

This project is focused on the development of a gene therapy approach to sickle cell disease using a γ -globin lentiviral vector with the capacity to permanently integrate into the genome of hematopoietic stem cells (HSCs), thereby providing the possibility of a lifelong cure. Our efforts will concentrate on satisfying two critical requirements for the eventual success of this approach. The first is achieving high-level, sustained erythroid-specific expression of a transferred γ -globin expression cassette. Recently, we have developed a γ -globin lentiviral vector with the capacity to achieve HbF at a level of 10% in the red cells of mice. Since it is likely that a therapeutic impact for sickle cell disease will require higher levels of HbF per cell, the first specific aim is centered on modifying our first generation vector to further increase expression. This will be done through a series of carefully planned alterations designed to boost both the level and persistence of γ -globin expression. Vectors modified to augment transcriptional activity and dampen position effect variegation and silencing will be evaluated in both *in vitro* and *in vivo* studies. These experiments will culminate in testing the therapeutic efficacy of optimized vectors in two murine models of sickle cell disease which we have acquired. The second specific aim focuses on developing a γ -globin vector also containing a selectable gene (methylguanine methyltransferase, MGMT), previously shown to enable *in vivo* selection of HSCs. We estimate that at least 10-20% of HSCs capable of giving rise to γ -globin expressing red cells will be required for a therapeutic effect in sickle cell disease. Therefore, it is likely that *in vivo* selection will be needed in a human therapeutic trial to increase the subtherapeutic, small proportion of transduced HSCs in recipients that will result from limited gene transfer efficiency and the preferable use of non-myeloablative conditioning. *In vivo* selection experiments in both normal mice and in the two sickle cell murine models are proposed in Specific Aim 2. The ultimate goal is to obtain therapeutic *in vivo* selection of γ -globin expressing cells in the sickle cell disease models. Progress in these two areas would have substantial impact on the planning of initial clinical trials and would bring gene therapy closer to being a potential treatment for sickle cell disease in the near future.