3. SCOPE AND PLAN

In the recent past, HIV research involving mitochondrial biology has drastically grown up. Much of its implications, has been in both the understanding the pathogenesis of HIV infection and its treatment related adverse events. Many lines of research findings have culminated in establishing the bottom-line of HIV infection that is chronic inflammation and immune activation. This has resulted in chronic oxidative stress that ultimately leads to loss of immune cells and thereby rapid disease progression. HIV proteins have been implicated in the impairment of various mitochondrial functions. Mitochondria being the major producer of intracellular ROS, in conditions of mitochondrial dysfunction are also prone to oxidative damage and depletion of mtDNA, mitochondrial membrane depolarization and impairment of mitochondrial respiratory chain thus causing a vicious cycle adding to mitochondrial dysfunction with the possible induction of apoptosis.

Coincidently, NRTI containing therapy also causes mitochondrial activity deficits. Thus HIV infected patients are prone to chronic mitochondrial derangements. In resource limited settings (RLS) where NRTI is prescribed as backbone of first line HAART, understanding mitochondrial dysfunction is very vital. Given the fact that there are differential effects of mtDNA variants on susceptibility to toxicity [Kampira E. et al., 2013] and even in the increased progression to AIDS [Hendrickson SL. et al., 2008] and these variants being categorized as sub-haplogroups which are characteristic of different ethnic groups and populations, mitochondrial dysfunction in south Indian patients has not yet been studied so far hence in the present study we had analysed the mitochondrial
parameters among the HIV infected NRTI treated and ART untreated patients in south India.

As NRTI can inhibit DNA pol $\gamma$, the only enzyme involved in mtDNA replication, mtDNA synthesis is affected thus mtDNA content may be potentially used as a biomarker of NRTI induced mitochondrial toxicity.

In order to estimate mitochondrial dysfunction caused by NRTI and HIV per se we have chosen the easily accessible PBMCs as the specimen from which the mitochondrial dysfunction can be measured in a multifaceted way so that the measured parameters could depict the wider aspects of mitochondrial activity deficits.

PBMC mitochondrial parameters included were quantification of mtDNA content and estimation of mtDNA mutations of $ND1$ gene along with TLA ($\%$) and $\text{TL} \Delta \Psi_m^{\text{low}}$ ($\%$). Oxidative stress was estimated by measuring the lipid peroxidation product, the plasma F2- IsoP. Moreover, all the PBMC mitochondrial parameters and plasma F2-IsoP levels were correlated with the clinical parameters known to be associated with mitochondrial abnormalities. As there is ethnic influence of mitochondrial toxicities attributed to mtDNA haplogroups we have estimated all the mitochondrial and clinical parameters in the LoRHC that would facilitate in gauging the mitochondrial abnormalities in the HIV infected individuals. Studies on NRTI induced mitochondrial dysfunction are limited due to a shorter duration of follow-up of patients on NRTI, whereas in our study the median duration of ART treatment was 85(69-93) months at the final visit, which would be an appropriate cohort to appreciate the complicated mechanisms involved in mitochondrial dysfunction.
Thus the overall aim of the study was to investigate mitochondrial dysfunction among both the ART-naïve and the NRTI-treated HIV-1 infected patients in south India with the following objectives:

3.1 To study the changes in the mtDNA content of PBMC of both the ART-naïve and the treated, who were treated with NRTI containing first-line regimen in comparison with the LoRHC and thereby to evaluate the utility of mtDNA as a marker of nucleoside related adverse events.

Previous studies have demonstrated that rather than other assays such as mitochondrial morphology and cell viability, quantifying mtDNA content would be a sensitive measure of NRTI induced mitochondrial toxicity. Thus, it would be appropriate to evaluate mtDNA content of the easily accessible PBMCs as a novel biomarker of NRTI related adverse events that would help in treatment monitoring for the emergence of adverse events among patients on NRTI.

3.2 To study the effects of HIV and NRTI in mitochondrial dysfunction with special emphasis on biochemical events such as apoptosis and mitochondrial membrane potential damage.

Intrinsic pathway of apoptosis involves mitochondria, during which there is mitochondrial membrane depolarization and subsequent release of cytochrome C. Addressing these mitochondrial parameters namely the lymphocyte mitochondrial
membrane potential damage and induction of lymphocyte apoptosis would thus bring out a comprehensive picture of mitochondrial dysfunction.

3.3 To study the influence of mitochondrial dysfunction in causing mutations in mtDNA among both the ART-naïve and the treated.

Mitochondria, in addition to be the major producer of ROS, are also more vulnerable to the damage caused by ROS. Hence a representative gene of mtDNA, $ND1$ gene was sequenced to identify whether there is an increased level of mutations during mitochondrial dysfunction.

3.4 To study the level of systemic oxidative stress in the ART-naïve and the treated and thereby its association with mitochondrial dysfunction.

Oxidative stress occurs both in the ART-naïve and the treated. Continuous systemic immune activation and inflammation may result in oxidative stress, weakened immune responses, pro-inflammatory cytokine production, and uncontrolled viral replication, in activated CD4+ T-cells. Mitochondria is a major producer of ROS, hence the level of systemic oxidative stress would probably give an estimate of the general mitochondrial dysfunction of the HIV infected which can be further correlated with the mitochondrial functions of PBMCs.