

## Scientific Abstract

At the present time, there are four FDA-approved pharmaceutical agents for the treatment of human immunodeficiency virus type I (HIV-1) infection. All the approved agents are nucleoside analog inhibitors of HIV-1 reverse transcriptase, including AZT, DDI, DDC and D4T. Other non-nucleoside analogs and inhibitors of retroviral protease are under development and may soon be approved for clinical use. Nevertheless, their effectiveness in inhibiting HIV-1 replication and altering the natural history of HIV-1 infection in humans are only limited. As well, due to the low fidelity of the reverse transcriptase enzyme, mutations developed quickly in the viral genome and resistant viral mutants have been a formidable problem in the treatment of HIV-1 infection. Most importantly, retroviruses, through their integrated proviral intermediate, appear to act as an acquired disease of DNA. The integration of the provirus in a relatively random fashion into the human genome, in billions of infected cells in the HIV-1-seropositive individual, constitutes a potential reservoir for viral replication over the lifetime of the infected-individual. In addition, recent studies on viral dynamics and turnover suggest that HIV-1 replication may occur throughout the clinical history of an infection, for years to over a decade from HIV-1-seroconversion. In addition, data have also accumulated which suggest that the lymphoid tissues may harbor high levels of viral replication at times when the peripheral blood has relatively little non-defective HIV-1 virions and viral replication appearing in peripheral blood mononuclear cells (PBMC). As such, based on these various findings, HIV-1 infection may best be treated using genetic therapy, to inhibit HIV-1 replication within the cells of infected-individuals, possibly in combination with standard pharmacological manipulations.

HIV-1 infects many cell-types *in vivo*, but the major cellular reservoirs include the CD4+ T-lymphocyte and the monocyte/macrophage. HIV-1 infection leads over a course of years to CD4 depletion based on a variety of poorly understood mechanisms. These include both direct viral destruction of HIV-1-infected cells, autoimmune processes leading to destruction of HIV-1-infected cells and, potentially, apoptotic cell death of both infected and uninfected cell-types.

Our laboratories have reported the development and study of a single chain variable fragment (SFv) constructed from a murine monoclonal antibody to the HIV-1 regulatory protein, Rev. HIV-1 Rev is absolutely essential for replication of this human retrovirus, by rescuing incompletely-spliced HIV-1 RNA from the nucleus of infected cells. When Rev is mutated in viral strains, viral replication is completely ablated. Utilizing a hybridoma cell-line expressing a monoclonal antibody which binds efficiently to the Rev activation or effector domain, an SFv was created with the same binding characteristics of its parent monoclonal. In a series of studies, this SFv was shown to be efficiently expressed in the cytoplasm of human cells, via various eukaryotic expression vectors. In initial studies, it was shown to potently inhibit HIV-1 replication in human epithelial cells. This was demonstrated to occur, with potent inhibitory abilities, regardless of the laboratory or primary viral strain utilized in the experiments. In follow-up studies, defective murine retroviral vectors were generated and characterized, expressing the anti-Rev SFv. These constructs were shown to protect T-lymphocytic cell-lines and PBMC, *in vitro*, from HIV-1 replication and cytopathic cell death. Both syncytia-inducing and non-syncytia-inducing primary isolates were inhibited by this intracellular immunization technique. No change in CD4 epitopes or cell proliferation was demonstrated in these studies, in which anti-Rev SFv was constitutively expressed intracellularly. In addition, studies have been generated from our laboratories which suggest that the anti-Rev SFv functions by changing the subcellular compartmentalization of Rev and also by decreasing its intracellular half-life. As such, these series of *in vitro* analyses, conducted over the last 2 1/2 years in our laboratories, suggest that this anti-Rev SFv construct may show significant promise for gene therapy of HIV-1-infected-individuals.

The present study is a Phase I clinical analysis, evaluating healthy HIV-1-infected asymptomatic individuals, with CD4+ lymphocyte counts between 200 and 500 cells/mm<sup>3</sup>. We will analyze the safety and feasibility of re-infusing autologous lymphocytes which have been transduced with murine retroviral vectors expressing the anti-Rev SFv. These lymphocytes will be transduced *ex vivo*, in two aliquots, using the retroviral vector expressing the anti-Rev SFv or a control vector lacking the SFv insert. Our team will compare and contrast the cell survival of those CD4+ lymphocytes expressing the anti-Rev SFv versus those expressing the control construct *in vivo*. As well, we will analyze the expression of the anti-Rev SFv in the transduced lymphocytes *in vivo*, using reverse

transcriptase-polymerase chain reaction (RT-PCR) technology. Studies will be conducted to evaluate potential immune responses to the anti-Rev SFv protein *in vivo*, evaluating cytotoxic T-lymphocyte (CTL) and antibody responses. In this study of somatic cell gene therapy, we will begin to analyze effects on viral RNA expression, studying both cell-free virions in the plasma, as well as alterations in spliced versus unspliced HIV-1 RNA expression in PBMC, during treatment with the anti-Rev SFv. Although this is primarily a safety and feasibility study, preliminary analysis of the effects of the anti-Rev SFv on alterations of CD4+ T-lymphocytes will also be obtained. As such, this protocol will assist us in the development of future gene therapeutic studies to combat HIV-1 infection and its effects on the human immune system.