4. Scope and plan of work

(As submitted to the University along with the application for the PhD program, shown here as a University requirement in the thesis)

In North America and parts of Europe where clade B is the predominant strain, HAART has helped in reduction of HIV morbidity, mortality and transmission. This however has led to the development of resistant strains. Advances in genotypic analysis have helped in identifying changes in sequences that have been associated with conferring resistance to antiretroviral drug. However very little data is available on how subtype diversity may affect drug susceptibility and resistance. Despite the fact that antiretroviral therapy has been specifically fine tuned to target the pol gene products (RT and protease), nucleotide divergence within this sequence as a function of HIV subtype is only now coming to light. Genotypic analyses of viruses of different clade show many nucleotide changes (silent mutations), polymorphisms and secondary mutations with RT and protease regions implicated in the emergence of resistance to NRTIs, NNRTIs and PIs used in HIV-1 treatment.

HIV-1 clade C strains exhibit polymorphisms at sites linked to resistance to Non Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) and Nucleoside Reverse Transcriptase Inhibitors (NRTIs) in the RT gene as well as protease polymorphism that facilitate the development of resistance to Protease Inhibitors (PIs). HIV-1 strains with drug resistant mutations are also frequently accompanied by a loss of replication capacity. HIV-1 tropism to coreceptor CCR5 & CXCR4 influences response to therapy.

Previous studies done on clade C strains in Africa have shown that some clade C isolates may show inherent resistance against NNRTIs due to the presence of a G190A mutation. In vitro
drug selection studies on clade C isolates (from Botswana and Ethiopia) have shown numerous baseline polymorphisms and silent mutations in treatment naïve individuals within RT at sites linked to resistance to NNRTIs and NRTIs as well as protease polymorphisms that facilitate resistance to PIs. Studies have also shown that novel resistance mutations can develop in clade C isolates. These have serious ramifications considering the fact that NNRTIs are relatively inexpensive and will be used widely in developing countries where non-clade B strains are prevalent. In one sentinel study from India published recently, a phenotypic analysis was carried out on treatment-naïve isolates. Primary resistance to reverse transcriptase inhibitors was 6.7% and that to protease inhibitors was 2.5%.

Most of the work done on clade C strains, with regard to drug resistance, has been in Africa. Clade C strains from India and Africa have shown divergence furthering our cause for an urgent need to study drug resistance in India.

It is proposed that the following shall be carried out:

1) To sequence the HIV –1 RT gene from plasma and assess the prevalence of polymorphism in the RT sequence of drug naïve Clade C infected population.

2) To sequence the HIV-1 protease gene from plasma and assess the prevalence of polymorphism in the protease gene of drug naive Clade C infected population.

3) To sequence the env region of HIV-1 for determination of co-receptor usage.

4) To investigate association between co-receptor usage and polymorphisms leading to drug resistance.

The study is designed as a cross-sectional study and will be done at Christian Medical College (CMC), Vellore, India. The study population will be drawn from the population of
HIV infected ART naïve individuals receiving care at the hospital. The study subjects should meet the following eligibility criteria

a. HIV positive by HIV rapid tests, ELISA and confirmatory western blot (WB)
   tests.

b. Viral load should have been done.

c. Come to HIV clinic for receiving care.

d. Should never have been on anti retroviral therapy.

e. Give consent for testing.

Patients or their attendants who do not give informed consent to participate in this study will be excluded

In an earlier published study from India, based on phenotypic drug resistance testing, the prevalence of primary resistance among treatment naive HIV infected individuals to RTI was 6.7% and to PIs was 2.5% (299). Taking this as the prevalence for the calculation of the sample size, with a worst acceptable rate of 2 % for RTIs and 0.2 % for PIs the calculated sample size was 109 for RTIs (95% CI) and 177 (95% CI) for PIs using Epi Info 6.04. Presently, in India there is wide spread usage of RTI, PIs are sparingly used and restricted mainly for salvage therapy. Based on this we have decided to take 180 individual blood samples for the study.

An aliquot of plasma sample will be used and RNA will be extracted from the sample. RNA extraction will be one using the QIAamp Viral RNA Extraction Kit. The extracted RNA will then be subjected to reverse transcription to form cDNA and subjected to amplification with primers directed to the RT region and the protease region of the HIV-1 genome. The amplified products will then be sequenced. The sequences will be analysed by sending
then to the Stanford drug resistance database for analysis. Study on prediction of co-receptor usage of HIV-1 will be done by sequencing of V1-V3 region.

We expect to detect the most prevalent polymorphism/mutation in the RT and protease region of clade C strains from India. We also expect to see co-receptor tropism influenced by drug resistance.