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- **Degree - Ph. D.**
- **Title of Thesis " REDUCING RESIDUAL GENOMIC INSERTION OF INTEGRATION-DEFICIENT LENTIVIRAL VECTORS (IDLV)"**

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ABSTRACT

Lentiviral vectors are very efficient for transgene delivery to important therapeutic target cells and are currently being tested for gene therapy in several diseases. They have a number of advantages, such as relatively high coding capacity, low immunogenicity and the ability to transduce nondividing cells. However, as all other retroviral vectors, they integrate in the target cell genome as proviruses. Such uncontrolled integration carries a risk of insertional mutagenesis, and mediates inherent position-effect variegation. Further enhancements can increase the bio-safety of lentiviral vectors, and one of them is to convert them into integration-deficient lentiviral vectors (IDLVs). IDLVs are most commonly generated through the use of mutations in the gene coding for integrase, the viral enzyme responsible for integration. The aim of this project was to reduce the residual integration frequency of lentiviral vectors, for which integrase sequence and/or function was affected.

In this study, we compared the effect of triple point mutation with a single point mutation of the catalytic core domain of integrase, the viral enzyme responsible for integration. We also created some integration-deficient lentiviral vectors with one or more mutations, including integrase mutation in the LEDGF/p75 binding domain and/or at the attachment sites (*att* sites) and compared them with a standard IDLV with a single point mutation. We found that there was not any significant difference between the residual integration of integration-deficient lentiviral vectors with triple point mutation and that of integration-deficient lentiviral vectors with a single point mutation. We found also that there was not any significant difference between the residual integration of any of the integration-deficient lentiviral vectors used in this study and that of the standard integration-deficient lentiviral vector.

It was also found that the residual integration of IPLV in the presence of INSTIs could be similar to or lower than that of the standard integration-deficient lentiviral vector. INSTIs may affect expression levels from IPLVs early in transduction of dividing cells. This effect was not observed in growth-arrested CHO cells, used as a model for naturally quiescent cells.

The summary not more than 500 words