting the Gag or Pol genes. Of great importance as well is the use of a system that accounts for the HIV genetic variant of global epidemiologic importance such as HIV-1 subtype C. On the other hand, there is need for assays appropriate for resource limiting settings, such as pseudoviruses, for the screening of molecules for anti-HIV properties. The pseudoviruses should also be genetically relevant to regions with high HIV endemicity.

The current thesis set out to (1) establish a PCR protocol that could be used on a routine basis to generate the complete GagPol gene of HIV-1 subtype C viruses containing resistance mutations of interest; (2) demonstrate the feasibility of cloning a complete HIV-1 subtype C GagPol into a viral vector so that harboured mutations of interests can be evaluated in a chimeric viral construct; (3) evaluate the application of an HIV-1 subtype C based pseudovirus system to screen plant derived molecules for anti-HIV properties.

### **Establishment of a PCR protocol for the complete HIV-1-C GagPol gene**

An optimized, sensitive and specific polymerase chain reaction (PCR) protocol to synthesize viral HIV-1 subtype C complete GagPol gene from naïve and drug experienced patients was established. The PCR protocol was able to amplify a 4.9 kb sequence either from viral RNA as starting material, through cDNA synthesis, or from viral DNA obtained from Peripheral Blood Mononuclear Cells (PBMCs). A 4.9 kb PCR



product was obtained from 5/6 (83.3%) of plasma samples with a viral load less than 50 copies/ $\mu$ l using RNA as the starting material. A higher success rate of 95.6% (22/23) was obtained when DNA from PBMC was used as the template. Of a subset of 12 nucleotide sequences from the PCR products, all except 1 (HIV-1 subtype B), were HIV subtype C. Sequencing showed that the unique restriction sites Xho I and Pac I were successfully incorporated through PCR mutagenesis at the 5' and 3' ends of the PCR product respectively. Restriction digestion demonstrated that flanking sequences could be removed to allow cloning of the patient-derived PCR fragment into a cloning vector (pCMV-RRE) for subsequent resistance evaluation of mutations contained in the viral gene. The PCR protocol was used to generate HIV-1 subtype C complete GagPol sequences. This enabled the evaluation of mutations associated with resistance to currently available and future protease, reverse transcriptase and integrase inhibitors (see below).

### **Construction of a chimeric vector system containing the complete HIV-1-C GagPol gene**

The goal was to construct a chimeric packaging vector for subsequent generation of an HIV-1 subtype C based pseudovirus. The pCMV-RRE vector was modified by inserting a multiple cloning site (MCS) containing XhoI and Pac I restriction sites by ligation. Successful insertion of the MCS was verified by restriction digestion and sequencing of clones from transformed DH5- $\alpha$  cells. A 5' splice site was also incorporated by ligation. An HIV-1 subtype C complete GagPol sequence obtained from a patient was cloned into pCR-TOPO-XL cloning vector, and subsequently transferred into pCMV-RRE vector. pLuc, and pEnv plasmids were transfected together with the pCMV-HIV1CGagPol-RRE constructs to generate virus-like particles capable of transducing luciferase. The amount of virus-like particles, as measured by p24 antigen levels were not as high as the values obtained for constructs derived from subtype B and C molecular clones (NL4-3 and Indie C). However, the approach in having a system with the complete GagPol gene geared for phenotypic assessment of known and putative resistance mutations is innovative and subsequent work will focus on improving the efficiency of expression from the constructs.



## Application of a pseudovirus system to screen molecules for anti-HIV properties

The use of medicinal plants has been of interest in the management of HIV/AIDS prior to and after the availability of antiretrovirals. A positive outcome from the use of a herbal preparation or plant extract on AIDS could be due to alleviation from opportunistic infections, stimulation of the immune system or direct antiviral effect. Efforts to identify plant derived molecules as leads of potential antiviral sources require a reproducible system for routine screening of selected plant compounds. This study evaluated the use of an HIV subtype C based pseudovirus system to screen plant compounds for potential anti-HIV-1 subtype C activity. Pseudoviruses were generated by transfection of human embryonic kidney cells (HEK 293T cells) and pseudovirions capable of transducing firefly luciferase were detected by p24 antigen measurement. This was followed by infection of CF2/CD4/CCR5 and CF2/CD4/CXCR4 cell lines. Selected plant substances were introduced at the infection stage to determine potential anti reverse transcriptase properties. Pseudoviruses were also subjected to the inhibitory properties of known anti reverse transcriptase compounds namely Zidovudine (AZT) and Nevirapine (NVP). AZT and NVP inhibited the pseudoviruses in a dose dependent manner. This demonstrated that the pseudoviruses were susceptible to antiviral compounds. On the other hand, only low levels of suppression were observed with AZT and NVP when resistant mutant strains of the pseudoviruses were tested. Among the plant-derived compounds used Catechin, obtained from stem-bark of *Peltophorum africanum* inhibited the HIV-1 subtype C pseudovirus with an IC<sub>50</sub> of 0.286 µg/µl. An IC<sub>50</sub> of 0.583 µg/µl and 1.523 µg/µl were obtained for Catechin against the NRTI and NNRTI resistant mutants respectively. Inhibition of RT even at the lowest concentration of 0.021 µg/µl and 0.008 µg/µl was observed with methanol extract of Pheko and *Eleadendron transvaalensis* respectively. Cytotoxicity was not observed for Catechin in an MTT assay with the CF2 cell line. The pseudovirus assay developed based on HIV-1 subtype C, the most common variant worldwide, can be used to identify molecules with anti-HIV activity. The system is also suitable for resource limited laboratories without a BSL3-containment facility.

## Overall conclusions and recommendations

The current study has demonstrated that it is feasible to generate a 4.9kb HIV-1 subtype C GagPol fragment from patients' plasma samples with viral loads at the limit of detection (<50 copies/ $\mu$ l) with a relatively high frequency of success. Furthermore, the PCR fragment can be cloned into a pCMV-RRE vector to create a construct that can be used to create pseudovirus particles when transfected with pLuc and pEnv plasmids. Since robust p24 antigen levels were not obtained from the constructs, further work will focus on improving the expression characteristics of the vector. In this way, a subtype C based pseudovirus system capable of carrying patients' viral GagPol gene and harbouring resistance associated mutations of interest can be fully characterized. Finally, a pseudovirus system that can be routinely applied to screen plant compounds for anti-HIV activity has been established; future work will entail the antiviral characterization of a library of pure compounds.