SUMMARY AND CONCLUSIONS

The Human Immunodeficiency virus type 1 (HIV-1) clade C is the dominant genetic subtype circulating in India, Sub-Saharan Africa and China (Ringe et al., 2010; Shankarappa et al., 2001). HIV-1 clade C predominantly circulating in India has been shown to be strictly CCR5 using irrespective of disease stages with rare evidences of usage of other coreceptors. In a typical clinical setting, early in the infection, HIV-1 env exclusively uses CCR5 and with progression towards symptomatic stages preferably tends to switch to exploit CXCR4 or become dual tropic in about 50% cases atleast with subtype B strains.

In the first section of my study, I generated a panel of full-length envs from plasma of five ART naive chronically infected Indian patients. Among the five patients, the plasma of two patients viz. LT-5 and LT-1 showed good neutralizing antibody response. Genetic characterization showed that all the patients except for the patient LT-5 were infected with pure subtype C strains. Envelope clones obtained from slow progressor LT-5 patient showed complex quasispecies B/C recombinants and pure clade B and C and indicated a case of dual infection with genetically distinct strains. Additionally, a multiregion hybridization assay (MHAbce v2) using LT-5 plasma also indicated recombination events in Env, suggesting that recombinant Env quasispecies constitute a major portion in the plasma. However, all the clones were found to be strictly CCR5 using. To study the genetic characteristics of Indian clade C envelope constituting distinct disease stages, we have assembled a large panel of envelope representing strictly clade C CCR5 using envelope clones isolated from Indian patients of disease stages ACUTE, EARLY & LATE. Comparison of important genetic traits like V3 loop charge, V1V2 loop length and number of PNGS did not uncover any unique pattern representing a particular disease stage.

In the next section I examined CCR5 and CD4 usage of the panel viruses by different cell based assays. The study revealed that the end stage viruses showed a higher median IC_{50} for TAK779 and Maraviroc suggesting most efficient use of CCR5 by this group. Acute, early and chronic envs were capable of utilizing CCR5
with same efficiency. As for CD4, all the patient derived viruses utilized the receptor with similar efficiency.

The sensitivities of the envs were checked against broadly neutralizing antibodies targeting vulnerable sites on gp120. The study indicated that the HIV-1 clade C envelopes obtained from the late stage patients were sensitive to b12 monoclonal antibody those obtained from other disease stages. When examined to another CD4 binding site antibody, VRC01, the acute envelopes were found to be significantly more sensitive when compared to chronic envelopes in case of antibody VRC01 that targets CD4 binding site of gp120 (p=0.01). Patient envelopes were also checked for their sensitivities to Griffithsin, an anti-viral lectin. The chronic envelopes were found to be most sensitive among the four groups and the acute envelopes showed a trend towards resistance. The patient derived envelopes were tested against fusion inhibitor T-20 and BNAab 4E10, both targeting gp41 domain of the envelope. The envelopes were found to have similar sensitivity to T-20 however, the chronic envelopes were found to be two fold more resistant to that of acute envelopes. The end stage viruses were found to be most sensitive among the four groups with respect of both T-20 and 4E10. The fusogenicity of the Patient derived envs of the assembled panel was examined at different time points post mixing of the cells. Our data indicated the acute and early had started to fuse within 4 hrs. This particular phenotype can help the virus of these two stages to cause faster disease progression. The chronic and the late stage envs showed evidence of fusion indicated by increase in RLU after 8 hrs post mixing of cells. However, when analysed no significant correlation was found between fusogenicity and sensitivity to fusion inhibitor T-20 or MAb 4E10 (P=0.6) of the envs.