

# Developing a Novel Retroviral Vector Capable of Inducible Knockdown in CD8 T Cells

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Memory CD8 T cells are immune cells that eliminate diseased cells in the body. Recent approaches to treating chronic illnesses involve bolstering their immune responses to target abnormal cells. However, the formation and regulation of memory CD8 T cells is unclear. The current investigation involved developing a doxycycline-dependent retroviral vector capable of inducing gene knockdown within a CD8 T cell. The tool would enable scientists to selectively silence a particular gene of interest using shRNA, whose expression is induced with the treatment of doxycycline. This would allow researchers to determine the dynamic role a gene plays during different stages of development. Five vector designs were created, and following transduction of Neuro-2A cells (a model cell line for CD8 T cells), samples were cultured in the presence or absence of doxycycline. Using flow cytometry, expression of Thy1.1 and GFP was used to determine if each vector was properly incorporated into cells' genomes and if the vector was capable of inducing gene knockdown in response to doxycycline treatment, respectively. One vector design resulted in a defined population of cells that expressed both Thy1.1 and GFP, indicating that the vector was successfully incorporated into cells' genomes and was capable of inducible knockdown. By isolating these cells, this vector shows promise as a research tool for the study of memory CD8 T cell formation and to help enhance immunotherapy treatments involving memory CD8 T cells. Further experimentation is required to test the effects of the vector design in mouse models.