

Scientific Abstract

Relapse of chronic myeloid leukemia (CML) after allogeneic bone marrow transplant (BMT) represents a significant cause of treatment failure and results from the persistence of the malignant disease despite the intensive chemoradiotherapy preparative regimen.

Donor T cells specific for recipient minor histocompatibility (H) antigens are thought to initiate both graft-versus-host disease (GVHD) and graft-versus-leukemia (GVL) reactions and contribute to the complete elimination of leukemia after allogeneic BMT. The infusion of unselected donor T lymphocytes to induce GVL activity - termed donor lymphocyte infusion (DLI) - has been evaluated as a treatment for relapsed CML. DLI therapy has induced complete remissions of the leukemia in the majority (50-75%) of patients but also caused significant morbidity and mortality related to GVHD and myelosuppression. Methods have been developed in our laboratory to introduce genes such as the HSV thymidine kinase (HSV-TK) gene into donor T cells. A retrovirus vector (termed HyTK) was developed in collaboration with Targeted Genetics Corporation for use in human studies and encodes hygromycin phosphotransferase and HSV-TK as a single fusion gene under the transcriptional control of the Moloney retrovirus LTR. The HyTK gene encodes for a bifunctional fusion protein incorporating hygromycin phosphotransferase (Hy) and herpes virus thymidine kinase (HSV-TK). This gene (HyTK) when introduced into human T cells provides two functions: (1) it serves as a selectable marker gene rendering the cell resistant to hygromycin *in vitro* and allowing the selective expansion of transduced cells and (2) confers upon the cell susceptibility to the cytotoxic effects of ganciclovir and acyclovir. The integrated HyTK proviral sequences are present in the DNA of transduced cells and PCR analysis of DNA from peripheral blood (PBL) using amplimers specific for proviral sequences can also be used as a rapid and sensitive indicator of the *in vivo* persistence of transferred cells. Thus, the introduction of HSV-TK into cells confers an inducible toxic phenotype and could potentially permit the ablation of transferred T cells in cases in which severe GVHD and/or myelosuppression result after donor lymphocyte therapy.

In the study proposed in this application, polyclonal peripheral blood T cells obtained from the patient's bone marrow donor will be transduced *in vitro* with HyTK. The HyTK transduced donor T cells will be infused into patients who have relapsed with Chronic Myelogenous Leukemia (CML) after allogeneic bone marrow transplantation. In the subset of patients developing severe graft-versus-host disease and myelosuppression after receiving HyTK-modified DLI we will determine if administering ganciclovir or acyclovir can eliminate the transduced T cells, resolve the toxicity and decrease the morbidity and mortality of DLI therapy.