

## 2.0 Scientific Abstract

This is a phase I/II study designed to determine, within an achievable dose range, the maximally tolerated dose (MTD) of donor derived T cells transduced with the onco-retroviral vector NIT. This bicistronic vector encodes a mutant LNGFR (mLNGFR) and a herpes simplex virus thymidine kinase under the transcriptional control of the Moloney long terminal repeat. Transduced T cells will be isolated early after sensitization to autologous Epstein-Barr virus transformed B lymphocytes cell lines (EBV BLCL). The transduced T cells will be isolated by immunoadsorption. After initial incubation of the transduced T cells with a monoclonal antibody directed against the mutant form of LNGFR that is biotinylated, the antibody coated T cells will be adsorbed to immunomagnetic beads coated with an anti-biotin monoclonal antibody. Using this approach, the NIT<sup>+</sup> CD3<sup>+</sup> T cells can be consistently isolated at high purity (>93%).

The patients to be entered on this trial must have biopsy proven EBV lymphoma and an HLA matched or haplotype matched EBV seropositive donor who consents to donate T cells for the generation of EBV sensitized vector transduced cells to be used for this therapy. Patients will be stratified by underlying condition and degree of histocompatibility (matched or HLA mismatched) based on their risk of developing GVHD, and treated with escalating doses of isolated NIT vector modified donor T cells. T lymphocytes will be transduced with the onco-retroviral vector NIT at 8 days following in vitro sensitization with autologous irradiated donor derived EBV transformed BLCL. Three days following transduction with the NIT vector, T cells expressing the mLNGFR will be isolated by initial incubation with a biotinylated monoclonal antibody specific for human mLNGFR and subsequent immunoadsorption to magnetic beads coated with anti-biotin antibody. After ascertainment of purity of the transduced populations and their microbiological sterility, the isolated transduced T cells will be infused.

Initially, 3-6 consenting patients in each of three disease groups will be treated at a starting dose of  $1 \times 10^5$  T cells/kg. Subsequent cohorts of 3-6 patients in each group will receive doses escalated to 3 and  $6 \times 10^5$  NIT<sup>+</sup> T cells/kg. The range of doses of T cells to be used in this trial  $1, 3$  and  $6 \times 10^5$  NIT<sup>+</sup> T cells/kg has been selected on the basis of the doses of unmodified peripheral blood T cells derived from seropositive marrow transplant donors that have been sufficient to and consistently induce regressions of EBV lymphomas in the post transplant period. Isolated populations of T cells transduced with NIT after 8 days of sensitization with irradiated autologous EBV transformed B cells have 4 – 6-fold increases in the concentration of EBV CTLp. The frequency of alloreactive T cells detected in these isolated transduced cells is also reduced by 5 – 15-fold below that detected in fresh BPMC. A conservative estimate of the dosing of major alloantigen reactive T cells that would be expected in an infusion of the starting dose  $1 \times 10^5$  NIT<sup>+</sup> T cells/kg would be equivalent to that provided in doses of  $0.6 - 2 \times 10^4$  fresh PBMC derived T cells/kg. These doses are lower than the doses of T cells provided in SBA<sup>-</sup> E<sup>-</sup> T cell depleted marrow grafts and CD34<sup>+</sup> E<sup>-</sup> peripheral blood stem cell transplants that have been administered either to HLA matched or HLA haplotype disparate donors. They are below the threshold dose for acute GvHD in HLA compatible related recipients ( $10^5$  T cells/kg) and have been associated with an 8 – 10% incidence of clinically significant GvHD in matched unrelated or 1-3 HLA allele disparate related recipients of T cell depleted marrow or peripheral blood stem cell transplants.

The patients will be monitored for toxicity, alterations in the growth of the EBV lymphoma and development of GvHD and its reversibility after parenteral treatment of ganciclovir. Patients also will be monitored for the survival, proliferation and, whenever possible, tissue and tumor localization of infused transduced NIT<sup>+</sup> T cells, alterations in EBV specific CTLp induced post-infusion and the contribution of NIT<sup>+</sup> T cells to these EBV specific CTLp populations. In patients developing GvHD, skin or other biopsies will be obtained to determine the representation of NIT<sup>+</sup> T cells in the T cell infiltrates that develop. In addition, patients who develop GvHD and are treated with ganciclovir, will be evaluated for the effects of this treatment on GvHD and the survival of NIT<sup>+</sup> T cells in blood and affected tissues. In addition, following the 30 month prescribed period of evaluation, the patients will be evaluated at yearly intervals as to their clinical status and will also be assessed for NIT<sup>+</sup> T cells among circulating T lymphocytes responding *in vitro* to stimulation with autologous EBV transformed B cells.

In this dose escalation trial, if no patients in the group of patients receiving the dose of  $10^5$  NIT<sup>+</sup> T cells/kg develop grade III or greater toxicity or grade II-IV acute GvHD that is not reversible with ganciclovir treatment at the starting dose, the next group of these patients will receive NIT<sup>+</sup> T cells at a dose of  $3 \times 10^5$  T cells/kg. If undue toxicity or GvHD again does not develop, the third group will be treated with a starting dose of  $6 \times 10^5$  T cells/kg/dose. The patients in each group will initially be observed and evaluated for 21 days after the first dose. Patients who achieve CR of their EBV lymphoma will receive no further doses. Patients with a PR and acceptable levels of toxicity and no or reversible GvHD may receive a second infusion of cells at the same dose. Patients with acceptable toxicity and no or reversible GvHD who have stable disease or disease progression may receive a third infusion at the same dose or will be given alternate therapy (Confer protocol for details). Evaluations and stopping rules for the trial are defined (Confer protocol, Section 14.0 "Biostatistics").