

## 2.0 Scientific Abstract

**Objectives:** The objectives of this trial are to assess the *in vivo* safety, biologic activity, and long-term survival of autologous T cells genetically modified to target the CD19 antigen either as a single therapeutic agent or in combination with prior lymphodepleting cyclophosphamide chemotherapy.

**Rationale:** Most patients with chronic lymphocytic leukemia (CLL) will either die of their disease or are incurable with current treatment modalities. We have developed a novel immunotherapy based on the genetic modification of patient T cells to recognize the B cell-specific cellular marker CD19. T cells can be genetically modified with a replication defective Moloney Murine leukemia virus (Mo-MuLV) retroviral vector containing a gene encoding a chimeric antigen receptor (CAR) specific for the CD19 antigen (19-28z). The 19-28z CAR consists of a single chain fragment-length antibody (scFv) derived from a murine CD19 specific monoclonal antibody (SJ25C1) fused to the extracellular, transmembrane and intracellular signaling domains of the co-stimulatory receptor CD28 and the cytoplasmic signaling domain of the T cell receptor (TCR) associated CD3  $\zeta$  chain. *In vitro*, retrovirally transduced 19-28z<sup>+</sup> T cells derived from both healthy volunteers as well as patients with advanced CLL lyse both allogeneic CD19<sup>+</sup> tumor cell lines and autologous CLL tumor cells. *In vivo*, transduced T cells from healthy donors eradicate established systemic Burkitt lymphoma in a SCID-Beige model of disease. Based on the hypothesis that prior lymphodepleting chemotherapy will enhance engraftment, expansion, and long-term survival of these adoptively transferred modified T cells, some patients on this trial will receive infusions of tumor targeted T cells *following* cyclophosphamide chemotherapy.

**Patient Population:** Patients with CLL refractory to purine analog chemotherapy who have evidence of refractory or relapsed disease are eligible. Patients who have received prior therapy with alemtuzumab, a T cell depleting therapy, are ineligible.

**Study Design:** This is a 2 step phase I dose escalation trial to assess the safety of treating purine analog-refractory CLL patients with autologous T cells genetically modified *ex vivo* to target the CD19 antigen expressed on most B cell malignancies. In the first step of this study, patients will be enrolled in cohorts of 3-6 patients to receive escalating doses of modified T cells. There will be three planned T cell doses studied in this initial step. In the second step of this trial, we will enroll patients in 3 planned cohorts of 3-6 patients who will be treated with escalating doses of cyclophosphamide chemotherapy prior to the infusion of the tumor targeted T cells at the maximum tolerated dose (MTD) established in step 1 of this study. In all cohorts, 3-6 patients will be treated at each dose level, and dose escalation will proceed if less than 33% of patients in a cohort experience unanticipated dose-limiting toxicity. If unacceptable toxicity is seen in the first patient at the first dose level, up to 6 patients will be treated at that dose using a conventional dose escalation scheme.

**Treatment Plan:** Patients will receive a single infusion of autologous T cells genetically targeted to CD19 either alone (step 1) or following cyclophosphamide chemotherapy (step 2). On the day of enrollment (day 0), patients will have blood drawn from which T cells will be isolated. Over the next 14-21 days, these T cells will be genetically modified and expanded in the Gene Transfer and Somatic Cell Engineering Facility at

MSKCC. In step 1, if a suitable number of T cells are obtained by day 12 following T cell isolation, the patient will be admitted to the hospital on day 13, modified T cells will be administered on day 14. If an insufficient number of T cells have been generated by day 12, hospital admission will be delayed by 1 week (day 20) to allow for further T cell expansion in the facility, and patients will receive their T cell infusion on day 21. In the second step of this protocol patients will be admitted to the hospital 3 days prior to planned T cell infusion (day 11 or 18 depending on the T cell number generated in the laboratory), receive cyclophosphamide chemotherapy on day 12 or 19, and T cell infusion on day 14 or 21. Serial sampling of blood and bone marrow will be performed following treatment to assess toxicity, therapeutic effects, and survival of the genetically modified T cells.

Time to Completion: At least 4 patients will be needed to complete this study. The amount of time required to complete this trial will depend on the number of dose levels studied and the number of patients accrued to each cohort. We anticipate enrolling on average 1-2 patients per month for a total accrual of 24-36 patients. Therefore, this trial will take 2-3 years to complete.