

2. Scientific Abstract

This human gene therapy protocol proposes to examine the feasibility and safety of intracavitary administration of autologous ex-vivo expanded CD8⁺ cytotoxic T lymphocyte (CTL) clones genetically modified to express the IL13R α 2-specific IL13-zetakine chimeric immunoreceptor for re-directed glioma targeting and the HyTK selection/suicide fusion protein to five adult patients with recurrent or refractory malignant glioma. The engineering of the IL13-zetakine was accomplished by PCR splice overlap extension and is composed of the mutant human IL-13 (E13Y) cytokine molecule tethered to the cell membrane by the human IgG4 immunoglobulin hinge-Fc domain and CD4TM which, in turn, is fused to the T-cell CD3 complex intracellular zeta chain. Pre-clinical studies have demonstrated redirected killing, cytokine production, and proliferation of primary human cytolytic T-cells upon stimulation with glioma targets. T cells present in peripheral blood mononuclear cells (PBMC) isolated from study subjects will be polyclonally activated with anti-CD3 antibody OKT3 then genetically modified by electroporation with linearized plasmid DNA vector encoding the IL13-zetakine and HyTK cDNAs under the transcriptional control of a modified human elongation factor-1 α promoter and the CMV immediate/early promoter, respectively. Genetically modified T cell clones generated from this procedure will be evaluated for cell surface phenotype by flow cytometry, chromosomal integration status of plasmid vector by Southern blot, receptor expression by FACS, and re-directed IL13R α 2-specific anti-glioma effector function by 4-hr chromium release assay. Clones meeting all quality-control criteria will be expanded by recursive stimulation with OKT3/rhIL-2 in the presence of irradiated feeder cells. Beginning as soon as T cell clones are expanded and following recovery from re-resection/intracavitary catheter placement, patients will receive a series of four cycles of every other day escalating cell dose infusions of their IL13-zetakine⁺HyTK⁺ CD8⁺ CTL clone. Each patient will be evaluated to establish the safety of this procedure with increasing cell doses of 10⁷ cells, 5x10⁷ cells, and 10⁸ cells. The secondary objectives of this protocol are to evaluate the anti-tumor activity of this strategy by serial brain MRIs, the immunogenicity of clones when administered in this fashion, and the efficacy of ganciclovir to ablate clones *in vivo* should toxicities warrant this maneuver. This pilot study will serve to provide safety and feasibility data to support the initiation of Phase I/II clinical trials for the treatment of malignant glioma.