A. Scientific Abstract

This is a phase I study with the primary endpoint to determine the safety and feasibility of adoptive transfer of CD8+ T cells genetically engineered to express the alpha and beta chains of a high affinity T cell receptor (TCR) specific for the HLA-A*0201-restricted MART-1 melanoma tumor antigen, together with the HSV1-sr39tk (sr39tk) suicide/PET imaging gene, to patients with locally advanced or metastatic melanoma. This gene transfer will be effected by a lentiviral vector pseudotyped with a vesicular stomatitis virus (VSV-G) envelope. The three transgenes genes are linked by picornavirus 2A sequences. Their expression is driven by the human ubiquitin promoter (FUW). The MART-1 TCR was cloned from a patient who received dendritic cell (DC) vaccines genetically-engineered with the MART-1 adenovirus vector. This MART-1 TCR was selected from several TCR isolated from this patient because of its high affinity and biological activity. This lentiviral construct was chosen because of its superiority in transduction efficiency and prolonged and stoichiometric expression of all three genes in human T cells.

Patients with MART-1-positive locally advanced or metastatic melanoma who are HLA-A*0201-positive, and HIV, hepatitis B and C seronegative, will receive a nonmyeloablative but lymphocyte depleting chemotherapy conditioning regimen consisting of cyclophosphamide and fludarabine, and then receive the adoptive transfer of CD8+ T cells transduced with the FUW-M1-TCR/sr39tk lentiviral vector. Cohorts of 3 patients will receive escalated doses of transduced CD8+ T cells. Following adoptive cell transfer, patients will receive MART-1_{26-35} peptide-pulsed DC vaccines and high dose interlukin-2 (IL-2).

Secondary study endpoints are transgenic T cell persistence in humans and their ability to home to MART-1 positive melanoma metastasis. Analysis will be performed by sampling of peripheral blood and tumor deposits for T cell persistence and by non-invasive metabolic imaging detecting r39tk positive transgenic cells using positron emitting tomography (PET) scans. These findings will be correlated with findings in tumor biopsies.

This somatic gene transfer clinical trial may represent one of the first-in-human uses of a lentiviral vector for a non-HIV clinical trial. This vector was chosen because its superiority over Moloney-based retrovirus vectors. The self-inactivating (SIN) lentiviral vector backbone is a type of gene transfer vector that has been previously tested in human subjects. The target disease, cancer, is a setting where most prior gene transfer clinical trials have been conducted. This trial is directed towards patients with an incurable malignancy. The application of gene transfer is the genetic engineering of T lymphocytes using TCR genes, which is an approach that is currently in clinical trials. Generation of the genetically modified cells involves a short (48 hour) *ex vivo* manipulation of CD8+ cells after their genetic modification, followed by cryopreservation to allow for full biosafety testing. The vector incorporates a suicide gene, which would allow transgenic cell elimination in case of untoward effects.