IRO received 10/04/21

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UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE

PROTOCOL 2639.00

Current Version: 06/01/2021
Previous Version: 09/19/2018

1. TITLE OF PROTOCOL: Phase I/II study of immunotherapy for advanced CD19+ chronic lymphocytic leukemia, acute lymphoblastic leukemia/lymphoma and non-Hodgkin lymphoma with defined subsets of autologous T cells engineered to express a CD19-specific chimeric antigen receptor.

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FHCRC IRB Approval
NOV 03 2021
Document Released Date

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2. INTRODUCTION

Patients with $CD19^+$ B cell malignancies such as chronic lymphocytic leukemia (CLL), indolent and mantle cell lymphoma, and diffuse large cell lymphoma that progress after first and second line conventional chemotherapy have a poor long-term prognosis, especially if they are not eligible for hematopoietic stem cell transplant due to the lack of a donor, or if stem cell transplant is contraindicated. This protocol will evaluate the safety and antitumor activity of adoptively transferring autologous T cells that are genetically modified with a self-inactivating (SIN) lentiviral vector to express a CD19-specific chimeric antigen receptor (CAR) to patients with advanced $CD19^+$ B-lineage malignancies.

3. BACKGROUND

3A. Chronic Lymphocytic Leukemia (CLL)

CLL is the most prevalent adult leukemia and patients may have an indolent or aggressive clinical course. Tumor cells nearly universally express the CD19 antigen and variable to low density of CD20. Asymptomatic patients without a large disease burden at diagnosis are usually observed until their disease progresses and requires therapy. Patients with high risk features including non-mutated immunoglobulin genes, expression of ZAP-70, CD38, and/or adverse genetic changes (11q23 or 17p-) detected by cytogenetics or FISH often progress more rapidly to symptomatic disease and require treatment to control disease burden and reduce symptoms [1].

For patients requiring antitumor therapy, the recent addition of monoclonal antibody therapy to combination chemotherapy has become standard of care. For medically fit patients less than 65 years of age, the CLL8 trial by the German CLL group demonstrated higher complete remission (CR) rates, longer progression free survival (PFS) and overall survival (OS) for patients randomized to fludarabine, cyclophosphamide, rituximab (FCR) chemotherapy compared to FC chemotherapy alone [2]. In this trial, lower levels of minimal residual disease (MRD) as detected by flow cytometry 2 months after the completion of therapy were correlated with improved PFS and OS [3]. Other approaches include combinations of fludarabine and rituximab or bendamustine and rituximab and are often used in older patients or those with comorbid medical conditions.

Unfortunately, current treatments for CLL are not curative and patients remain at a continuous risk of relapse. The prognosis for patients relapsing after first line therapy is variable but largely dependent on the duration and depth of first remission and on the interim development of adverse genetic risk features such as 17p deletion. For patients with long remissions (> 2 years), the initial treatment can often be repeated. However, the response rate, depth and duration of remission are usually shorter with subsequent therapy. Patients with short remissions after initial aggressive chemoimmunotherapy or who have developed fludarabine refractory disease (less than partial response (PR) to therapy or relapse within 6-12 months) have a poor prognosis with median OS of < 2 years and are candidates for aggressive treatments, including allogeneic hematopoietic cell transplantation (HCT).

Patients beyond first or second relapse are in need of novel experimental treatments. Current approaches include the use of new agents targeting the B cell Ig receptor signaling pathway including PI3kinase-delta with GS-1101 (CAL-101) and the Brutons Tyrosine kinase (BTK) inhibitor, ibrutinib (PCI32765) [4, 5]. Allogeneic HCT using HLA matched related or unrelated donors following non-myeloablative conditioning may provide a curative option, however there is significant risk of non relapse mortality of approximately 25% at 1 year due to

GVHD and/or infections [6]. The recent demonstration that adoptive T cell therapy with autologous T cells engineered to express a chimeric antigen receptor specific for CD19 induced complete and sustained remissions in 2 of 3 patients with refractory CLL provides an additional treatment option for these patients [7, 8].

3B. Indolent Lymphoma and Mantle Cell Lymphoma

The indolent lymphomas represent a wide group of tumors predominately comprising follicular, small lymphocytic, marginal cell and lymphoplasmacytic histologies that often have a long natural history characterized by continuous relapses. Patients with asymptomatic disease at presentation and with low tumor bulk are usually observed and not treated until the development of symptoms or significant disease progression. Several recent studies presented in abstract form have demonstrated that single agent rituximab, given for short or extended schedules has a high response rate and delays the time to requiring next therapy [9, 10]. However, these studies have not yet shown an impact on OS and this practice is not standard. For medically fit patients requiring therapy, combination chemo-immunotherapy is emerging as the standard of care. Multiple studies have demonstrated that the addition of rituximab to combination or single agent chemotherapy (CHOP, CVP or Bendamustine) results in longer PFS and in some trials prolonged OS [11-13]. Following initial therapy, many patients are treated for up to 2-years with maintenance rituximab using a variety of schedules. The PRIMA study in patients with follicular non-Hodgkins lymphoma (NHL) demonstrated a decrease in the risk of progression at 2 years by 50% by this strategy, although there was no difference in OS [14].

As in CLL, patients with indolent lymphoma who relapse following initial therapy may be treated again with the same or similar regimen. Patients with early relapse, or those who progress on rituximab maintenance are considered rituximab refractory. There is no standard of care for the treatment of patients with relapsed indolent NHL, and the usual approaches include the use of agents singly or in combination that had not been given as initial therapy (such as bendamustine and rituximab in patients failing R-CVP or R-CHOP), or more aggressive salvage therapies and autologous HCT. Unfortunately, these treatments are generally not curative.

Patients with recurrent or progressive indolent lymphoma may be candidates for allogeneic HCT, which can result in long-term disease free survival [15]. However, as in CLL, the treatment is dependent on the identification of an appropriate donor and is associated with significant risks of acute and chronic GVHD and infections. For transplant eligible patients, the decision to proceed to transplantation is often a matter of personal choice of risk-vs-benefit. Patients without donors, those who are not transplant candidates, and those who elect not to undergo allogeneic HCT are in need of novel therapies.

Mantle cell lymphoma (MCL) represents a chronic relapsing NHL with a more aggressive clinical course. Current standard of care includes induction chemo-immunotherapy with regimens such as R-CHOP or HyperCVAD/Mtx/Ara-C [16]. Patients with responsive disease are considered for high-dose therapy and autologous HCT as consolidation in first remission. A study by the German low-grade lymphoma group demonstrated improved PFS and OS with autologous HCT in first remission compared with interferon maintenance therapy [17, 18]. Data from the MD Anderson Cancer Center suggest that patients receiving full course HyperCVAD may have similar outcomes without autologous HCT [16]. Even with the use of autologous HCT, patients appear to remain at risk of relapse with no evidence of a plateau on the PFS curve. Patients who relapse following autologous HCT, or those unable to receive autologous HCT

have a poor outcome and are considered for experimental approaches and for allogeneic HCT. Surprisingly, allogeneic HCT appears to provide powerful graft-versus-tumor effect against MCL and several studies have demonstrated high CR rates and a low rate of relapse [19]. This suggests MCL is susceptible to T cell recognition however as discussed above, the considerable risk of morbidity and mortality from GVHD and infection makes proceeding to an allogeneic HCT a difficult decision for many patients. Patients unable to find a donor or unwilling to accept the risk of allogeneic HCT are usually treated with single agent or combination chemotherapy with palliative intent.

3C. Diffuse Large Cell and Other Aggressive B Cell Lymphomas

The most common histologic type of lymphoma is diffuse large B cell (DLBCL), representing approximately 30% of the annual incidence of NHL. In contrast to the indolent NHL's, these lymphomas require immediate treatment with curative intent. The addition of anti-CD20 antibody therapy with rituximab to standard CHOP chemotherapy has improved the outcome for patients with DLBCL [20]. The goal of treatment is to obtain a CR as demonstrated by a negative positron emission tomography (PET) scan. Current standards for patients with limited stage disease include a short course of R-CHOP followed by involved field irradiation or full course R-CHOP. Patients with advanced stage disease usually receive 6-8 cycles of R-CHOP or 6 cycles of dose adjusted EPOCH-R. Patients who fail to achieve a PET negative CR or those who relapse following CR are treated with salvage therapy with plans for high-dose chemotherapy and autologous HCT for those who respond to the salvage regimen. High dose therapy with regimens such as BEAM or CY/TBI/VP-16 or BuMelTT can cure 20-50% of chemotherapy responsive patients and is considered the standard of care for patients responding to salvage chemotherapy who are medically fit for transplant. Unfortunately, patients who relapse early after primary therapy with a regimen containing rituximab, have a worse prognosis even with salvage high-dose therapy and autologous HCT [21].

Patients who are unable to undergo autologous HCT or those who relapse following autologous HCT have a poor prognosis with a median survival of 6 to12 months. Allogeneic HCT may be considered in patients who have appropriate donors and chemotherapy sensitive disease that can be rendered into CR or near CR prior to the transplant [22]. Evidence for a graft-versus-lymphoma effect for DLBCL is more limited, perhaps because class I MHC is absent on a high proportion of DLBCL [23], and long-term survival is achieved in only 30-40% of patients who have minimal disease at the time of allogeneic HCT. Thus, patients with relapsed aggressive lymphoma who have previously had an autologous HCT or those medically unable to receive autologous HCT have a poor prognosis and are in need of novel treatments.

3D. Adoptive T cell therapy for human malignancy

The potential for T cells to eradicate human malignancies is illustrated by the graft-versus-leukemia effect of allogeneic T cells administered as part of a stem cell transplant [24, 25]; and in melanoma patients who receive in vitro expanded, autologous T cells derived from the tumor infiltrate and administered after lymphodepleting chemotherapy [26]. These approaches have antitumor efficacy, but have been associated with some toxicity related to recognition of normal cells that express the antigen(s) targeted by the infused T cells. Patients that receive allogeneic HCT or donor lymphocyte infusions often develop graft-versus-host disease as a consequence of donor T cell recognition of epithelial cells that express minor H antigens [27], and melanoma patients treated with tumor infiltrating lymphocytes (TIL) may develop vitiligo due to recognition of normal melanocytes [26]. Despite these complications, T cell therapy has cured a

subset of patients with advanced malignancy that was resistant to conventional chemotherapy and/or radiotherapy [26, 27].

3E. Transfer of tumor targeting receptors into T cells to confer tumor-specificity

An obstacle for applying T cell therapy more broadly is the difficulty isolating tumor reactive T cells from patients with cancer. An approach to overcome the low frequency of tumor-reactive T cells in patients is to redirect the specificity of T cells by expressing a transgene that encodes a receptor specific for a tumor-associated antigen (TAA). Vector systems to deliver transgenes into primary human T cells have been developed and clinical trials in which autologous T cells are modified to express a tumor-reactive T cell receptor (TCR) have been initiated with transient clinical responses [28, 29]. However, redirecting T cells to recognize tumor antigens through TCR gene transfer is inherently constrained because of the requirement for major histocompatibility complex (MHC) restricted peptide presentation by tumor cells, and because many tumors including DLBCL express low levels of MHC molecules to avoid T cell recognition [23].

An alternative method for targeting T cells to tumor cells is to express an artificial chimeric non-MHC restricted antigen receptor (CAR) that recognizes a tumor cell surface molecule [30, 31]. A CAR is typically comprised of a fusion gene that encodes a monoclonal antibody-derived single chain variable fragment (scFv), consisting of heavy (V_H) and light (V_L) chains joined by a flexible linker, and then fused through a transmembrane domain to a cytoplasmic signaling moiety consisting of CD3ζ alone, or CD3ζ combined with activation domains from costimulatory molecules such as CD28, 4-1BB or OX40 [31, 32]. CARs with specificity for tumor cell-surface epitopes are "universal" in that they bind antigen in an HLA independent fashion, and one receptor construct can be used to treat all patients with tumors that express the molecule targeted by the CAR. T cells obtained from the blood of cancer patients can be modified with CARs to generate anti-tumor effector cells (CAR-T cells) for adoptive therapy that can recognize tumor cells that have downregulated HLA molecules, thereby avoiding the need to isolate rare HLA-restricted tumor-reactive T cells. CARs have been constructed for many tumor-associated cell surface molecules including CD19, CD20, EGFR, Her2neu, GD2, PSMA, mesothelin, CAIX and ROR1 [31-36]. In vitro studies have demonstrated that both $CD4^+$ and $CD8^+$ T cell effector functions can be triggered via CARs, and studies in animal models and in small numbers of patients have demonstrated the capacity of adoptively transferred CAR-T cells to eradicate established tumors [7, 8, 37, 38].

3F. Rationale for targeting CD19 on B cell malignancies with CAR-T cells

Efforts to target B cell malignancies with CAR-T cells have focused predominantly on introducing receptors that recognize surface molecules such as CD19 and CD20 that are restricted in their expression to the B cell lineage. CD20 is present on B cell lymphomas and CLL, and has been targeted effectively with rituximab [39, 40]. However, rituximab is now routinely administered to most patients with B cell malignancies and the presence of antibody bound to CD20 can block target recognition by T cells engineered to express a CD20-specific CAR. CD19 is a type I transmembrane protein that associates with CD21, TAPA-1, and Leu13 to form a signal transduction complex that participates in regulating B cell proliferation [41]. CD19 is expressed on all human B cells beginning from the initial lineage commitment until terminal differentiation into plasma cells, and is expressed homogeneously on B cell CLL, indolent and mantle cell lymphoma, and DLBCL. Importantly, CD19 is not expressed on any normal tissue

apart from B lymphocytes, therefore depletion of normal B cells is the only anticipated on-target toxicity to normal cells of CD19 CAR-T cells [7, 8].

3G. Clinical experience with the adoptive transfer of T cells genetically modified to express a CAR

The initial human trials of CAR-modified T cells employed first generation constructs that linked the scFv to the CD3ζ or FcR gamma epsilon chain as the only intracellular signaling domain. T cells engineered with first generation CARs specific for CAIX were administered to patients with renal cell cancer, and caused elevations in liver enzymes possibly due to expression of CAIX on bile duct epithelium [42]. T cells specific for CD171 (L1CAM) were administered to patients with neuroblastoma without toxicity [43]. However, in each of these studies, T cell persistence was poor and sustained antitumor effects were not observed.

Patients with advanced B-cell malignancies have also been treated with first generation CARs that were specific for CD20 or CD19. These patients experienced little or no toxicity, but the CAR-T cells persisted poorly and sustained antitumor efficacy was not observed [28, 44]. The suboptimal results in these initial trials may reflect several factors including the absence of signaling domains to provide costimulation necessary for sustained T cell proliferation and survival in first generation CARs; the methods used to engineer and expand T cells for adoptive transfer that required long term culture and favored infusing terminally differentiated effector cells; and the variability in phenotype of the starting population, which can influence the capacity of T cells to persist and function long term in vivo [44-48].

To overcome the limitations identified in the initial trials and enhance potency, costimulatory endodomains were added to CD3ζ to enhance signaling through the CAR [7, 8, 49-51], and improved methods for selecting and transducing T cells of defined phenotype have been developed that do not require long-term culture [7, 8].The antitumor efficacy of T cells engineered to express a CD19-specific CAR and derivatives that include CD28 and/or 4-1BB signaling domains in series with CD3ζ have been studied in vitro and in pre-clinical in vivo models, and receptors that provide costimulation have been superior to those that do not [49, 52] (Hudecek M, Riddell SR, unpublished data). Moreover, potent and sustained antitumor activity has been observed in a small number of patients with CLL and NHL treated with autologous CD19 CAR-T cells that include either a 4-1BB or CD28 signaling domain in the CAR design [7, 8, 51, 53]. In these studies, lymphodepleting chemotherapy was administered to enhance T cell persistence, and in some cases, IL-2 was administered after the T cell infusion, although the most impressive antitumor responses were observed in patients that did not receive IL-2 [7]. The major toxicities of CAR-T cells include cytokine release and tumor lysis syndromes that occurred approximately 2 weeks after infusion of CD19 CAR-T cells, and persistent depletion of normal $CD19^+$ B cells, necessitating intravenous immunoglobulin treatment. There was no obvious correlation between efficacy, toxicity, T cell persistence and the T cell dose administered to patients in these initial trials [7, 8, 51, 53]. The inconsistency in the behavior of the therapeutic products may be because polyclonal T cells from the patient were transduced to express the CAR, and the resulting cell products administered to each patient varied greatly in phenotypic composition (CD4, CD8) and subset derivation.

3H. Lymphodepleting chemotherapy improves persistence of transferred T cells

Clinical trials of T cell therapy for melanoma at the National Cancer Institute demonstrated that administering lymphodepleting chemotherapy such as fludarabine and

Cytoxan, or fludarabine, cytoxan and total body irradiation, prior to the transfer of 10^{10}-10^{11} polyclonal melanoma-specific T cells improved the survival of a subset of the transferred T cells and therapeutic efficacy [26, 54, 55]. The size of the T cell pool is subject to homeostatic regulation, and the induction of lymphopenia results in less competition for cytokines such as IL-15 and IL-7 that promote lymphocyte proliferation and survival, and leads to the proliferation of residual T cells including those that are adoptively transferred. Lymphodepleting chemotherapy may also eliminate $CD4^+$ $CD25^+$ regulatory T cells, and activate antigen presenting cells that may promote the function of transferred T cells. Studies in murine models subsequently confirmed the human data that lymphodepletion improves the persistence and antitumor efficacy of transferred T_E cells [56].

3I. Heterogeneity of T cell products in clinical adoptive transfer studies

Clinical trials of genetically modified T cells have typically utilized peripheral blood mononuclear cells derived from the blood as the starting population for transduction. The T cell pool is characterized by marked heterogeneity in the phenotype and function of cells, and this is further altered by age and prior chemotherapy. T cells can be broadly divided into $CD45RA^+$ antigen inexperienced naïve T cells (T_N) that express $CD62L^+$ and $CCR7^+$ to enable their transit through lymph nodes where they survey for foreign antigens; and $CD45RO^+$ memory T cells that have clonally expanded in response to prior antigen encounter, and can be subdivided into $CD62L^+$ central memory (T_{CM}) and effector memory (T_{EM}) subsets [57]. A subset of memory T cells that is intermediate between that naïve and memory cells has also been described, and suggested to represent a "memory stem cell" based on the ability to self-renew, and give rise to effector (T_E), and T_{CM} and T_{EM} subsets [58]. As a consequence of heterogeneity in the distribution of T cell subsets in the blood of different individuals, particularly cancer patients who may have had markedly different exposures with prior chemotherapy, the composition of T cell products obtained after gene transfer is often dramatically different, and the consequences of this variability for interpreting, cell persistence, and the toxicity and efficacy of infused T cell products are not known.

3J. Rationale for deriving CAR-T cell products from defined T cell subsets

It is possible to generate genetically modified T cells from a defined starting population of T cells that are enriched from PBMC based on the expression of distinct cell surface markers that define their lineage and differentiation. Studies in animal models including immunodeficient mice that were administered human $CD8^+$ T cells showed that cells from defined subsets have markedly different capacity to survive and function in vivo [45, 59]. For example, effector $CD8^+$ T cells derived from purified T_{CM} have been shown to persist long term in vivo, migrate to memory cell niches in the bone marrow and lymph nodes, and establish functional populations of T_{CM} and T_{EM} [45]. Studies from our group have also demonstrated that human $CD4^+$ T cells modified with a CAR enhance the proliferation of $CD8^+$ CAR-T cells derived from T_{CM} in response to tumor cells (Hudecek M, Riddell SR, unpublished data). These results suggest that preparing CAR T cells from purified $CD8^+$ T_{CM} and $CD4^+$ T cells and combining these subsets to achieve a defined product composition may provide a more reproducible safety profile of transferred T cells and improve therapeutic efficacy.

3K. Development of a self-inactivating (SIN) lentiviral vector encoding a CD19-specific CAR

We have constructed a CD19-specific CAR consisting of an scFv derived from the murine IgG1 monoclonal antibody (mAb) FMC63. The scFV is fused to the human IgG4 hinge region and the human 4-1BB costimulatory domain in tandem with CD3ζ (Figure 1). The construct also encodes a truncated epidermal growth factor receptor (tEGFR) downstream of a T2A sequence to provide coordinate expression of tEGFR, which can serve both as a marker for cell selection and for tracking transduced T cells in vivo [60]. We have compared the activity of different CD19 CARs in vitro and in vivo using murine xenograft models, and found that the CD19 CAR containing 4-1BB as a co-stimulatory domain exerts superior antitumor activity compared with a CD19 CAR that contains an IgG4 hinge and CH2-CH3 spacer with CD28 as the costimulatory domain. This data is consistent with uncontrolled results of other groups in which a small number of patients have been treated with CD19 CAR-T cells containing CD28 or 4-1BB costimulatory domains [7, 8, 50, 51, 53]. Based on the practical and theoretical advantages of lentiviral vectors, a SIN lentiviral vector ([ZRX-014-LV provided by ZetaRx Biosciences, Inc), Seattle] that encodes the CD19-4-1BB-CD3ζ-tEGFR CAR under transcriptional control of the elongation factor 1 alpha (EF1α) promoter was produced under GMP conditions, and the virus supernatant subjected to quality control analysis for clinical applications. Exposure of primary human T cells to CD19-4-1BB-CD3ζ-tEGFR SIN lentiviral vector results in efficient transduction and expression of the CAR and tEGFR, and confers recognition of $CD19^+$ target cells including primary CLL, and mantle cell and large cell lymphoma cell lines.

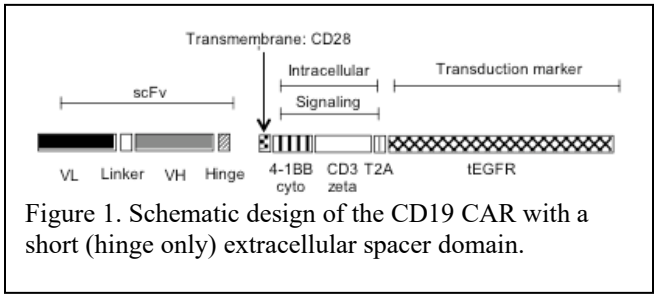


Figure 1. Schematic design of the CD19 CAR with a short (hinge only) extracellular spacer domain.

3L. Safety considerations for adoptive therapy with genetically modified T cells

There are several potential toxicities of adoptive therapy with CD19 CAR-T cells. The first potential toxicity is particular to targeting cells that express the CD19 molecule, as re-directed T cells will recognize and eliminate non-malignant CD19+ B-cells, potentially resulting in a long-term B-cell immunodeficiency [7, 8, 50, 51, 53]. Prolonged suppression of normal B cells in patients receiving rituximab therapy is common and does not appear to result in significant complications, providing immunoglobulin levels are maintained by IVIG therapy [61]. Thus, in patients with advanced B cell malignancies that have failed conventional chemotherapy and already have a B-cell deficiency from rituximab therapy, B-cell lymphopenia may be an acceptable side effect of T cell therapy if it is accompanied by a significant antitumor effect.

A second potential toxicity relates to the possibility of transformation of the adoptively transferred gene-modified T cells as a consequence of insertional mutagenesis. The development of T cell leukemia has been reported in a subset of patients on two gene therapy trials for X-linked severe combined immunodeficiency syndrome (SCID), in which bone marrow derived CD34 cells were transduced with a retroviral vector encoding the common cytokine receptor gamma chain [62-66]. Currently, five cases of T cell leukemia resulting from retroviral insertional mutagenesis have been described after successful correction of X-linked SCID. Four of the cases were associated with activation of the LMO2 oncogene [62-66]. Animal studies have shown that

mature T cells are resistant to transformation after retroviral integration [67], and leukemia has never been observed in clinical trials involving gene transfer into mature T cells, despite more than 10 years of follow-up in some studies. Our study differs from the X-linked SCID trial in several important aspects: *(a)* we will use a lentiviral vector and not a retrovirus to genetically modify cells, which reduces the risk of integrating into a transcriptionally active site; *(b)* the target of the genetic modification will be a mature T cell and not hematopoietic progenitor cells; and *(c)* the genetically modified CD19-specific T cells do not constitutively express a functional growth factor receptor as was the case with transduced cells in the X-linked SCID trial.

A third potential toxicity is that CD19 CAR-T cells might cause cytokine release after engagement of target cells or induce rapid tumor cell death resulting in a tumor lysis syndrome. Both cytokine release and tumor lysis syndromes have been observed in small clinical trials of CD19 CAR-T cell therapy in patients with high tumor burdens, but there has not been a clear correlation with T cell dose, with tumor burden or with the costimulatory domain in the CAR [7, 51]. We anticipate that toxicity from cytokine release may be more predictable with T cell products of a defined composition. In this trial we will use a dose escalation of CAR-T cells that consist of a 1:1 mix of $CD8^+$ T_{CM} and $CD4^+$ T cells in cohorts of patients to determine if a T cell dose can be defined that provides acceptable toxicity, long-term persistence, and reproducible depletion of $CD19^+$ B cells. To minimize the risk of tumor lysis syndrome, we plan to administer standard chemotherapy prior to the T cell infusion to reduce tumor burden and induce lymphopenia to improve T cell persistence.

3M. Overview of the Study

This is a Phase 1/2, open-label, nonrandomized study that will evaluate the safety and potential antitumor activity of adoptively transferring autologous CD19 CAR-T cells transduced with a lentiviral vector to express a CD19-specific CAR to patients with advanced $CD19^+$ B cell malignancies. To provide for greater reproducibility of T cell products and to facilitate safety and efficacy evaluation, we will enrich $CD8^+$ T_{CM} and $CD4^+$ T cells separately from the leukapheresis product or blood of each patient, transduce and expand each cell subset with the CD19 CAR lentivirus independently, and pool T cells in a 1:1 ratio to achieve the specified cell dose for each cohort of patients. The trial will consist of two stages – an initial dose escalation/de-escalation stage to define a cell dose that has acceptable toxicity, and then an analysis of the safety of that cell dose in expanded cohorts of up to 45 patients each with ALL and NHL and up to 15 patients with CLL.

3N. Modification to extend Stage 1 of the trial:

1. Analysis of disease specific cohorts for determination of optimal dose for ALL, NHL and CLL.

Experience from the initial 16 patients treated on the dose escalation and de-escalation Stage 1 of the trial revealed differences in toxicity based on disease histology and tumor burden. Patients with ALL and high tumor burden have had toxicity attributed to CD19 CAR-T cell therapy that has required dose de-escalation per protocol guidelines. Two patients with high tumor burden ALL (≥ 15% bone marrow involvement) had dose limiting toxicity (one transient CNS toxicity and one patient death from cytokine storm) at dose level 3. In contrast, none of the 5 (2 at dose level 1 and 3 at dose level 2) patients

treated with low tumor burden ALL (< 15% bone marrow involvement at pre treatment evaluation) had dose limiting toxicity. After discussions with the FDA we propose to extend the Stage 1 portion of the trial by evaluating ALL patients in separate high tumor burden or low tumor burden cohorts to determine the optimal and safe dosing for the stage 2 portion of the trial for patients with ALL.

In contrast to the experience in ALL, none of the 9 patients with NHL/CLL treated at any of the dose levels (2 at dose level 1, 4 at dose level 2, and 3 at dose level 3) experienced a dose limiting toxicity regardless of their tumor burden (marrow or nodal disease); therefore, we will not separate NHL/CLL patients into disease burden cohorts, but will evaluate NHL and CLL patients in separate disease-specific stage 1 cohorts.

Stage 1 will be extended to further examine dosing in disease specific cohorts outlined in **3N.1.a** and the results used to identify a cell dose for Stage 2 evaluation to determine safety and efficacy in larger disease/ patient specific cohorts, as described in **3N.2.a**. Each of the Stage 1 cohorts will follow the dose escalation and de-escalation rules based on dose limiting toxicities as defined in **14C.1.b**.

a. Stage 1 cohorts:

A) ALL-High tumor burden defined as ≥ 15% ALL in bone marrow at staging prior to treatment. Treatment will start at Dose level 1 and then escalate or de-escalate in a 3 x 3 design as described in Section **14C.1.b**. This cohort could consist of 3-18 patients.

B) ALL: Low tumor burden defined as < 15% ALL in bone marrow at staging prior to treatment. Treatment will start at Dose level 2 and then escalate or de-escalate in a 3 x 3 design as described in Section **14C.1.b**. This cohort could consist of up to 3-12 patients; however, we have seen no dose-limiting toxicity in ALL patients with low tumor burden and do not anticipate that enrollment on this cohort will be prolonged before proceeding to stage 2.

C) NHL: Patients will be initially treated at Dose level 2 and then escalate or de-escalate in a 3 x 3 design as described in Section **14C.1.b**. This cohort could consist of up to 3-12 patients; however, we have seen no dose-limiting toxicity in the 9 NHL patients previously treated, and do not anticipate that enrollment on this cohort will be prolonged before proceeding to stage 2.

D) CLL: We have limited experience with the treatment of patients with CLL. Based on the differences observed in ALL and NHL, we propose to formally examine dosing in a cohort of patients with CLL to determine the optimal dose for Stage 2. Treatment will start at Dose level 2 and then escalate or de-escalate in a 3 x 3 design as described in Section **14C.1.b**. This cohort could consist of up to 3-12 patients; however, we have seen no dose-limiting toxicity in patients with low grade NHL and with concurrent DLBCL/SLL and do not anticipate that enrollment on this cohort will be prolonged.

2. Modification of Stage 2 cohorts:

At the completion of the Stage 1 dose finding in disease specific cohorts, the identified dose/s will be used for treatment of patients in the specific Stage 2 cohorts. Stage 2 for a given cohort/disease may begin immediately on identification of the optimal dose from the Stage 1 cohorts.

a. Stage 2 cohorts:

A) ALL patients with low (< 20% marrow disease) or high (≥ 20% marrow disease or bulky extramedullar disease) are eligible, n=45.

B) Non Hodgkin Lymphoma. All histologies expressing CD19 are eligible including Diffuse Large B cell, Follicular, Marginal cell, Mantle cell or other histologies, n=45.

C) CLL or small lymphocytic lymphoma, n=15

3O. Modification to Refine Dose in NHL and CLL (following Cy/Flu lymphodepletion)

1. DL3 was selected for Stage 2 for patients with NHL based on no DLT in 3 patients treated in Stage 1. However, poor CAR-T cell persistence and expansion were observed in part due to the development of immune responses to the CAR modified T cells. The use of Cy/Flu for lymphodepletion improved CAR-T cell expansion and persistence and clinical response rate, but was unexpectedly associated with greater toxicity at DL3 in NHL patients. While protocol stopping rules (see section 14C2) were not met, there were 2 treatment related deaths occurring on day 13 and day 23 after treatment. In discussions with the DSMB and FDA we elected to dose de-escalate to DL1 in patients with NHL and to treat 3 patients with re-escalation to DL2 if no DLT observed. Either DL1 or DL2 will then be used for the remainder of the Stage 2 cohort. Until a dose level is cleared, these patients will be treated at no less than 2 week intervals. All patients will count toward safety and efficacy as before and there is no change to overall study numbers.

2. Five patients with CLL have been treated (4 DL2, 1 DL3) in Stage 1. However we observed a DLT at dose level 3 in a patient with Cy/Flu conditioning so we will also return to dose escalate patients with CLL from DL1 prior to starting Stage 2 for this cohort.

3P. Planned Dose Dense Cohort Expansion for NHL:
DL2 was found to be well tolerated and selected following dose refinement as discussed in section 3O with the use of Cy/Flu lymphodepletion and will be used for the completion of Stage 2 in NHL. We observed that patients with prolonged T cell persistence and expansion had better clinical responses and outcome suggesting that enhancing the area under the curve with an early second infusion may further improve outcome. We have experience with second CAR-T cell infusions following Cy/Flu lymphodepletion to date in 4 patients with residual NHL at the same or next higher dose level, as outlined in section **9B**. These infusions were given around or after day 28 with minimal toxicity

and no severe CRS or neurotoxicity suggesting that earlier treatment with a second infusion of CAR-T cells at the same or higher dose may boost levels and clinical activity with minimal increased toxicity. Three of these four patients had evidence of additional anti-tumor activity. Upon completion of Stage 2 for NHL we propose to evaluate an earlier sequential dosing strategy aimed to increase the peak levels and persistence of the CAR-T cells. Patients will receive a planned second infusion at DL2 ($2x10^6$ $EGFR^+$ cells/kg) of CAR-T cells between day 10-21 following the first infusion ***without*** additional lymphodepletion and after initial toxicity including CRS and neurotoxicity has abated. Up to 20 patients will be treated.

3Q. Dose Revision for Low- Tumor Burden ALL for the remainder of Stage 2:
Following treatment of 17 patients with low-tumor burden ALL (<20% bone marrow involvement) the protocol is being modified after discussions with the FDA to treat these patients at a lower initial CAR-T cell dose, Dose Level 1 (2×10^5/kg). This was in response to the death of a patient due to severe neurotoxicity and CRS. Following this modification, all subsequent ALL patients (all tumor burdens) will receive Dose Level one (2×10^5 $EGFRt^+$ cells/kg).

3R. Dose Revision for Low-Tumor Burden ALL for expanded Stage 2: After the administration of the lower dose described in section 3Q, 3 of 5 patients (60%) with ≤10% marrow blasts who received Cy/Flu lymphodepletion and DL1 (2×10^5 $EGFRt^+$ cells/kg) failed to achieve CR. In contrast 6 of 6 patients (100%) with ≤10% marrow blasts who received Cy/Flu lymphodepletion and DL2 (2×10^6 $EGFRt^+$ cells/kg) prior to the dose reduction in section 3Q achieved CR without excessive toxicity attributed to CAR-T cell infusion. We propose an increase in CAR-T cell dose for patients with **≤5%** marrow blasts, such that they receive CAR-T cells at DL2 (2×10^6 $EGFRt^+$ cells/kg).

3S. Cohort expansion for CLL patients with concurrent Ibrutinib: In Stage 2 for patients with CLL we observed a high rate of clearance of CLL cells from the blood and the bone marrow, but a slower clearance or failure to clear tumor in bulky LN (ASH CLL reference). This often resulted in less than complete remissions. In our study to date, the majority of patients have been previously treated with ibrutinib, but stopped the drug prior to T cell collection and were off of the drug during therapy. Discontinuation of ibrutinib is often associated with significant disease progression and the rapid reoccurrence of bulky lymphadenopathy, while treatment with ibrutinib often rapidly shrinks lymph nodes by mobilizing CLL cells from the LN and spleen into the blood[70]. In addition, recent data suggest that the quality of T cells collected from patients who are on ibrutinib may be better for the manufacturing of CAR-T cells and for the function of the CAR-T cells when used to treat CLL patients[71]. Further, ibrutinib may have additional activity by reducing the severity of cytokine release syndrome (either by direct action on the T cells or by inhibition of the flare or inflammation that can occur upon discontinuation of ibrutinib). Lastly, multiple ongoing clinical trials support the ability to administer ibrutinib safely concurrently with chemotherapy including fludarabine, cyclophosphamide and rituximab[72]. All of these observations support the continuation of ibrutinib for CLL patients considering treatment with CD19 specific CAR-T cells rather than stopping it prior to CAR-T cell therapy.

We propose a pilot study of 20 CLL patients total who will be treated with concurrent ibrutinib throughout T cell collection and then for up to 3 months following CAR-T cell infusion. This group will consist of two cohorts of patients- cohort 1 (n=5-15): those who are continuing on ibrutinib, but with significant residual disease, and cohort 2 (n=5-15): those who have previously failed ibrutinib in the past and who are now on subsequent treatments. Both cohorts will be treated with ibrutinib (continue or restart) for at least 2 weeks prior to leukapheresis and will continue on ibrutinib for up to 3 months after the proceeding CAR-T cell infusion. Ibrutinib dosing will be per the FDA label for patients with CLL. Patients who required dose reduction of ibrutinib for toxicity will continue or resume at the reduced dose of ibrutinib that was tolerated. We will continue with the current Stage 2 CAR-T cell dosing for CLL at DL2 (2×10^6 EFGRt+ cells/kg). We will evaluate toxicity and response rates and compare them to our earlier experience. This data will be used for the development of a multicenter CLL trial.

3T. Special Consideration for Patients Previously Leukapheresed but NOT Treated.
Over the conduct of this clinical trial, several patients with a variety of CD19 positive B cell malignancies were eligible for and underwent leukapheresis and had CAR- T cell products manufactured and cryopreserved, but were unable or not eligible to receive their CAR T cell treatment during the conduct of the Stage 1 or Stage 2 portions of the study. The major reason for this was that they were unexpectedly found to be in complete remission at the time of planned treatment and had CAR-T cell treatment deferred or were having a good response to other therapy. Ten of these patients are still alive and may become eligible for treatment upon relapse or disease progression. As part of our ongoing commitment to these patients who had cells collected for this study, we propose to include treatment of these patients as additional numbers to the Stage 2 cohorts of each disease if they become eligible for therapy during the remaining conduct of this trial (maximum of 12 months) from the treatment of the last patient.

4. OBJECTIVES

4A. Primary objectives

1. To evaluate the feasibility and safety of adoptive T cell therapy using *ex vivo* expanded autologous $CD8^+$ and $CD4^+$ CD19 CAR-T cells for patients with advanced $CD19^+$ B cell malignancies.

4B. Secondary objectives

1. To determine the duration of *in vivo* persistence of adoptively transferred T cells, and the phenotype of persisting T cells.

2. To determine if adoptively transferred T cells traffic to the bone marrow and function *in vivo*.

3. To determine if the adoptive transfer of CD19 CAR-T cells results in depletion of $CD19^+$ B cells *in vivo* as a surrogate for functional activity.

4. To determine if the adoptive transfer of CD19 CAR-T cells has antitumor activity in patients with measurable tumor burden prior to T cell transfer.

5. To determine if the adoptive transfer of CD19 CAR-T cells is associated with tumor lysis syndrome.

5. PATIENT SELECTION

5A. Inclusions for Screening and Leukapheresis

1. Patients with CD19 expressing ALL, CLL or NHL
2. Male or female subject, greater than or equal to 18 years of age.
3. Ability to understand and provide informed consent.
4. Not HIV infected.

5B. Inclusions for CAR-T cell Therapy

1. Patients with

a. CLL who are beyond first remission and who have failed combination chemoimmunotherapy with regimens containing a purine analogue and anti-CD20 antibody or who were not eligible for such therapy. Patients with CLL for whom ibrutinib is now standard first line therapy, must have progressed on ibrutinib. Patients with fludarabine refractory disease are eligible. Patients may be treated following allogeneic HCT.

For the concurrent ibrutinib cohort, patients must agree to continue on or be restarted on ibrutinib and must not have had prior intolerance to ibrutinib that would prevent this. Patients managed with prior dose reductions for toxicity will continue at the reduced dose for the remainder of this study.

b. Indolent NHL or Mantle cell NHL who are beyond first remission and previously treated with chemoimmunotherapy or who were not eligible for such therapy. Patients who have relapsed following autologous or allogeneic HCT are eligible. Aggressive NHL such as DLBCL, who have relapsed or have residual disease following treatment with curative intent. Patients should have relapsed following, or not be eligible for high-dose therapy and autologous HCT. Patients with chemotherapy refractory disease or marrow involvement or comorbidities precluding successful autologous HCT are eligible. Patients may be treated following allogeneic HCT.

c. Patients with CD19 expressing, relapsed or refractory ALL.

d. Patients with one of the above diagnoses whose disease state does not qualify but who have prognostic indicators that suggest a high risk of progression of disease may be screened and undergo leukapheresis. Enrollment for T cell therapy would require meeting the full disease state eligibility.

2. Confirmation of diagnosis

3. Evidence of CD19 expression by immunohistochemistry or flow cytometry on any prior or current tumor specimen or high likelihood of CD19 expression based on disease histology.

4. Karnofsky performance status ≥ 60% (**Appendix B**)

5. All patients of childbearing potential must be willing to use a contraceptive method before, during, and for at least two months after the T cell infusion.

6. Ability to understand and provide informed consent.

5C. Exclusions for CAR-T cell Therapy

1. Patients requiring ongoing daily corticosteroid therapy at a dose of >15 mg of prednisone per day (or equivalent). Pulsed corticosteroid use for disease control is acceptable.
2. Active autoimmune disease requiring immunosuppressive therapy is excluded unless discussed with the PI.
3. Major organ dysfunction defined as:
   a. Serum creatinine > 2.5 mg/dL
   b. Significant hepatic dysfunction (SGOT > 5x upper limit of normal; bilirubin > 3.0 mg/dL)
   c. Patients with clinically significant pulmonary dysfunction, as determined by medical history and physical exam should undergo pulmonary function testing. Those with an FEV1 of < 50 % of predicted or DL_{CO} (corrected) < 40% will be excluded.
   d. Significant cardiovascular abnormalities as defined by any one of the following: NYHA class III or IV congestive heart failure, clinically significant hypotension, uncontrolled symptomatic coronary artery disease, or a documented ejection fraction of <35%.
4. Uncontrolled active infection.

6. EVALUATION AND COUNSELING OF PATIENT

Patients will be seen at the Seattle Cancer Care Alliance or the Fred Hutchinson Cancer Research Center (FHCRC) for consideration of treatment options for their disease. The protocol will be discussed thoroughly with the patient and other family members if appropriate, and all known and potential risks to the patient will be described. The procedure and alternative forms of therapy will be presented as objectively as possible, and the risks and hazards of the procedure explained to the patient. Consent from the patient will be obtained using forms approved by the Institutional Review Board (IRB) of the FHCRC. A summary of the clinic visit detailing what was covered will be dictated for the medical record.

7. PROTOCOL REGISTRATION

Eligible subjects will be identified and registered into the system by the Clinical Coordinators Office (CCO) (Intake Office) and assigned a UPN (Unique Patient Number). The CCO will register the subject on to the protocol through the Data Management Office.

Patients are initially screened and undergo an apheresis procedure. Enrollment to the therapy portion of the study occurs at the conclusion of the pre T cell work up when data is reviewed for all inclusion and exclusion criteria by the Immunotherapy attending physician and the patient signs Consent A.

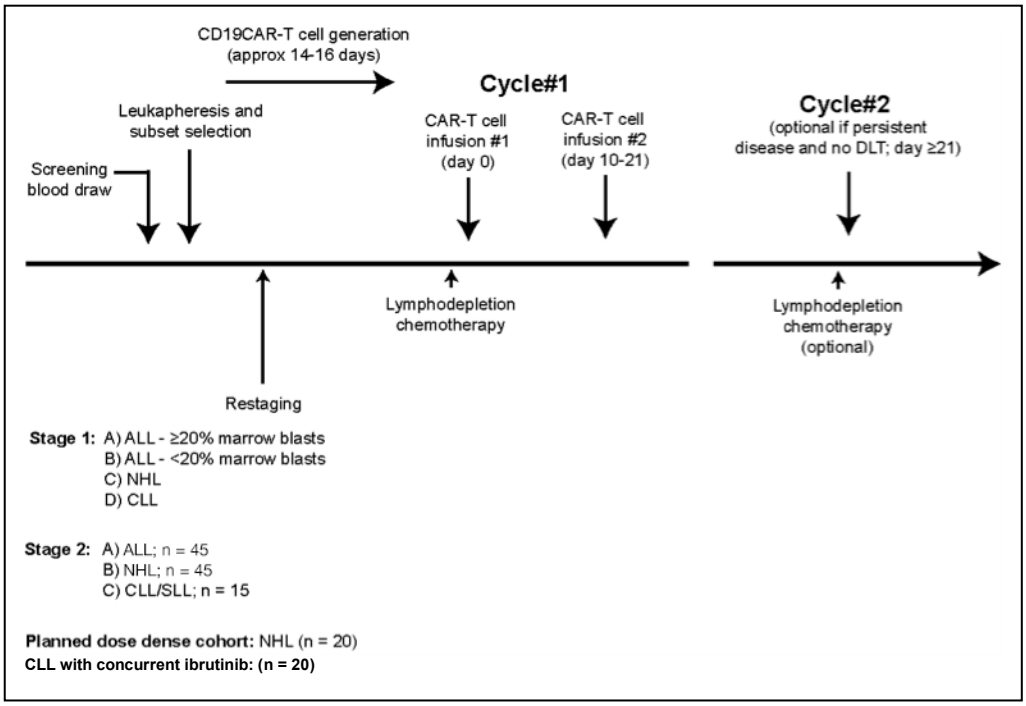
8. STUDY AGENT

8A. CD19 CAR-T cells

1. The methods employed to derive CD19 CAR-T cells from the patient's $CD8^+$ and $CD4^+$ T cells enriched from PBMC, and release tests of the cell products prior to infusion are outlined in the Chemistry, Manufacturing and Controls (CMC) section of the Investigational New Drug (IND) application to the FDA. Modifications to the CMC section during the course of the study must be submitted for FDA review.

2. The autologous T cell product will be prepared and administered to the patient by intravenous infusion. Patients will be pretreated with lymphodepleting chemotherapy and receive the T cell infusions **36-96 hours after completing chemotherapy (Section 9.B)**.

3. A schematic of the modified treatment plan including expanded stage 1 and 2 cohorts is shown in Figure 2.



9. PLAN OF TREATMENT

9A. CLL patients will be treated with ibrutinib (continue or restart) for at least 2 weeks prior to leukapheresis and will continue on ibrutinib for up to 3 months after CAR-T cell infusion. Ibrutinib dosing will be per the FDA label for patients with CLL, except as noted above where patients with prior dose reductions for toxicity, will continue or

resume the tolerated dose. Otherwise, patients will receive treatment at 420 mg daily (3 tablets), unless toxicity requires dose reductions or discontinuation of therapy as outlined in section 10.F

9.B. Leukapheresis or blood-draw to obtain T cells for CD19 CAR-T cell manufacturing

1. A leukapheresis will be performed on each patient by the SCCA Apheresis Unit using standard operating procedures for obtaining peripheral blood mononuclear cells. Should a technical issue arise during the procedure or in the processing of the product, or insufficient CD19 CAR-T cells be manufactured for the prescribed CD19 CAR-T cell dose, a second procedure may be performed.

   Patients ineligible for a vein to vein apheresis may elect to have a percutaneous central venous access catheter inserted to support this collection.

   Patients ineligible for leukapheresis and who have a hematocrit of >38 and a total non-malignant (normal) lymphocyte count of >2000 may undergo phlebotomy of 400 ml of blood to obtain PBMCs necessary to establish the T cell cultures.

2. The leukapheresis or phlebotomy product will be delivered to the Cell Processing Facility (CPF) at the FHCRC or the Cell Therapy Laboratory (CTL) at the SCCA. Cell selection may be performed in the FHCRC CPF or SCCA CTL. PBMC not required for cell selections may be archived for research.
   If lymphocyte subset counts are considered adequate, the product will be divided into two aliquots. One aliquot will be enriched for $CD8^+$ T_{CM} cells and the second aliquot will be enriched for $CD4^+$ T cells using clinical grade reagents and SOPs developed at the FHCRC/SCCA. Subsequent processing, after selection of $CD4^+$ and $CD8^+$ T_{CM} cells and cryopreservation (if required), is performed in the FHCRC CPF.
   If processing of $CD8^+$ T_{CM} and $CD4^+$ cells is not considered suitable due to lymphopenia, low $CD8^+$ T_{CM} and $CD4^+$ cell counts or other reasons, we may use other strategies to select and manufacture CD19 CAR-T cells that are approved for use in this protocol by the FDA.

3. Quality control and release testing will be performed on the CD19 CAR-T cell product prior to its release for patient infusion**.**

9C. Lymphodepleting Chemotherapy

1. Prior to the first infusion of CD19 CAR-T cells, the patients will receive lymphodepleting chemotherapy that is appropriate for their underlying disease as determined by the referring physician in consultation with the protocol Principal Investigator (PI). The objectives of administering chemotherapy are to reduce the tumor burden prior to infusion of CD19 CAR-T cells and to provide lymphodepletion to facilitate T cell survival.

2. Refer to **Appendix A** for suggested chemotherapy regimens based on disease cohort. For Stage 2, unless the clinical situation dictates changes, the preferred regimen will be with fludarabine and cyclophosphamide.

9D. Clinical and/or Laboratory Exclusion Criteria for T Cell Infusions

1. CD19 CAR-T cells should be administered between 36 and 96 hours after the completion of lymphodepleting chemotherapy providing the patient does not have one or more of the following exclusion criteria:
   - Pulmonary – Patients requiring mechanical ventilation
   - Cardiovascular – Patients requiring pressor support
   - Renal function – Patients with rapidly deteriorating renal failure; unless due to malignancy.
   - Hepatic function – Patients with an elevated total bilirubin of >3.9 or transaminases of > 5x the upper limit of normal values unless due to malignancy
   - Neurologic – Patients with encephalopathy or new focal neurologic deficits unless due to malignancy
   - Uncontrolled, active and serious infection.
   - Treatment with other investigational agent(s) within 30 days of T cell infusion.

2. The criteria listed in **Section 9C.1** must be met for each T cell infusion in patients who are eligible to receive more than one T cell infusion.

3. Patients with one or more exclusion criteria in **Section 9C.1** above may be eligible to receive a T cell infusion with or without chemotherapy at a later time if they subsequently resolve the clinical and/or laboratory abnormalities.

9E. Infusions of CD19 CAR-T cells

1. Stage 1: Identification of a cell dose for evaluation in expanded cohorts: In Stage 1, cohorts of three or more patients will receive a single intravenous infusion of CD19 CAR-T cells at one of three escalating dose levels beginning with dose level 1. Dose escalation or de-escalation is determined by the incidence of toxicity in each cohort, as described in **Section 14C.1.**

Dose level 0:	up to $2x10^4$ $EGFR^+$ cells/kg
Dose level 1:	up to $2x10^5$ $EGFR^+$ cells/kg (Starting dose level)
Dose level 2:	up to $2x10^6$ $EGFR^+$ cells/kg
Dose level 3:	up to $2x10^7$ $EGFR^+$ cells/kg

   The weight used for cell dose determination is the current weight in kg as documented on the clinical anthropometry report.

   Treatment of patients in the dose-escalation/de-escalation cohorts will be staggered with a minimum of a 14-day interval following infusion between each patient within each disease cohort.

2. Stage 2. Evaluation of the safety and efficacy of a single dose of CD19 CAR-T cells in an expanded cohort stratified by disease histology:
   After the toxicity data has been obtained from each dose escalation cohort in Stage 1, we will examine the laboratory data on T cell persistence, antitumor activity, and B cell depletion, and if appropriate, proceed with an expanded cohort of up to 45 patients

with ALL, 45 patients with NHL and 35 patients with CLL treated at the highest dose level established to be safe. See section 3O for dose re-escalation in Stage 2. The objective of Stage 2 is to derive data on safety of a single dose of CD19 CAR-T cells of a defined composition and an estimate of antitumor activity in patients with 1) ALL (both high and low tumor burden treated at the corresponding doses re-defined in stage 2, 2) NHL, and 3) CLL with and without concurrent ibrutinib therapy.

3. Cell administration. Individual aliquots of $CD4^+$ and $CD8^+$ CD19 CAR-T cells will be prepared for each T cell infusion according to protocols established in the GMP Cell Processing Facility and formulated to provide a single cell product at the specified cell dose. The specified T cell dose refers to CAR^+ T cells determined by the expression of the truncated EGFR transduction marker, which is expressed coordinately with the CAR in the vector. Each T cell infusion should be administered intravenously over approximately 20 – 30 minutes at the specified T cell dose.

4. Products that cannot be formulated to meet target cell dose specification. The intent for each infusion is to provide a cell product that contains approximately 50% (+/- 15%) of CD19 CAR-modified $CD4^+$ T cells and $CD8^+$ T cells (i.e. CAR-modified T cells in 1:1 $CD4^+/CD8^+$ ratio). If a T cell product cannot be formulated to meet this target cell dose specification because of low transduction efficiency, suboptimal growth of one of the subsets, or failure of either the $CD4^+$ or $CD8^+$ T cell product to meet release criteria, the cell product should be infused at or as close as possible to the specified phenotype allocation and total T cell dose. In the Stage 1 dose escalation component, such patients will not be considered evaluable for safety analysis in the assigned dose cohort and a replacement subject will be added to the cohort. In Stage 2, such patients will be included in analysis of each patient/tumor histologic cohort.

5. Retreatment of patients on the study. Patients enrolled in either stage of the study may be eligible to receive another CD19 CAR-T cell infusion with or without additional lymphodepleting chemotherapy at the same (for those that received the highest cell dose) or up to the next highest dose level if adequate CD19 CAR-T cells can be produced and the following criteria are met:
   i) There is evidence of persistent/relapsed disease at any time after the previous T cell infusion.
   ii) There were no toxicities attributed to the prior infusion/s that were dose-limiting or required dose de-escalation (**Section 14.C.1**).
   iii) The patient is ≥ 21 days from the previousT cell infusion.
   iv) There are no clinical and/or laboratory exclusion criteria (**Section 9C.1**).
6. Planned Dose Dense Cohort Expansion for NHL. Patients enrolled in this cohort will be accrued following the completion of stage 2 for NHL. An additional cohort of up to 20 patients will receive a planned second CD19 CAR-T cell infusion without additional lymphodepleting chemotherapy at the same dose level if adequate CD19 CAR-T cells can be produced and the following criteria are met:
   a. There were no toxicities attributed to the first infusion that were dose-limiting or required dose de-escalation (Section 14.C.1)
   b. The patient is 10-21 days from the first CAR-T cell infusion.

c. There are no clinical and/or laboratory exclusion criteria (Section 9C.1).
d. Afebrile for greater than 24 hours
e. Non-hematologic events thought to be probably or definitely RELATED to the first CAR-T cell infusion have resolved to less than or equal to grade 2 with the exception of related neurotoxicity which must be resolved to less than or equal to grade 1.

7. Patient monitoring during T cell infusions. All patients will be monitored during each T cell infusion with vital signs and O_2 saturation being monitored and recorded at the following approximate time points: before, 15 mins after starting, at the end of the infusion and approximately hourly for 2 hours after.

10. MANAGEMENT OF TOXICITIES AND COMPLICATIONS

Acute infusional toxicity may occur during or shortly after T cell infusion. In addition, cytokine release syndrome, tumor lysis syndrome, and neurologic toxicity have been reported specifically after CD19 CAR-T cells. Management of these complications is addressed in **10A-10E**. A table of proposed grading criteria for cytokine release syndrome is given in Appendix G.

10A. Management of acute toxicity associated with T cell infusion

The results of our prior studies of adoptive immunotherapy for CMV, HIV, lymphoma, and melanoma suggest that serious acute infusional toxicities resulting simply from infusing the numbers of T cells proposed in this study are unlikely to occur. However, transient constitutional symptoms have been observed with T cell infusions.

Examples of potential symptoms and signs due to T cell infusions and their initial management are listed below:

1. Fever, chills, and temperature elevations $\geq$38.3°C may be managed with acetaminophen 650 mg p.o. q 4-6 hrs. All subjects who develop fever or chills should have a blood culture drawn.
2. Headache may be managed with acetaminophen.
3. Nausea, vomiting may be managed with diphenhydramine 25-50 mg IV or other antiemetics (excluding corticosteroids).
4. Hypotension should be managed initially by fluid administration.
5. Hypoxemia should be managed initially with supplemental oxygen.

If the following signs appear during T cell infusion, the infusion should be paused and the patient assessed. If after, assessment by the PI/designee the patient's condition is stable then the infusion may be resumed.

Systolic BP < 80 mmHg AND > 30 mmHg fall from baseline
Heart rate > 140/min AND increase from baseline > 40/min (confirmed by palpation or EKG)
Respiratory rate > 35/min AND increase from baseline of > 10/min
Arterial O_2 saturation < 88% on air AND fall from baseline > 5%

If a T cell infusion is terminated due to acute toxicity, the residual T cells should be returned to the Turtle Lab for analysis. Investigation of possible causes of observed signs should proceed and, if necessary, additional medical treatment will be instituted.

Patients requiring discontinuation of T cell infusion may be eligible for re-treatment if the cause is deemed not related to the T cell infusion.

10B. Management of cytokine release syndrome

If patients become febrile or develop symptoms of cytokine release, we may measure cytokine levels, serum ferritin, C-reactive protein and markers of tumor lysis syndrome (e.g. chemistry, uric acid, LDH), and evaluate persistence and phenotype of the transgene-expressing cells, as clinically indicated.

Any patient who develops clinical evidence of symptoms related to cytokine release will have a work-up to exclude infection or other causes. Initial treatment should consist of supportive measures as dictated by the clinical and laboratory findings, and may include fluid replacement, medications to support blood pressure, antipyretics, oxygen supplementation, and broad-spectrum antibiotics if infection cannot be excluded as a potential etiology for the signs and symptoms. Patients with Grade $\geq$ 3 CRS (severe CRS; sCRS) and or Grade 2 CRS with progressive symptoms and signs should be treated with tocilizumab (4-8 mg/kg IV) and corticosteroids (dexamethasone 10 mg IV every 12 hours). Higher doses of steroids may be given after discussion with the Principal Investigator or designee and repeated doses of tocilizumab may be given if necessary. See Appendix G for cytokine release syndrome grading and Appendix H for recommended management guidelines.

10C. Management of tumor lysis syndrome

1. All patients will be considered at risk for tumor lysis and should receive allopurinol prophylaxis before chemotherapy begins, unless contraindicated. Allopurinol should be continued for as long as the medical team determines appropriate after the T cell infusion. They may receive additional hydration and urine alkalinization for the first 2 weeks after the T cell infusion.

2. If tumor lysis syndrome develops, as defined by the Cairo Bishop criteria [68], the Attending Physician will direct patient management with guidance from the study staff [69]. Conservative therapy, including allopurinol, urinary alkalinization, and IV fluid hydration may be instituted immediately for suspected tumor lysis syndrome. Hyperkalemia may be treated with potassium binding resins, diuresis, or insulin/dextrose therapy. Hyperphosphatemia may be treated with phosphate binding resins. In severe cases, rasburicase (in non-G6PD-deficient individuals) or renal dialysis may be necessary.

10D. Management of neurotoxicity

1. Neurotoxicity, manifest as delirium, seizures and/or focal neurologic deficits, has been reported after CD19 CAR-T cell therapy. Patients with high tumor burden ALL (>20% blasts) should receive prophylactic treatment with Keppra 500 mg bid (or similar regimen) starting at least 1 day prior to T cell infusion. Keppra can be discontinued when clinically appropriate. Neurotoxicity that is attributed to the T cell infusion/s should be treated with corticosteroids (e.g.

dexamethasone 10 mg IV q 4-12 hours). Tocilizumab may also be given, preferably after discussion with the Principal Investigator or designee. See Appendix H for recommended management guidelines.

10E. Management of other toxicities

1. If a new onset CTCAE v4.0 grade ≥ 3 toxicity is observed following any T cell infusion, the patients will receive investigation and medical treatment appropriate for the physiological abnormalities.

2. Grade ≥ 3 toxicity that is attributed to the T cell infusion/s, and is unresponsive to supportive measures or persists for > 7 days may be treated with corticosteroids (e.g. dexamethasone 10 mg IV q 4-12 hours) or tocilizumab after discussion with the Principal Investigator or designee.

3. Uncontrolled proliferation of CD19 CAR-T cells has not been observed in clinical trials to date. However, in the unlikely event uncontrolled proliferation of CD19 CAR-T cells occurred in a study subject, initial therapy may involve treatment with corticosteroids (e.g. methylprednisolone 1 g IV). Anti-lymphocyte globulin or cytotoxic drugs would also be considered in serious cases. If we observe an increase in CAR positive cells to greater than 10% of T cells at more than 3 months after last infusion we will analyze for clonal expansion by deep sequencing of the TCR beta gene (Adaptive Biotechnology).

10.F. Management of ibrutinib dosing

Patients with CLL who are enrolled in the expanded CLL cohort will receive oral ibrutinib at 420 mg daily (3 tablets). Ongoing dose reductions of 140mg may be considered as needed for patients who are intolerant of side effects. As ibrutinib can be associated with increased risk of bleeding, it should be held during severe thrombocytopenia (platelet count unable to be sustained > 20,000 by transfusion), and then restarted upon platelet recovery.

11. SCHEDULE OF PATIENT EVALUATIONS (Appendix C - Table of Evaluations)

Please note that results of tests and/or procedures conducted as per standard of care purposes may be used for research purposes if conducted within the protocol-defined window prior to screening/leukapheresis (11.A.) and/or T-Cell Therapy (11.B.).

11A. Evaluation for Screening/Leukapheresis

1. Informed consent and HIPPA signing

2. Laboratory evaluation
   a. CBC, differential and platelet count

   b. Hepatic function panel with LD and Renal function panel with Mg

   c. Virology Panel

   d. Pregnancy test for females of child-bearing potential within 14 days

e. Recipients of allogeneic HCT should have documentation of donor chimerism.

f. ABO blood typing and antibody screen

3. Research blood sample (30 ml, heparin/green top tubes)
This sample should be obtained at the time of initial evaluation for research and sent to the Turtle Lab at the FHCRC. If the PI/designee feel that the PBMC immunophenotype may have changed in the interval between initial screening and the time of leukapheresis or blood draw for PBMC collection then subsequent screening samples may be repeated as necessary to confirm feasibility and/or facilitate cell selection.

4. Medical history including:
   a. Hematologic, cytogenetic, flow cytometric, and histologic findings at diagnosis

   b. Prior therapies and response to therapy

5. Physical Exam and Karnofsky Performance Status

11B. <u>Evaluation for T cell therapy: Should be completed within 21 days of enrollment unless otherwise specified</u>

1. Signing of informed consent for T cell therapy

2. Confirmation of diagnosis at any time point prior to enrollment by internal pathology review of initial or subsequent biopsy or other pathologic material at the FHCRC/SCCA

3. Updated history and physical exam

4. Laboratory evaluation
   a. CBC, differential and platelet count

   b. Renal function panel with Mg and hepatic function panel with LD
   c. Uric acid.

   d. Serum ferritin

   e. PT, PTT, fibrinogen and D-dimer

   f. G6PD screening

   g. C-reactive protein

h. Pregnancy test for females of child-bearing potential. Chimerism testing, obtained at any time point post transplant, for any patient who has had an allogeneic transplant

j. Peripheral Blood B cell Immunophenotyping. If clinically indicated, 5 ml blood in sodium heparin should be sent to SCCA Hematopathology Laboratory for analysis of circulating normal and/or malignant B cells. This may be omitted if previously performed within 30 days of the planned T cell infusion AND the patient has not received anti-tumor therapy in the interim.

5. A bone marrow aspirate/biopsy should be performed with pathology, flow cytometry, karyotyping, FISH and other molecular studies as indicated by the disease. This may be omitted if done within 30 days of planned lymphodepletion AND the patient has not received anti-tumor therapy in the interim.

6. A CT scan (preferably diagnostic quality) and, if possible, a PET scan should be performed to evaluate disease status in patients with lymphoma and CLL. Patients with B-ALL should undergo CT +/- PET imaging if clinically indicated. Imaging studies may be omitted in patients who have had recent imaging within 30 days of planned lymphodepletion AND have not received anti-tumor therapy in the interim.

7. Lumbar puncture with CSF evaluation is required for any patient with a history of CNS disease or signs and symptoms of CNS or epidural disease. This may be omitted if a lumbar puncture performed within 30 days of planned lymphodepletion did not show evidence of CNS disease.

8. Baseline CXR within 21 days of enrollment

9. Baseline EKG within 21 days of enrollment

10. Quantitative immunoglobulins (IgG, IgA and IgM)

11. Research samples:
    a. A blood sample (30 ml, heparin/green top tubes) should be obtained for baseline T cell persistence and sent to the Turtle Lab at the FHCRC.

    b. A blood sample (up to10 ml, serum separator tube) should be obtained for measurement of serum inflammatory cytokine levels. Samples should be sent as soon as possible to the Turtle Lab at the FHCRC.

    c. If a bone marrow is being done clinically then an additional 5 - 10 ml of marrow aspirate should be obtained and sent in heparin/green top tubes to the Turtle Lab at the FHCRC.

    d. If biopsy or sampling of tissues other than bone marrow; ie cerebral spinal fluid (CSF), pleural fluid. etc. is performed for clinical indications then additional

sample may be obtained during the same procedure for research studies. Please discuss the planned procedure with the PI.

11C. Evaluation on the day of each T cell infusion

1. Toxicity
   a. CBC, differential and platelets prior to the T cell infusion

   b. Hepatic function with LD and Renal function panel with Mg prior to the T cell infusion

   c. Uric acid, C-reactive protein, and serum ferritin prior to the T cell infusion

   d. PT, PTT fibrinogen and D-drimer

   e. Vital signs at the approximate times: before starting , every 15 minutes during the T cell infusion, and hourly for 2 hours following the T cell infusion

   f. O_2 saturation should be monitored continuously by pulse oximetry during the T cell infusion. Values should be recorded at these approximate times: prior to initiating the infusion, every 15 minutes during the T cell infusion and hourly for 2 hours post infusion.

2. Serum cytokines
   Blood samples (approximately 10 ml, serum separator tube) should be obtained prior to the T cell infusion for measurement of serum inflammatory cytokine levels. Samples should be sent as soon as possible to the Turtle Lab at the FHCRC.

3. Evaluation of persistence and phenotype of CD19 CAR-T cells
   Blood samples (approximately 30 ml, heparin/green top tubes) should be obtained prior to the T cell infusion for baseline analysis of the presence of transferred T cells by q-PCR for vector sequences and/or for expression of the EGFR and CD19 CAR transgenes on $CD8^+$ and $CD4^+$ T cells by flow cytometry, if sufficient PBMC can be obtained.

11D. Patient Evaluations After Each T Cell Infusion

The following evaluations will be performed after each T cell infusion. If a patient receives a second T cell infusion, the day of the second infusion will be designated ‘day 0’ for evaluations thereafter.

1. Toxicity
   a. Record new findings on history and physical exam 1 day after the T cell infusion and at least weekly for four weeks.

   b. CBC, differential and platelet count at least twice weekly for two weeks then weekly until four weeks after each T cell infusion.

c. Hepatic function panel with LD and Renal function panel with Mg twice weekly for two weeks then weekly until four weeks after each T cell infusion.

d. Uric acid, C-reactive protein, and serum ferritin twice weekly for two weeks then weekly until four weeks after each T cell infusion.

e. PT, PTT, fibrinogen and D-dimer twice weekly for two weeks then weekly until four weeks after each T cell infusion.

f. If patients become febrile or develop symptoms of cytokine release or tumor lysis between the indicated time points, we may measure serum ferritin, C-reactive protein and tumor lysis markers at additional times, as clinically indicated.

2. Serum Cytokines
   a. A blood sample (approximately 10 ml, serum separator tube) should be obtained on approximately days 1, 3, 7, 10, 14, 21, 28 after each T cell infusion for measurement of serum inflammatory cytokine levels. Samples should be sent to the Turtle Lab at the FHCRC.

   b. If patients become febrile, develop symptoms of cytokine release, or assessment of cytokines is clinically appropriate at times other than those indicated, we may measure cytokine levels at additional times.

3. Peripheral Blood B cell Immunophenotyping
   a. Blood, 5 ml in sodium heparin should be sent to SCCA Hematopathology Laboratory for flow cytometry immunophenotyping on approximately days 14, 28, 60, 90, 180, and 365 after the last T cell infusion unless the patient initiates other non-CAR T cell systemic therapy. If a deficiency of B cells persists at 1 year, we may continue to monitor B cell recovery every 6 months.

4. Immunoglobulin levels
   a. Patients receiving CAR T cells are at risk for chronic B cell depletion and may have IgG deficiencies. Recommendations will be made for monitoring of IgG levels and administering intravenous immunoglobulin (IVIG) as clinically indicated.

5. Evaluation of persistence and phenotype of CD19 CAR-T Cells
   a. Blood samples (approximately 30 ml, heparin/green top tubes) should be obtained on approximately days 1, 3, 7, 10, 14, 21, 28, 60, 90, 180, and 365 after the T cell infusion for analysis of the persistence of transferred T cells. Additional samples may be collected at other times than those indicated if required for evaluation of persistence of CAR-T cells. Persistence monitoring may be discontinued beyond day 28 in patients who do not have detectable transgene-expressing T cells on two consecutive occasions. Samples should be sent to the Turtle Lab at the FHCRC.

b. A subset of blood samples obtained after the infusion should also be analyzed by multiparameter flow cytometry for the phenotype of persisting $CD8^+$ and $CD4^+$ CD19 CAR-T cells. Markers that may be analyzed include CD62L, CCR7, CD28, and CD127.

c. If patients become febrile, develop possible signs of toxicity, or assessment of CAR-T cell persistence is clinically appropriate at times other than those indicated, we may measure the persistence of transferred T cells at additional times.

6. Evaluation of transgene immunogenicity

To evaluate for antibody or T cell-mediated transgene immune responses the following samples may be collected.

a. Blood (approximately 10ml, serum separator tube) around days 28, 60, 90, 180, and 365, after the final T cell infusion and serum extracted for evaluation of antibody-mediated immune responses. Samples should be sent to the Turtle Lab at the FHCRC.

b. Blood (approximately 20 ml, sodium heparin/green top tubes) around days 28, 60, 90, 180, and 365 after the final T cell infusion) and PBMC isolated for evaluation of cellular immune responses. Samples should be sent to the Turtle Lab at the FHCRC.

7. Optional archival samples for future studies of T cell function

a. Blood (approximately 60 ml, sodium heparin/green top tubes) may be obtained from patients once between days 0 and 21 after CD19 CAR-T cell infusion and once between days 21 and 90 for archival purposes.

b. Blood (approximately 20 ml, sodium heparin/green top tubes) may be obtained from patients around days 90, 180, and 365 after the final T cell infusion for archival purposes. Samples should be sent to the Turtle Lab at the FHCRC.

c. Serum (approximately 10 ml, serum separator tube) may be obtained from patients at around days 90, 180, and 365 after the final T cell infusion for archival purposes. Samples should be sent to the Turtle Lab at the FHCRC.

8. Evaluation of migration of adoptively transferred CD19 CAR-T cells

a. If aspirations and/or biopsies of bone marrow are performed for evaluation of tumor response or other clinical indications then additional aspirates (approximately 5 - 10 ml in heparin/green top tubes) may be obtained and sent to the Turtle Lab, FHCRC.

b. If biopsy or sampling of tissues other than bone marrow; ie CSF, pleural fluid. etc. is performed for clinical indications then additional tissue may be obtained during the same procedure for research studies. Please discuss the planned procedure with the PI.

9. Evaluation of tumor response
    a. Bone marrow aspiration and biopsy.
        i. Because the timing of disease response to CD19 CAR-T cells has been variable in reported studies a bone marrow aspirate and biopsy should be performed if clinically appropriate between 14 days and 3 months after each T cell infusion, and should be performed before the patient receives additional conventional anti-tumor therapy. Evaluation should include pathology analysis, flow cytometry, karyotyping, FISH studies and other molecular studies, according to disease-specific guidelines.
        ii. A bone marrow aspirate and biopsy may also be performed at approximately 6 and at approximately 12 months after the first T cell infusion, as clinically indicated.

    b. Imaging studies
        i. A CT scan (preferably diagnostic quality) and, if possible, a PET scan of the neck, chest, abdomen and pelvis should be obtained between 3 weeks and 3 months after each T cell infusion, and should be performed before the patient receives additional conventional anti-tumor therapy. Patients with B-ALL do not require CT/PET imaging, unless it is clinically indicated.
        ii. PET/CT scanning may also be performed at 6 and 12 months after the first T cell infusion, as clinically indicated.
        iii. CT/PET imaging will be read using Cheson 07 criteria for response assessment.

    c. Serum chemistry panel and LDH should be performed concurrently with restaging by biopsy or imaging, as clinically indicated.

    d. Peripheral blood flow cytometry should be performed concurrently with restaging by biopsy or imaging, as clinically indicated.

    e. Standard criteria (as detailed in **Appendix E**) will be used to define tumor response.

    f. Evaluation of tumor response may be discontinued in patients who proceed to other systemic non-CAR-T cell therapies.

10. Long Term Follow-Up (**Appendix F**)

    Enrolled patients who receive CD19 CAR-T cells will be asked to participate in long-term follow-up (LTFU) according to the guidelines set forth by the FDA's Biologic Response Modifiers Advisory Committee that apply to gene transfer studies. Current recommendations from the FDA suggest a minimum of 15 years of follow-up. In addition, unexpected late medical problems including information on hospitalizations and medications may be collected through the Oncology Clinic at SCCA. Recommendations will be made for an autopsy to be conducted if the research participant dies.

12. PROTOCOL ENROLLMENT

12A. *Projected Target Accrual*

In stage 2, 2 cohorts of 45 patients each with ALL and NHL and 1 cohort of 35 patients with CLL will be enrolled. For the planned multiple dose cohort, 20 additional patients will be treated. The total projected accrual will allow for treatment of 189 patients. The anticipated study duration is 5 years.

ETHNIC AND GENDER DISTRIBUTION CHART

TARGETED / PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex / Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	3	5	8
Not Hispanic or Latino	73	108	173
Ethnic Category Total of All Subjects*	76	113	189
Racial Categories			
American Indian / Alaska Native	3	1	4
Asian	4	5	9
Native Hawaiian or Other Pacific Islander	1	1	2
Black or African American	2	4	6
White	66	102	168
Racial Categories: Total of All Subjects*	76	113	189

13. RECORDS

The medical record containing information regarding treatment of the patient will be maintained as a confidential document, within the guidelines of the Fred Hutchinson Cancer Research Center, the University of Washington Medical Center, and the Seattle Cancer Care Alliance. The investigators will ensure that data collected conform to all established guidelines for coding collection, key entry and verification. Each patient is assigned a unique patient number to assure patient confidentiality. Patients will not be referred to by name or by any other personal identifier in any publication or external presentation. The Clinical Statistics Departments at FHCRC maintain a patient database to allow storage and retrieval of patient data collected from a wide variety of sources. The licensed medical records departments, affiliated with the institution where the patient receives medical care, maintains all original inpatient and outpatient chart documents. The primary research records are kept in access controlled office spaces or password protected computer based applications. Information gathered from this study regarding patient outcomes and adverse events may be made available to the Federal Drug Administration, NIH and Juno Therapeutics. All precautions to maintain confidentiality of medical records will be taken.

14. EVALUATION AND STATISTICAL CONSIDERATIONS

14A. Type of study

This is a phase 1/2 study to assess the safety and antitumor activity of adoptive T cell therapy with autologous $CD4^+$ T cells and $CD8^+$ T_{CM} cells transduced to express a CD19-specific CAR for patients with advanced $CD19^+$ B cell malignancies.

14B. Definition of endpoints

1. Safety assessment (relevant data being obtained)
    a. General Toxicity Assessment
        - History and physical exam before and at intervals after T cell infusions.
        - Pulse oximetry before and during the infusion
        - Chemistry battery before and at intervals after the T cell infusion
        - Toxicity grading according to NCI CTCAE Version 4.0
        - Serum cytokine levels
        - B cell reconstitution
        - Replication competent lentivirus
        - Adverse event reporting

2. Efficacy Assessment
    a. Evaluation of the duration of persistence of adoptively transferred CD19 CAR-T cells
    b. Evaluation of the migration of adoptively transferred CD19 CAR-T cells
    c. Evaluation of antitumor activity of adoptively transferred CD19 CAR-T cells by evaluating the objective response rate of complete remission and partial remission, and determining progression free survival, and overall survival.

14C. Endpoint Evaluation

1. Safety Assessment – Stage 1

In Stage 1 of the study, patients will be treated in cohorts of three or more patients at one of four dose levels of CD19 CAR-T cells starting at dose level 1 (**Section 9D.1**) to determine a single cell dose to evaluate in an expanded cohort of patients in Stage 2.

a. Dose limiting toxicities

1. Death within 8 weeks of the study cell infusion thought to be definitely or probably related to CAR T cell therapy.

2. Other dose limiting toxicities will be defined as follows:

i. Grade ≥ 3 non-hematologic toxicity in any major organ system that is probably or definitely attributed to T cell infusion AND is unresponsive (does not improve to < grade 3 toxicity) to treatment with dexamethasone 10 mg q 12 hours IV for ≥ 7 days (or an equivalent corticosteroid dose) or tocilizumab 8 mg/kg IV for ≥ 3 doses OR of greater than 28 days duration. Hematologic toxicity is an expected complication of chemotherapy, and other than B cell depletion, has not been observed in prior trials of CAR T cell therapy, and therefore will not be considered for altering T cell dose.

ii. CTCAE grades 3-5 allergic reactions related to the study cell infusion.

iii. CTCAE grades 2-5 autoimmune reactions, other than expected B cell depletion.

3. Patients receiving CD19 CAR-T cells may develop serious toxicity due to T cell activation, proliferation and cytokine secretion after encounter with tumor antigen. Cytokine release syndrome, macrophage activation syndrome and neurotoxicity may occur and require intensive care support, and will not be considered DLTs unless they meet criteria as outlined in 14.C.1.a.2.i.

b. Dose escalation or de-escalation

If toxicity defined above (**Section 14C.1.a.**) develops in 0 of 3 patients at any cohort-specified dose level, as described in **9D.1**, the next 3 patients may receive the next higher dose of T cells. If such toxicity develops in 2 of 3 patients at any dose level, the next 3 patients will be treated at one dose level lower. If such toxicity develops in 1 of 3 patients, up to an additional 3 patients will be treated at the same dose level. If such toxicity occurs in 1 of 6 patients at a given dose level, subsequent patients may be treated at the next higher dose.

If toxicity developed in 2 of 3 patients at the lowest dose level (dose level 0), the PI and DSMB would review the data to determine if evaluation of even lower doses of T cells was appropriate.

The highest dose of T cells that is estimated to result in toxicity in < 1/3 patients within each Stage 1 cohort will be the dose selected for Stage 2 evaluation where patients will be stratified into one of 3 cohorts based on the histology of their disease.

c. Dose de-escalation for the second CAR-T cell infusion for the "Planned Dose Dense Cohort Expansion for NHL"

Eligible patients will be treated with a second infusion at DL2. Up to twenty patients will be treated in this expanded cohort. If there ever exists sufficient evidence to suggest that the true toxicity rate after the second infusion exceeds 30%, where toxicity is as defined in Section 14C.1.a, consideration will be given to reducing the dose (DL1) and enrollment of patients will continue at this reduced dose. We would analyze the basis for the toxicity (cytokine production, in vivo cell proliferation, tumor lysis) to determine if treating additional patients at this lower T cell dose was appropriate.

Sufficient evidence for this rule will be defined as any observed outcome whose lower 80% confidence limit exceeds 30%. Operationally, any of the following ratios of toxicities to patients treated would trigger such a rule: 2/2, 3/3-5, 4/6-8, 5/9-10, 6/11-13, 7/14-16, 8/17-19, 9/20. If the true probability of toxicity is 0.20, then the probability of this achieving this trigger after 20 patients is approximately 0.12. If the true toxicity rate is 0.50, then the probability after 20 patients is approximately 0.94.

If all patients in this cohort are treated without this stopping rule being met, then we will consider the true toxicity rate associated with this treatment to be consistent with an acceptable level (i.e. ≤ 30%) in this cohort and we may proceed to a larger trial that would formally analyze the antitumor effect of CAR-modified T cells as well as continue to assess the safety of this approach.

Additionally, we will assess the AUC (between day 0-28) of T cell persistence in this cohort, as the goal of the 2nd infusion is to increase this relative to what we have seen before. The benchmark that we will use for assessment of this improvement is a mean of 715,000 copies/ug based on results observed in 11 patients treated with Flu/Cy and given DL2 CAR-T cells. All 20 patients treated will be included in this assessment, regardless of the dose of the 2nd infusion. If the true AUC is 0.58 standard-deviation units away from the benchmark of 715,000, then 20 patients will provide 80% power to detect a statistically significantly increased AUC (at the one-sided significance level of .05).

2. Safety Assessment – Stage 2

Stopping and Suspension Rules

1. If during the Stage 2 evaluation there ever exists sufficient evidence to suggest that the true toxicity rate exceeds 30% in any of the cohorts, where toxicity is as defined in **Section 14C.1.a**, enrollment of patients in that disease cohort will be suspended for safety reasons, pending a detailed review by the PI, study monitor and statistician. We would analyze the basis for the toxicity (cytokine production, in vivo cell proliferation, tumor lysis) to determine if treating additional patients in that cohort at a lower T cell dose was appropriate.

   Sufficient evidence for this stopping rule will be defined as any observed outcome whose lower 80% confidence limit exceeds 30%. Operationally, any of the following ratios of toxicities to patients treated would trigger such a rule: 2/2, 3/3-5, 4/6-8, 5/9-10, 6/11-13, 7/14-16, 8/17-19, 9/20-22, 10/23-25, 11/26-28, 12/29-31, 13/32-34, 14/35-37, 15/38-40, 16/41-43, 17/44-45.. If the true probability of toxicity is 0.20, then the probability of stopping after 25 patients is approximately 0.12. If the true toxicity rate is 0.50, then the probability of stopping after 25 patients is approximately 0.94.

   If all patients in a particular cohort are treated without this stopping rule being met, then we will consider the true toxicity rate associated with this treatment to be consistent with an acceptable level (i.e. ≤ 30%) in this cohort and we may proceed to a larger trial that would formally analyze the antitumor effect of CAR-modified T cells as well as continue to assess the safety of this approach.

2. Study pause for treatment related mortality: If in the combined Stage 1 and Stage 2 portions of the study there is evidence of CAR-T cell related mortality whose lower limit of one-sided 90% confidence limit exceeds 10% the study

will be paused and evaluated by the DSMB and with the FDA. Operationally, any of the following ratios of CAR-T cell related mortality to total patients treated would trigger such a rule: 18/130, 20/140, 21/150, 22/160, 23/170, 24/180, 25/190 and 27/200. This will be looked at after every 10th patient is treated.

3. Suspending enrollment for lack of efficacy: If in the Stage 2 component of the study, none of the first 10 patients in any disease cohort exhibit an antitumor response, the PI and DSMB would review the data and make a determination whether the cohort should be discontinued or if a modification of dose and or treatment regimen considered.

4. The occurrence of a malignancy attributed to lentiviral modified T cells or detection of replication competent lentivirus (RCL) in either stage would result in termination of the protocol.

3. Assessment of efficacy of transferred T cells

Data should be collected for persistence, migration and efficacy of transferred T cells and descriptive statistics will be used to summarize the changes from baseline where possible. For those patients with measurable disease at the time T cell therapy commences, responses will be evaluated using standard response criteria based on CT or PET imaging and histologic analysis of bone marrow or other tissue samples.

15. GUIDELINES FOR REPORTING ADVERSE EVENTS AND DATA SAFETY AND MONITORING PLAN.

15A. General issues and IRB reporting requirements

1. Definitions associated with reportable events and reporting requirements can be found on the FHCRCs Institutional Review Office (IRO) extranet website (Table 1).
2. The NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40) will be used for grading and analysis of adverse events. All grade 3 or greater adverse events will be collected during and for 48 hrs post leukapheresis and again from the day of lymphodepletion through day 28 after each T cell infusion.

The collection of adverse events will stop at the time of commencement of new systemic anti-tumor therapy.

To ensure that investigative treatment-related conditions are distinguished from disease-related conditions, attribution of causality will be established in grading adverse events. For each event, the Principal Investigator or designee, in conjunction with the physician or research nurse who examined and evaluated the research participant, will assign the attribution. Data managers who are removed from the clinical assessment of the research participant should not perform this. Attribution of adverse events attributed to the infused genetically modified T cells should be determined using the following criteria:

Definite - the adverse event is clearly related to the infused T cells
Probable - the adverse event is likely related to the infused T cells
Possible - the adverse event may be related to the infused T cells
Unlikely - the adverse event is doubtfully related to the infused T cells
Unrelated - the adverse event is clearly not related to the infused T cells

3. The FHCRC IRB will be notified of reportable events by the FHCRC Principal Investigator (PI) or study nurse according to current reporting obligations as found on the FHCRC Institutional Review Office extranet website.
4. The review and reporting of AEs will be in accordance with the Cellular Immunotherapy AE reporting for separate FH sponsor and Investigator SOP.
5. Reporting of unanticipated adverse effects to the FDA will be the responsibility of the sponsor.
6. The FHCRC PI and Research Nurse and study personnel should meet regularly (in person or via teleconference) to review all reported events.

Table 1: FHCRC IRB Policies for Reportable Events.
(Relevant FHCRC policies include, but are not limited to the following documents. Please also refer to the FHCRC IRO website.)

IRB Policy 2.6	Adverse Events and Other Unanticipated Problems Involving Risks to Subjects or Others	http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html
IRB Policy 1.9	Noncompliance with the Office of the Director's Human Research Protection Program Policy	http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html
IRB Policy 1.1	Reporting Obligations for Principal Investigators	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html
IRB Policy 2.2	Continuing Review	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html
IRB Policy 1.13	Investigational New Drugs (IND), Biologics and Investigational Device Exemptions (IDE)	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html

Table 2: FHCRC IRB Forms for Reporting

Adverse Event Reporting Form	http://extranet.fhcrc.org/EN/sections/iro/irb/forms/index.html
Unanticipated Problem Reporting Form	http://extranet.fhcrc.org/EN/sections/iro/irb/forms/index.html
Noncompliance Reporting Form	http://extranet.fhcrc.org/EN/sections/iro/irb/forms/index.html

15B. Data and Safety Monitoring Plan

1. Definition of Risk Level

This phase I/II trial involves genetic modification of somatic cells and requires an IND. At the FHCRC, this type of trial has independent monitoring twice each year through the Clinical Trials Support Office at the FHCRC, and has a Data and Safety Monitoring Board (**Section 16B.3**).

2. Monitoring and Personnel Responsible for Monitoring

a) The Principal Investigator (P.I.) is responsible for every aspect of the design, conduct and final analysis of the protocol. Regulations defining the responsibilities for assessment and reporting of adverse events (AE), serious AE and unexpected AE are defined by the Code of Federal Regulations: 21 CFR 312.32 and Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 published by the Cancer Therapy Evaluation Program (CTEP), a division of the NCI/NIH.

b) This clinical study will rely upon the monitoring of the trial by the P.I. in conjunction with a Study Physician(s), Physician Assistant(s) (PA) or Nurse Practitioner(s), Research Nurse(s), Research Coordinator(s), statistician, and an independent Study Monitor assigned by the FHCRC Clinical Research Support Office (CRS).

c) Continuous monitoring of the data and safety of this study should be performed by the Protocol Management Team (PMT), which consists of the Principal Investigators, Research Nurse, and study staff.

d) A Case Report Form (CRF) should be completed for every patient that was registered for participation in the study.

- Forms should be completed as information becomes available on a visit-by-visit or course-by-course basis.
- The Principal Investigator or a Co-Investigator will sign and date the indicated places of the CRF. This signature will indicate that thorough inspection of the data therein has been made, and will certify the contents of the form.

e) The PMT should meet at least monthly to review the clinical status of events and follow up information for each patient. The PMT will be responsible for implementation of the stopping rules for safety if necessary.

3. Data and Safety Monitoring Board

The study will be monitored by the Immunotherapy Integrated Research Center (IIRC) DSMB. The DSMB will be responsible for safeguarding the interests of trial participants and assessing the safety and efficacy of the interventions during the trial. This responsibility will be exercised by providing recommendations about stopping or continuing the trial. To contribute to enhancing the integrity of the trial, the DSMB may also formulate recommendations relating to the selection, recruitment and retention of participants and their management; adherence to protocol-specified regimens; and the procedures for data management and quality control.

The DSMB will be advisory to the study Sponsor and the PI, who will be responsible for prompt review of the DSMB recommendations to guide decisions regarding continuation or termination of the trial and whether amendments to the protocol or changes in study conduct are required.

The external DSMB is an independent, multidisciplinary group consisting of clinical experts and a statistician who collectively have experience in leukemia, lymphoma, hematology, biostatistics, and the conduct and monitoring of clinical trials. The DSMB will meet approximately every 6 months to review data. The current members are listed in the IIRC DSMB charter.

16. TERMINATION OF THE STUDY

The study may be terminated at any time by the Protocol PI, the FHCRC IRB, or the FDA.

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18. APPENDICES

APPENDIX A - Suggested Cytoreduction/Lymphodepletion Chemotherapy Regimens

The following is a general guide to accepted chemotherapy regimens to be used immediately prior to adoptive T cell therapy. The selection of appropriate chemotherapy regimen to provide tumor cytoreduction and lymphodepletion is based on the tumor histology and on the types of prior treatment that the patient has undergone and should be discussed with the study PI's. Chemotherapy regimens that employ prolonged administration of corticosteroids that would continue after the T cell infusion cannot be used for prior to T cell infusion. Patients should be scheduled for T cell infusions starting 36-96 hours after the last dose of chemotherapy. Patients should not receive Alemtuzumab or other T cell depleting antibodies in the 6 months prior to T cell therapy.

The preferred regimen for Stage 2 of the trial is the combination of Fludarabine and Cyclophosphamide for all diagnoses unless the patients' clinical situation dictates otherwise. Suggested regimens include Cyclophosphamide up to 60 mg/kg day 1, followed by fludarabine 25 mg/m^2 daily x 3 days.

Alternative regimens would include Cyclophosphamide 300 mg/m^2 day 1-3 and Fludarabine 30 mg/m^2 day 1-3.

Depending on the patients' clinical situation a number of additional regimes could be used after discussion with the study PI. Possible regimens may include the following as a general guide.

1) CLL
    a. High dose cyclophosphamide (2-4 grams/m^2) with MESNA with or without anti-CD20 antibodies such as rituximab or ofatumumab
    b. OFAR
    c. Bendamustine alone or with rituximab or ofatumumab
    d. Fludarabine with rituximab or ofatumumab +/- cyclophosphamide
2) Indolent NHL and Mantle Cell NHL
    a. One or two cycles of aggressive salvage regimen such as ICE, DHAP, ESHAP alone or in combination with an anti-CD20 antibody.
    b. High dose cyclophosphamide (2-4 grams/m^2) with mesna alone or in combination with an anti-CD20 antibody.
    c. High dose etoposide alone or in combination with an anti-CD20 antibody.
3) Aggressive NHL
    a. One or two cycles of aggressive salvage regimen such as ICE, DHAP, ESHAP alone or in combination with an anti-CD20 antibody.
    b. High dose cyclophosphamide (2-4 grams/m^2) with mesna alone or in combination with an anti-CD20 antibody.
    c. High dose etoposide alone or in combination with an anti-CD20 antibody.
4) ALL
    a. High dose cyclophosphamide (2-4 grams/m^2) with MESNA
    b. Any aggressive ALL regimen.

APPENDIX B - Karnofsky Performance Scale

The Karnofsky Performance Scale Index allows patients to be classified as to their functional impairment. This can be used to compare effectiveness of different therapies and to assess the prognosis in individual patients. The lower the Karnofsky score, the worse the survival for most serious illnesses.

KARNOFSKY PERFORMANCE STATUS SCALE DEFINITIONS RATING (%) CRITERIA

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	**70**	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

Appendix C. Patient evaluations over the course of the study

Approximate days post T cell infusion

Study Assessments/ Testing	Screen	Enrolled	0	During and following each T cell infusion	1	3	7	10	14	21	28
History and Physical[1]	X	X		Day 1 and weekly x 4	X		X		X	X	X
Karnofsky performance status	X										
Vitals including O2 sats			X	q 15 min during then hourly x 2							
Automated CBC with differential, platelet count	X	X	X	2 x per week in first two weeks then weekly x 2	X		X	X	X	X	X
Hepatic function with LD and renal function with Mg	X	X	X	2x per week in first two weeks then weekly x 2	X		X	X	X	X	X
uric acid, ferritin, LDH, C-reactive protein		X	X	2 x per week in first two weeks then weekly x 2	X		X	X	X	X	X
PT, PTT, fibrinogen and D-dimer		X	X	2 x per week in first two weeks then weekly x2	X		X	X	X	X	X
Pregnancy Test[14]	X	X[14]									
Virology Panel	X										
G6PD screening		X									
ABO blood typing + antibody screen	X										
Quantitative Immunoglobulins		X									
EKG		X									
CXR		X									
Lumbar Puncture with CSF evaluation[4]		X									
Bone marrow aspirate[5]		X									
CT/PET scan[6]		X									
PBMC collection	X										
Chimerism testing[15]	X										
Peripheral blood 30 ml to Turtle lab	X										
Cytokine levels[8,13]		X	X		X	X	X	X	X	X	X
Peripheral Blood B cell Immunophenotyping		X							X		X
T cell persistence[9, 13]		X	X		X	X	X	X	X	X	X

Transgene immunogenicity_serum											X
Transgene immunogenicity_PBMC											X
PBMC archive[11]				0-21 and 21-90							

1. History to include hematologic, cytogenetic flow cytometric and histologic findings at diagnosis and time of enrollment as well as prior therapies and response to therapy.
2. If positive, complete antigen quantitation by PCR.
3. Should be performed concurrently with restaging by biopsy or imaging, as clinically indicated.
4. Required for any patient with a history of CNS disease or signs and symptoms of CNS or epidural disease, unless a negative lumbar puncture was performed within 30 days prior to scheduled T cell infusion.
5. Bone marrow aspirates/biopsies should be sent for pathology analysis as clinically indicated and according to the protocol. A 5-10 ml aliquot of the bone marrow aspirate in sodium heparin should be sent to the Turtle Lab for research.
6. Diagnostic CT/PET scan to include neck chest, abdomen and pelvis, as clinically indicated by disease and status and according to the protocol. Staging evaluations may be ceased if the patient proceeds to other anti-tumor therapy.
7. PB for flow cytometry in patients with possibility of circulating tumor cells in the blood.
8. In addition to these times, blood samples should be sent to the Turtle Lab if there is a suspicion of cytokine storm or macrophage activation syndrome. Discuss with PI or designee.
9. In addition to these times, blood samples should be sent to the Turtle Lab if there is a significant clinical event. Discuss with PI or designee.
10. PB B cell immunophenotyping studies may be omitted after day 28 in patients who proceed to other systemic therapies, with a recommendation to be made to the treating physician to follow IgG levels and replace if necessary.
11. Blood for archival purposes is optional and may be collected as follows: day 0-21 and 21-90, blood 60 mL
12. The volume of all research samples is approximate and may vary based on patient condition and clinical situation.
13. Samples may be collected if clinically indicated based on PI request.
14. Preganancy test only needs to be repeated if than 21 days from enrollment
15. Chimerism tesing only for post allogeneic HCT patients

T cell infusion time points represent guidelines for performance of required evaluations. Due to numerous factors influencing scheduling (pt and provider availability, testing services limitations etc), variation in evaluation performance dates is anticipated and acceptable to the protocol (e.g., within +/- 7 days of time points ≤ day 30; +/- 90 days for time points > day 30).

APPENDIX D: Research sample checklist

RECIPIENT RESEARCH EVALUATIONS BEFORE T CELL INFUSION

SAMPLE	TIME	TEST	TUBE	VOL (Approx)	LAB
Blood	Time of initial Screening	Research	Sodium heparin (green)	30 ml	Turtle, D3-313
Blood	At Enrollment	Baseline T cell persistence	Sodium heparin (green)	30 ml	Turtle, D3-313
Blood	At Enrollment	IFN-γ, TNF-α, MIP-1α, IL-2 levels	Serum separator	10 ml	Turtle, D3-313
Bone Marrow	At Enrollment	Migration	Sodium heparin (green)	5-10 ml	Turtle, D3-313
Blood	At Enrollment	B cell immunophenotyping	Sodium heparin (green)	5 ml	Hemepath SCCA
Blood	Day 0	IFN-γ, TNF-α, MIP-1α, IL-2 levels	Serum separator	10 ml	Turtle, D3-313
Blood	Day 0	T cell baseline persistence, function	Sodium heparin (green)	30 ml	Turtle, D3-313

RECIPIENT RESEARCH EVALUATIONS AFTER T CELL INFUSION.

SAMPLE	TIME AFTER FINAL INFUSION	TEST	TUBE	VOL. (Approx)	LAB
Blood	Day 1	IFN-γ, TNF-α, MIP-1α, IL-2 levels	Serum separator	10 ml	Turtle, D3-313
Blood	Day 1	T cell persistence	Sodium heparin (green)	30 ml	Turtle, D3-313
Blood	Day 3	IFN-γ, TNF-α, MIP-1α, IL-2 levels	Serum separator	10 ml	Turtle, D3-313
Blood	Day 3	T cell persistence	Sodium heparin (green)	30 ml	Turtle, D3-313
Blood	Day 7	IFN-γ, TNF-α, MIP-1α, IL-2 levels	Serum separator	10 ml	Turtle, D3-313
Blood	Day 7	T cell persistence	Sodium heparin (green)	30 ml	Turtle, D3-313
Blood	Day 10	IFN-γ, TNF-α, MIP-1α, IL-2 levels	Serum separator	10 ml	Turtle, D3-313

Blood	Day 10	T cell persistence	Sodium heparin (green)	30 ml	Turtle, D3-313
Blood	Day 14	IFN-γ, TNF-α, MIP-1α, IL-2 levels	Serum separator	10 ml	Turtle, D3-313
Blood	Day 14	T cell persistence	Sodium heparin (green)	30 ml	Turtle, D3-313
Blood	Day 14	B cell immunophenotyping	Sodium heparin (green)	5 ml	Hemepath SCCA
Blood	Day 21	IFN-γ, TNF-α, MIP-1α, IL-2 levels	Serum separator	10 ml	Turtle, D3-313
Blood	Day 21	T cell persistence	Sodium heparin (green)	30 ml	Turtle, D3-313
Blood	Day 0-21 (optional)	PBMC archive	Sodium heparin (green)	60 ml	Turtle, D3-313
Blood	Day 28	IFN-γ, TNF-α, MIP-1α, IL-2 levels	Serum separator	10 ml	Turtle, D3-313
Blood	Day 28	T cell persistence	Sodium heparin (green)	30 ml	Turtle, D3-313
Blood	Day 28	B cell immunophenotyping	Sodium heparin (green)	5 ml	Hemepath SCCA
Blood	Day 28	PBMC transgene immunogenicity	Sodium heparin (green)	20 ml	Turtle, D3-313
Blood	Day 28	Serum transgene immunogenicity	Serum separator	10 ml	Turtle, D3-313
Blood	Day 35-optional	IFN-γ, TNF-α, MIP-1α, IL-2 levels	Serum separator	10 ml	Turtle, D3-313
Blood	Day 35-optional	T cell persistence	Sodium heparin (green)	30 ml	Turtle, D3-313
Blood	Day 42-optional	IFN-γ, TNF-α, MIP-1α, IL-2 levels	Serum separator	10 ml	Turtle, D3-313
Blood	Day 42-optional	T cell persistence	Sodium heparin (green)	30 ml	Turtle, D3-313
Blood	Day 49-optional	IFN-γ, TNF-α, MIP-1α, IL-2 levels	Serum separator	10 ml	Turtle, D3-313
Blood	Day 49-optional	T cell persistence	Sodium heparin (green)	30 ml	Turtle, D3-313
Blood	Day 60	T cell persistence	Sodium heparin (green)	30 ml	Turtle , D3-313

Blood	Day 60	B cell immunophenotyping	Sodium heparin (green)	5 ml	Hemepath SCCA
Blood	Day 60	PBMC transgene immunogenicity	Sodium heparin (green)	20 ml	Turtle, D3-313
Blood	Day 60	Serum transgene immunogenicity	Serum separator	10 ml	Turtle, D3-313
Blood	Day 21-90 (optional)	PBMC archive	Sodium heparin (green)	60 ml	Turtle, D3-313
Blood	Day 90	T cell persistence	Sodium heparin (green)	30 ml	Turtle, D3-313
Blood	Day 90	B cell immunophenotyping	Sodium heparin (green)	5 ml	Hemepath SCCA
Blood	Day 90	PBMC transgene immunogenicity	Sodium heparin (green)	20 ml	Turtle, D3-313
Blood	Day 90	Serum transgene immunogenicity	Serum separator	10ml	Turtle D3-313
Blood	Day 90	PBMC archive	Sodium heparin (green)	20 ml	Turtle, D3-313
Blood	Day 90	Serum archive	Serum separator	10 ml	Turtle, D3-313
Blood	Day 180	T cell persistence	Sodium heparin (green)	30 ml	Turtle, D3-313
Blood	Day 180	B cell immunophenotyping	Sodium heparin (green)	5 ml	Hemepath SCCA
Blood	Day 180	PBMC transgene immunogenicity	Sodium heparin (green)	20 ml	Turtle, D3-313
Blood	Day 180	Serum transgene immunogenicity	Serum separator	10 ml	Turtle, D3-313
Blood	Day 180	PBMC archive	Sodium heparin (green)	20 ml	Turtle, D3-313
Blood	Day 180	Serum archive	Serum separator	10 ml	Turtle, D3-313
Blood	Day 365	T cell persistence	Sodium heparin (green)	30 ml	Turtle, D3-313
Blood	Day 365	B cell immunophenotyping	Sodium heparin	5 ml	Hemepath SCCA

			(green)		
Blood	Day 365	PBMC transgene immunogenicity	Sodium heparin (green)	20 ml	Turtle, D3-313
Blood	Day 365	Serum transgene immunogenicity	Serum separator	10 ml	Turtle, D3-313
Blood	Day 365	PBMC archive	Sodium heparin (green)	20 ml	Turtle, D3-313
Blood	Day 365	Serum archive	Serum separator	10 ml	Turtle, D3-313
Blood	Clinical events	T cell persistence	Sodium heparin (green)	Up to30 ml	Discuss with study staff
Blood	Clinical events	IFN-γ, TNF-α, MIP-1α, IL-2 levels	Serum separator	10 ml	Discuss with study staff
Bone marrow	Day 14-90	Migration	Sodium heparin (green)	5-10 ml	Turtle , D3-313
Bone Marrow	Day 180	Migration	Sodium heparin (green)	5-10 ml	Turtle , D3-313
Bone Marrow	Day 365	Migration	Sodium heparin (green)	5-10 ml	Turtle, D3-313
Bone marrow or other tissue biopsy	Any time as clinically indicated	Research	Discuss with study staff	Discuss with study staff	Discuss with study staff

APPENDIX E – Response criteria for CLL and NHL

Chronic lymphocytic leukemia (CLL)

Complete remission (CR): Normal imaging studies (X-ray, CT, MRI) (nodes, liver, and spleen), peripheral blood by flow cytometry has no clonal lymphocytes, bone marrow by flow cytometry has no clonal lymphocytes, bone marrow by morphology has no nodules (or if present, nodules are free from CLL cells by immunohistochemistry), and the duration is at least 2 months.

CR with minimal residual disease (CR-MRD): CR by above criteria except peripheral blood or bone marrow by flow cytometry with >0 - <1 CLL cells/1000 leukocytes (0.1%)

Partial remission (PR): Absolute lymphocyte count in peripheral blood at least 50% decreased and physical exam/Imaging studies (nodes, liver, and/or spleen) at least 50% decreased. Duration is at least 2 months.

Progressive disease (PD): at least one of: Physical exam/imaging studies (nodes, liver, and/or spleen) >50% increase or new, circulating lymphocytes by morphology and/or flow cytometry >50%increase, and lymph node biopsy with Richter's transformation

Stable disease (SD): Did not meet any of the above criteria for complete or partial remission or progression.

Non-Hodgkin Lymphoma (NHL)

Complete response (CR): Disappearance of all clinically detectable disease.

Partial response (PR): >50% reduction of the sum of the products of the perpendicular diameters of marker lesions, no progression of any existing lesions, and no new lesions.

Stable disease (SD): Stabilization of all existing lesions with no new lesions (i.e. a <25% increase or <50% decrease in disease parameters defined above throughout the treatment period).

Progressive disease (PD): >25% increase in the sum of the products of the perpendicular diameters of marker lesions, or the appearance of new lesions.

Acute lymphoblast leukemia (ALL)

Remission status will be determined by restaging of bone marrow and other involved sites by morphology, flow cytometry and molecular studies as appropriate.

APPENDIX F - Long Term Follow-Up

Study participants should be asked to participate in long term follow-up, as directed by the FDA Guidance for Industry – Gene Therapy Clinical Trials: Observing Subjects for Delayed Adverse Events. (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm072957.htm#5).

Long term follow-up should commence one year after the final T cell infusion. The planned recommendations for follow-up are as follows:

Years 1 - 15:

1. Recommendation that patients undergo at least annual history and physical examination with their primary physician:
    - Adverse event screening guidance for the primary physician in the form of a gene therapy LTFU-directed screening survey may be available.
    - A request for the study team to be notified of all new malignancies and unexpected illnesses.
    - The primary physician may be provided with a blood draw courier kit to enable samples to be returned to the Turtle Lab for archival purposes, and for analysis for transgene and vector persistence, and RCL, as dictated by studies of transferred T cell persistence.

2. Annually participants will complete the "***Immunotherapy Long Term Follow-up questionnaire***" to screen for adverse events.

3. Offer the opportunity to return to FHCRC for an annual LTFU clinic visit.

4. Compliance with 21 CFR 312.32 in adverse event reporting.

5. Research studies
    - 5.i. 30 ml of blood in sodium heparin for evaluation for transgene vector sequence by PCR of PBMC every 6 months for years 1 to 5 and every year for years 6-15 until the transgene becomes undetectable.
    - 5.ii. If > 1% of cells express the transgene or if clonality is suggested, vector integration sites or TCRB sequence utilization may be analyzed in PBMC, CAR-T cells or other tissue. If clonality is suggested, repeat testing may be performed 3 months later. Persistent monoclonality, clonal expansion or vector integration near a known oncogenic locus should precipitate careful attention to the possibility of malignancy. However, the need for additional intervention should be guided by the clinical circumstances and not solely by the presence of these factors.
    - 5.iii. 10 ml of blood in sodium heparin for annual testing of PBMC for RCL by VSVG QPCR. If there is no evidence of transgene persistence, RCL assays may be suspended after one year and samples may be archived.

APPENDIX G – Grading Criteria for CRS

Grade	Description of Symptoms
1: Mild	Not life-threatening, require only symptomatic treatment such as antipyretics and anti-emetics (e.g., fever, nausea, fatigue, headache, myalgia, malaise)
2: Moderate	Require and respond to moderate intervention: • Oxygen requirement < 40%, or • Hypotension responsive to fluids or low dose of a single vasopressor, or • Grade 2 organ toxicity (by CTCAE v4.03)
3: Severe	Require and respond to aggressive intervention: • Oxygen requirement ≥ 40%, or • Hypotension requiring high dose of a single vasopressor (e.g., norepinephrine ≥ 20 μg/min, dopamine ≥ 10 μg/kg/min, phenylephrine ≥ 200 μg/min, or epinephrine ≥ 10 μg/min), or • Hypotension requiring multiple vasopressors (e.g., vasopressin + one of the above agents, or combination vasopressors equivalent to ≥ 20 μg/min norepinephrine), or • Grade 3 organ toxicity or Grade 4 transaminitis (by CTCAE v4.03)
4: Life-threatening	Life-threatening: • Requirement for ventilator support, or • Grade 4 organ toxicity (excluding transaminitis)
5: Fatal	Death

Adapted from Lee et al., 2014 (Lee 2014)

APPENDIX H – Recommended management guidelines for CRS and neurotoxicity