I. Scientific Abstract

This human gene therapy protocol proposes to examine the safety of administering autologous *ex-vivo* expanded $CD8^+$ cytotoxic T lymphocyte (CTL) clones genetically modified to express the CE7R chimeric immunoreceptor (scFvFc: ζ for re-directed neuroblastoma specificity and the HyTK selection/suicide fusion protein to ten children with recurrent or refractory disseminated neuroblastoma. T cells present in peripheral blood mononuclear cells (PBMC) isolated from study subjects at the time of relapse will be polyclonally activated with anti-CD3 antibody OKT3 then genetically modified by electroporation with linearized plasmid DNA vector encoding the CE7R scFvFc: ζ and HyTK cDNA's under the transcriptional control of a modified human elongation factor-1alpha promoter in a dicistronic format utilizing the EMCV interna1 ribosome entry site (IRES). 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 Western blot and re-directed anti-neuroblastoma effector function by chromium release assay. Clones meeting all quality-control criteria will be expanded by recursive stimulations with OKT3/rhIL-2 in the presence of irradiated feeder cells. Beginning as soon as T cell clones are expanded or following recovery from salvage chemotherapy/ radiation, patients will receive a series of three escalating cell dose infusions at two-week intervals of their genetically-modified $CE7R^+HyTK^+$ $CD8^+$ CTL clones. Each patient will be evaluated to establish the safety of this procedure with increasing cell doses of 10^8 cells/m^2, 10^9 cells/m^2, and 10^{10} cells/m^2 of body surface area. The research participants will be divided into two 5-patient cohorts. Cohort 1 will consist of the first five enrolled children: This cohort will not receive rhlL-2 with their T cell infusions. Cohort 2 will receive with T cell doses #2 and #3 subcutaneously administered low-dose IL-2. The secondary objectives of this protocol are to study the *in vivo* persistence of transferred cells by Q-PCR of peripheral blood samples for vector-specific sequence and the impact of exogenous IL-2 on maintaining persistence, Additionally, patient peripheral blood samples will be obtained following adoptive therapy and evaluated for evidence of humoral and cellular immune reactivity against the infused genetically-modified clones. Finally, when applicable, anti-neuroblastoma responses will be assessed by tracking tumor markers, disease burden in bone marrow, and radiographic responses. This phase I study will serve to provide safety data to support the initiation of larger Phase II clinical trials in the treatment of poor prognosis neuroblastoma prior to overt relapse.