Chapter 7 Summary & Conclusions

HIV-1 poses a tremendous challenge to the researchers worldwide in developing effective therapeutics. Currently, the best approach to address this problem emerges as a combinatorial therapy. The viral life cycle involves the entry into host cells and subsequent production of the viral proteins utilizing the host machinery to produce more infectious particles. Therefore, a combinatorial approach utilizing a host factor and a viral factor may aptly address the current problem. The most suitable host candidate appears to be the chemokine receptors essential for virus entry. CCR5 and CXCR4 have been long identified as the major co-receptors for viral entry. Also, presence of natural deletion mutation in the CCR5 gene was able to confer resistance to a certain population. Since then many researchers including Dr Banerjea’s group at NII, New Delhi, identified such Δ-32 mutations in Indian population. The fact that individuals harbouring CCR5Δ-32 mutation lead a normal life, makes it an ideal host gene against which multiple anti-HIV-1 approaches can be developed. Therefore CCR5 has been suitably identified as one of the candidates for effective drug design against HIV. But, there are T-tropic HIV which exclusively utilizes the CXCR4 co-receptor for infection. Also, there have been reports where patients normally resistant to HIV progression by R5-tropic viruses have shown susceptibility to infection with T-tropic viruses. Therefore there is an urgent requirement to develop strategies to knockdown CXCR4 co-receptor as well.

HIV-1 Vpr is one important viral candidate which can be exploited to develop anti-HIV therapeutics. HIV-1 Vpr is a small accessory gene involved throughout the viral life cycle. It is important for several of the important viral functions such as the fidelity of reverse transcription, incorporation of the pre-
integration complex, transactivation of viral and several host factors, apoptosis and cell cycle arrest.

In the present study, we utilized several nucleic acid based approaches to downregulate these two identified targets, the cellular co-receptor CXCR4 and the viral gene Vpr. We utilized catalytic nucleic acids, Ribozymes (Rzs) and DNA-enzymes (Dzs) which have a catalytic core and two hybridizing arms complementary to the target RNA sequence. The catalytic nucleic acids are advantageous over protein based therapeutics in reducing the toxic effects of antiviral approaches and undesirable immune responses expected with any foreign antigen. Also, their catalytic capability makes these molecules better suited to achieve target specific gene inhibition. These molecules can be developed further for therapeutic purposes against HIV-1.

We were able to obtain efficacious catalytic nucleic acids, shRNA and antisense that downregulated the cell surface expression of CXCR4 co-receptor and also prevented virus infection. Although the Dz was very effective in downregulating the surface expression of CXCR4 (nearly 50%), the Rz showed significantly reduced capability to do so (only 20%). But, remarkably the use of antisense molecules together with both these catalytic nucleic acids, enhanced their efficiency tremendously. Another unique bispecific construct containing a shRNA and the previously described Rz was designed. This construct also resulted in nearly 50% downregulation of CXCR4 co-receptor which was enhanced significantly with a specific antisense oligonucleotide. Among all the antiviral approaches used in the study we identified the Dz and its antisense ODN as the best approach in inhibiting viral infection by 80%. The bispecific construct along with the antisense to siRNA also gave encouraging results in inhibiting the virus infection by nearly 50%. Based on our findings, we report for the first time, that by complementing siRNA or Dz with specific antisense molecules, it is
possible to increase the efficacy of either approaches and achieve more potent gene inhibition.

Several Dzs were synthesized against the HIV-1 Vpr gene isolated from pNL4-3 (subtype B) and their efficacy was checked on Vpr gene isolated from the Indian isolate subtype C. It was relevant to identify the Dz which would be effective against both these subtypes as the major form of HIV-1 present in India is subtype C. We were able to successfully identify an extremely efficacious Dz-94 that was capable of cleaving both Subtype B and C Vpr RNA with equal efficiency. Also this Dz was extremely potent at reducing the intracellular levels of protein.

For the development of effective gene based treatments it is essential to determine what functional differences might arise due to sequence variation among different subtypes, in particular Subtypes B and C. Also, how variations within a subtype and emergence of new recombinant forms affect HIV pathogenesis were few of the questions we tried to address using HIV-1 Vpr as the candidate gene. We reasoned that genetic differences between subtype B and the Indian isolate sequences may impact on the known functions of Vpr. Indeed we found that our subtype C Vpr gene showed significant functional differences with respect to the two known functions (Transacivation and Apoptosis). We also showed an increased potential of subtype C Vpr to induce G2 cell cycle arrest which was reversed by using the earlier identified Dz-94.

Further, we identified variants and recombinants at the Vpr locus from HIV infected individuals from North India by performing certain sequence and phylogenetic analysis. The Vpr protein from these patients showed almost comparable capability to cause apoptosis, but they showed varied transactivating capabilities due to the presence of mutations at critical
residues involved in transactivation. We found that most of them possessed a conserved L64P mutation and they had conserved C-terminal sequences. Approximately 10% of our Vpr sequences turned out to be novel B/C/D recombinants with multiple cross over points using the modern genotypic tools. This observation highlighted the importance of co-circulation of multiple subtypes and propensity for creation of novel recombinants.

The purpose of the current study was to identify the functional differences arising due to sequence variations among subtypes and subtype variants to elucidate their role in HIV pathogenesis. This would further provide valuable insight in the development of effective gene based therapies. We were able to shed some light on the functional differences with respect to their ability to transactivate and cause apoptosis between various subtypes of the accessory gene Vpr. We also experimentally showed that it was possible to achieve gene-specific suppression with nucleic acid based antiviral approaches against both the cellular and viral factors essential for the progression of HIV/AIDS. The study suggests that using combination of multiple antiviral approaches (siRNA, Rz, Dz) optimally; it is possible to achieve long term gene suppression of HIV-1.