

## 2. THE SCIENTIFIC ABSTRACT

This Phase I open-label human gene therapy protocol tests the safety and feasibility of using autologous T cell clones that have been genetically modified to be specific for CD19 and expanded *ex vivo*, in five "pediatric" patients between the ages of 2 and 18 and five "adult" patients between the ages of 19 and 60 with high risk acute lymphoblastic leukemia (ALL) of B cell origin, who have undergone an autologous hematopoietic stem cell transplant (HSCT). Research participants are identified as high risk if any of the following occur: (a) there is a failure to eliminate leukemia in the bone marrow by four weeks of induction therapy, (b) there are high risk cytogenetics defined as t(9;22) or BCR/ABL by PCR analysis, t(4;11) or other re-arrangements of the *MLL* gene, balanced t(1;19), -7, or 8q24 abnormality, (c) hypodiploid karyotype, (d) there is B-lineage ALL with white blood count  $>30,000/\mu\text{L}$  at time of initial presentation, (e) the patient is in second or third CR. The autologous T cells are rendered specific for CD19 by electroporating with a naked DNA plasmid construct coding for a chimeric immunoreceptor (CD19R) and a bifunctional fusion gene that combines hygromycin phosphotransferase and herpes virus thymidine kinase (HyTK) permitting *in vitro* selection of genetically modified T cells with hygromycin and potential *in vivo* ablation of transferred cells with ganciclovir. The specificity of the scFvFc: $\zeta$  chimeric immunoreceptor is derived from the variable regions of a mouse mAb specific for CD19 that are tethered to the T cell via the human IgG4 Fc region and CD4 transmembrane domain. Upon binding CD19, the genetically modified T cells are activated by the cytoplasmic CD3- $\zeta$  chain attached to the chimeric immunoreceptor. Genetically modified T cells are selected for growth in hygromycin, cloned by limiting dilution, evaluated for specific lysis of CD19<sup>+</sup> targets and expanded to large numbers in compliance with current Good Manufacturing Practice (GMP). The T cells will be given once the research participant meets the eligibility requirement for T cell infusion. For each research participant, a series of four escalating T cell doses will be administered with the same autologous genetically modified cytolytic clone beginning at  $1 \times 10^9$  cells/m<sup>2</sup> and cumulating at  $4 \times 10^9$  cells/m<sup>2</sup>. Each T cell infusion will only be given on the COHNMC campus. The research participant will be hospitalized at COHNMC for at least the night following the T cell infusion. Research participants must meet eligibility requirements before receiving an increased cell dose. Cytolytic T cells require IL-2 to achieve their proliferative potential and long-term survival. In this study, patients will be eligible for exogenous low-dose ( $5 \times 10^5$  IU/m<sup>2</sup>/dose q 12-hrs) sub-cutaneous (s.c.) self-administered recombinant human interleukin 2 (IL-2) to support the *in vivo* persistence of transferred T cell clones. Patients without significant toxicity attributed to the T cell infusions will receive low-dose s.c. IL-2 for 5 days following T cell infusion #2 and #3 and for 10 days following infusion #4. In addition to safety and feasibility data, information will be collected on the emergence of a host immune response directed against the genetically modified T cells as well as survival of the infused T cells. It is likely that additional studies will be necessary to establish the ability of the CD19-specific T cells to eradicate ALL.