Conclusions
6. Conclusion:

- HVR1 consensus sequence was obtained from 49 HCV genotype 3 patients. Amino acids at positions 2, 6, 7, 12, 14, 15, 17, 18, 19, 20, 22, 23, and 26 were conserved (13/27, 48.2% amino acids) when compared with HVR1 consensus sequences published earlier.

- The percent reactivity with HCV positive human serum samples for various HVR1 consensus variants was: 79.1% (Variant-1), 67.0% (variant-2), 87.8% (variant-3), 92.2% (variant-4), 72.2% (variant-pool), and 42.6% (variant-tetramer).

- PCR amplified, cloned, bacterially expressed and HPLC purified recombinant NS3 protein reacted with anti-HCV positive sera (genotypes 3a and 1b).

- Upon immunization with liposome encapsulated individual peptides in mice, HVR1 variant 2 was found to be non-immunogenic (Variant 2 differed from consensus sequence by 5 amino acids).

- Antibody responses to individual variants 1, 3 and 4 were comparable to the HVR1 variant pool and HVR1 tetramer, and could be detected by all the variants individually, indicating cross-reactivity in ELISA.

- Even though the tetramer induced high antibody titres with different formulations, the lower efficiency (42.6%) in detecting anti-HCV antibodies in human serum samples argues against its suitability as a vaccine candidate.

- Addition of individual variants, variant pool or tetramer to recombinant pNS3 in Cad-B yielded optimum antibody titres.

- Isotyping analysis documented involvement of both Th1 and Th2 type immune responses with NS3 and predominantly Th2 type immune response with HVR1 component.
• Al(OH)$_3$ gave balanced Th1/Th2 response while Th2 response was observed with CadB adjuvant with tetramer.

• Results of cytokine bead array for mouse splenocytes were not conclusive.

• Both Th1 and Th2 genes were up regulated in TLDA indicating a balanced immune response and its enhancement upon liposome encapsulation of HVR1 pool-NS3-cadB group when compared with HVR1 pool-NS3 without adjuvant group.

• Upon performing immune capture RT-PCR, mouse anti-HVR1 antibodies were able to bind viruses of genotypes 3a and 1b from HCV positive human serum samples.

• The study clearly showed that HVR1 variant pool-pNS3 cadB combination was the ideal vaccine candidate.