

### Non-Technical Abstract

Human immunodeficiency virus type I (HIV-1) infects a number of cell-types *in vivo*, including CD4+ T-lymphocytes and monocyte/macrophages. This infection of CD4+ T-lymphocytes leads to immune suppression and opportunistic infections and opportunistic tumors. This clinical syndrome has been called the acquired immune deficiency syndrome (AIDS). The mechanisms by which CD4+ lymphocytes are destroyed in HIV-1 infection is still poorly characterized, but it appears that both direct infection of CD4+ lymphocytes with the HIV-1 virus and autoimmune destruction may be important in the depletion of these critical cells for the human immune system.

This proposed study will test a form of genetic therapy to combat HIV-1 infection. Gene therapy utilizes the introduction of new molecules into cells to either stimulate or inhibit a process in the human body. The use of gene therapy to combat certain infectious agents, including retroviruses which represent the group of viruses including HIV-1, is called "intracellular immunization". In the present study, portions of a mouse antibody will be constructed into a new molecule called a single chain variable fragment (SFv). This portion of an antibody will be introduced into CD4+ T-lymphocytes of infected humans to inhibit HIV-1 growth in those cells. This SFv fragment has been especially designed to function intracellularly, unlike the original antibody which works outside the cell and in the bloodstream. Studies in the laboratory has shown that this particular SFv molecule greatly inhibits HIV-1 production in human cells. It functions by blocking a HIV-1 regulatory protein, called Rev. Once Rev is blocked by this intracellular antibody fragment, HIV-1 growth is greatly inhibited. The use of this anti-Rev SFv should block the virus before it is released from cells infected with HIV-1 in the human body.

In the present clinical study, our team will evaluate the safety and efficiency of introducing this gene which expresses the anti-Rev SFv into the CD4+ T-lymphocytes of six individuals infected with HIV-1. These individuals will not be in the late stages of disease but will have HIV-1 infection without expression of opportunistic infections or tumors. It is suggested that patients properly treated with CD4+ lymphocytes harboring this anti-Rev SFv may lead to decreased

production of the virus in the body, and hopefully, decrease the viruses' negative effects on the human immune system.

In this study, our team will isolate CD4+ T-lymphocytes from the bloodstream of HIV-1-infected-individuals and introduce the anti-Rev SFv into these cells using a virus which has been crippled so that it can only introduce this gene but cannot itself reproduce. This will be done in the laboratory, in cells removed from the study subjects. After these cells have been genetically manipulated to express the anti-Rev SFv, the cells will be placed back in the patient. We will then investigate, over a one year period, how well these cells live and survive in the individuals and whether HIV-1 replication is decreased in these cells within the infected-individuals. As well, any positive or deleterious effects on the patient from these cells will be monitored and investigated. These studies will assist in demonstrating whether the anti-Rev SFv-manipulated CD4+ T-cells may be used in the treatment of people with HIV-1 infection. As well, this initial study will help in determining how to develop future investigations potentially using the anti-Rev SFv in gene therapy to fight HIV-1 disease states.