CHAPTER V

CONCLUSION

The Cys_2His_2 ZFP is the most common form of DNA binding motifs in humans, which provides an ideal scaffold for designing ZFP to target novel sequences. These are superior in folding in the cytoplasm and homing to nucleus. Therefore, a designed ZFP could be an alternative scaffold for conferring long-lasting protection against viral infection. This study goal was to construct a ZFP for use as an intracellular immunization to provide a steric barrier to the processing of partially 2-LTR circle junctions which is the integrase recognition sequences of HIV-1 DNA before integration.

Herein, 2LTRZFP-GFP was designed from the web-based design program "Zinc Finger Tool" (Mandell and Barbas, 2006). The designed protein was a six-finger contiguous ZFP. Assessment of binding affinity was determined by surface plasmon resonance (SPR) which showed a similar nanomolar binding affinity on level HIV-1 IN (Bugreev et al., 2003; Deprez et al., 2004). The specificity of this binding was also demonstrated with competitive SPR. The results indicated that 2LTRZFP-can specifically bind to its target ds-DNA, whereas binding of a control protein GFP in the C-terminal part was not facilitated. This binding was also confirmed by electrophoretic mobility shift assay (EMSA). To investigate the intracellular activity of 2LTRZFP-GFP, it was first stably expressed in 293T cells and challenged with VSV-G pseudotyped RFP compared to cells expressing control ZFP (Aart-GFP). A dramatic suppression of RFP expression was observed in 293T cells expressing

2LTRZFP-GFP that were inoculated with VSV-G pseudotyped RFP lentivirus at both 1 and 10 MOI. Next, to determine whether 2LTRZFP-GFP inhibited viral replication, a multiple-round infectious virus (HIV-1_{NL4-3}) was used for the challenge. A third-generation lentiviral vector (Dull *et al.*, 1998; Zufferey *et al.*, 1998) was used for delivery of the 2LTRZFP-GFP gene into the human T-lymphocytic cell line (SupT1). The results demonstrated that HIV-1 integration was inhibited in cells expressing 2LTRZFP-GFP but not in those expressing the control ZFP, and inhibition depended on the amount of transgene expression.

Moreover, stable T-cell lines expressing 2LTRZFP-GFP were generated by non-viral transduction and challenged with HIV-1_{NL4-3}. 2LTRZFP-GFP successfully inhibited viral integration and replication as measured by an *Alu-gag* qPCR and p24 antigen assay, respectively. These findings indicated that viral integration can be inhibited by intracellular immunization with 2LTRZFP-GFP. The findings may be applied for limiting viral integration to maximize the impact of HIV gene therapy in the future.