Abbreviated Title: Phase I Anti-CD 19 CAR

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Title: Phase I Study of T Cells Expressing an Anti-CD19 Chimeric Receptor in Children and Young Adults with B Cell Malignancies

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Investigational Agents:

Drug Name:	Autologous peripheral blood lymphocytes (PBL) cultured with OKT3 and Interleukin-2; Transduced with retroviral vector (PG13-CD19-H3) expressing Anti-CD19 chimeric antigen receptor gene
IND Number:	14985
Sponsor:	Center for Cancer Research, NCI
Manufacturer:	Clinical Center Department of Transfusion Medicine (DTM), Cell Processing Section (CPS)

Commercial Agents: Fludarabine, Cyclophosphamide, Cytarabine, Etoposide, Ifosfamide, Filgrastim

PRÉCIS

Background:

- Chimeric antigen receptors (CAR) that recognize the CD19 antigen have been constructed and are in clinical trials at several institutions. In this trial, the POB will utilize a chimeric receptor containing the signaling domains of CD28 and CD3-zeta, currently under study in the CCR in adults, for children and young adults with CD19 expressing malignancies.
- In co-cultures with CD19-expressing acute lymphoblastic leukemia cells, anti-CD19-CAR-transduced T cells show robust killing, and in xenograft models, can rapidly clear CD19-expressing ALL cell lines.

Objectives:

1) To determine the safety and feasibility of administering escalating doses of anti-CD19-CAR engineered peripheral blood lymphocytes in two strata (prior allogeneic stem cell transplant [SCT] vs. no prior SCT) of children and young adults with B cell malignancies following a cyclophosphamide/fludarabine preparative regimen. **COMPLETED March 2014**.
2) To determine the safety of administering cells in two groups of children and young adults with B-cell malignancies expressing CD19:
    - Arm 1 – Patients without high-burden disease or patients for whom chemotherapy toxicity is a concern will receive standard preparative regimen.
    - Arm 2 – Patients with high-burden disease who receive standard chemotherapy to reduce burden, (defined as patients with ALL who have M3 bone marrow blasts and/or presence of peripheral blood blasts on routine CBC, or patients with lymphoma).
3) To determine the feasibility of administering anti-CD19 CAR transduced T cells within 21 days of the target date in children and young adults with B-cell malignancies expressing CD19 enrolled on arm 2: Patients with high-burden disease who receive standard chemotherapy to reduce burden.

Eligibility:

Patients 1-30 years of age, at least 15 kg, with a CD19-expressing B-cell malignancy that has recurred after or not responded to one or more standard chemotherapy-containing regimens for their malignancy and is deemed incurable by standard therapy. Patients with a history of allogeneic SCT who meet all eligibility criteria are eligible to participate.

Design:

- PBMC will be obtained by leukapheresis. Anti-CD19 CAR T cells will be manufactured from fresh or frozen PBMCs. On Day -7, PBMC will be enriched for CD3+ cells and cultured in the presence of anti-CD3/-CD28 beads followed by retroviral vector

supernatant containing the anti-CD19 CAR. Total culture time is approximately 7-14 days.

- Patients will be divided into the 2 groups listed above.
    - Arm 1: Patients will begin preparative regimen comprising fludarabine 25 mg/m2 on Days –4, –3 and –2 and cyclophosphamide 900 mg/m2 on day –2.
    - Arm 2: Patients with high-disease burden will be treated with intensive standard of care chemotherapy to decrease disease burden during cell manufacturing.
- All patients: The CD19-CAR cells will be infused on Day 0, with up to a 72h delay allowed for fresh cells or a 21 day delay if cells are cryopreserved, if needed for resolution of clinical toxicities or to generate adequate cell numbers.
- The previously determined maximum tolerated dose (MTD) of 1 X 106 will be administered intravenously.
- Patients will be monitored for toxicity, response and T cell persistence.

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1. INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objectives

1.1.1.1 To determine the safety and feasibility of administering escalating doses of anti-CD19-CAR engineered peripheral blood lymphocytes in two strata (prior allogeneic stem cell transplant [SCT] vs. no prior SCT) of children and young adults with B cell malignancies following a cyclophosphamide/fludarabine lymphodepletion regimen. The following cell dose escalation scheme will be used: **COMPLETED**

- 1 x 10^6 transduced T cells/kg (± 20%)
- x 10^6 transduced T cells/kg (± 20%)
- x 10^7 transduced T cells/kg (± 20%)

1.1.1.2 To determine the safety of administering cells in two groups of children and young adults with B-cell malignancies expressing CD19:

- Arm 1 – Patients without high-burden disease or patients for whom chemotherapy toxicity is a concern will receive standard preparative regimen.
- Arm 2 – Patients with high-burden disease who receive standard chemotherapy to reduce burden (defined as patients with ALL who have M3 bone marrow blasts and/or presence of peripheral blood blasts on routine CBC, or patients with lymphoma).

1.1.1.3 To determine the feasibility of administering anti-CD19 CAR transduced T cells within 21 days of the target date in children and young adults with B-cell malignancies expressing CD19 enrolled on arm 2: Patients with high-burden disease who receive standard chemotherapy to reduce burden (defined as patients with ALL who have M3 bone marrow blasts and/or presence of peripheral blood blasts on routine CBC, or patients with lymphoma)

1.1.2 Secondary Objectives

1.1.2.1 Explore whether the administration of anti-CD19-CAR engineered peripheral blood lymphocytes can mediate antitumor effects in children with B cell malignancies who 1) do NOT have high-burden disease and receive standard preparative regimen, or 2) have high-burden disease and receive standard cytotoxic chemotherapy to reduce disease burden.

1.1.2.2 To evaluate the ability of CRS treatment algorithm to reduce the incidence of Grade 4 Cytokine Release Syndrome (CRS) to ≤ 10% of patients receiving anti-CD19 CAR engineered peripheral blood lymphocytes at the maximum tolerate dose (MTD).

1.1.2.3 Measure persistence of adoptively-transferred anti-CD19-CAR-transduced T cells in the blood and where possible the bone marrow and CSF of patients.

1.1.2.4 To describe the toxicity of administration of anti-CD19-CAR engineered peripheral blood lymphocytes in children and young adults with CNS disease.

1.1.2.5 To assess cognitive functioning (memory, attention, processing speed, and executive functioning [primary outcome = CogState One Card Learning score]) pre- and post-infusion to examine the cognitive effects of the anti-CD19 CAR infusion.

1.1.2.6 Define the cardiovascular toxicity associated with CD-19 CAR therapy infusion and associated cytokine release syndrome (CRS). Explore the relationship between cardiac function, inflammatory and cardiac biomarkers and cardiac imaging to evaluate cardiac function pre- and post-CAR to determine whether specific markers could provide an early signal for subjects at risk of developing cardiac dysfunction post-infusion.

1.1.3 Exploratory Objectives

1.1.3.1 Describe the safety, feasibility and clinical impact of administration of cryopreserved anti-CD19-CAR engineered peripheral blood lymphocytes in patients who received clinical benefit from the initial infusion. Explore the relationships between cognitive test scores and serum cytokine levels (e.g. IFN-γ, IL-2, IL-6, and TNFα).

1.1.3.2 Explore whether sequence-based genomic analysis can detect minimal residual disease (MRD) in patients who are MRD negative by flow cytometry.

1.2 BACKGROUND AND RATIONALE

1.2.1 Pediatric B-cell Malignancies

Between infancy and 15 years of age, cancer is the leading cause of death by disease in U.S. children[1]. Acute lymphoblastic leukemia (ALL) represents the most common pediatric malignancy, accounting for approximately 25% of childhood cancer. Although disease-free-survival rates are high for children with ALL, approximately 20% still die of their underlying disease, making ALL the leading cause of cancer death in childhood. For those who relapse, long-term prognosis is poor. Even with attainment of a second remission and despite allogeneic stem cell transplant, long-term disease-free survival rates of <50% are expected[2]. New treatment approaches are needed to overcome resistance to standard therapy.

1.2.2 Adoptive Cell Therapy using Chimeric Antigen Receptors (CARs)

Adoptive transfer of donor T cells following bone marrow transplantation has shown activity in recurrent leukemia[3,4] and adoptive therapy with tumor infiltrating lymphocytes (TIL) has shown activity in some cancers[5]. More recently, adoptive transfer of T cells transduced with the genes of tumor-antigen-specific T cell receptors caused regressions of melanoma and synovial cell sarcoma in some patients[6]. Another approach to adoptive T cell therapy is to engineer T cells to express chimeric antigen receptors (CARs). CARs are made up of a single chain variable fragment (scFv) coupled to signaling molecules that can activate T cells expressing the CAR[7-9]. The earliest preclinical work conducted with CARs was performed in the late 1980s[10,11] and more recently, CARs have been designed to target many different tumor antigens and many important questions have been addressed using CARs in mouse models. Murine models have shown that syngeneic T cells transduced with retroviruses encoding CARs protected mice from tumor challenges *in vivo*[12,13]. Murine models indicated that inclusion of CD28 signaling domains enhanced tumor cytotoxicity and persistence of CAR-transduced T cells[14,15]. Increasing the

number of CAR-transduced T cells administered to mice consistently enhanced tumor protection as well[15-17]. One murine study reported that administration of high-dose IL-2 enhanced tumor protection by CAR-transduced T cells[18].

In the last 5 years, several clinical trials of CAR based therapies have been initiated including CARs targeting carbonic anhydrase IX in kidney cancer[19], anti-GD2 CARs in neuroblastoma[20,21], anti-CD20-CAR T cells in B cell lymphoma, anti-Her2Neu CAR therapy[22], as well as anti-CD19 CAR therapy (discussed in greater detail below). From this early clinical experience, several general conclusions are emerging. First, chimeric antigen receptor expressing T cells can mediate antitumor activity as tumor shrinkage has been demonstrated in neuroblastoma[20] and lymphoma[23]. Second, the CAR engineered T cells can induce substantial toxicity primarily related to cytokine storm[24], as on-target but off-tumor toxicity resulting from antigen expression on normal tissues[19]. Thirdly, antitumor effects appear to correlate with cell persistence and lymphopenia inducing therapies, such as chemotherapy administered prior to CAR therapy, which also appears to increase the incidence and degree of CAR-cell persistence. Another factor potentially modulating persistence is anti-CAR immune responses as recently demonstrated in patients with lymphoma and renal cell carcinoma[19,25]. Finally, recent data demonstrates that 2nd generation chimeric antigen receptor T cells that incorporate a co-stimulatory domain, such as CD28, have superior persistence and expansion compared to 1st generation CAR T cells that have only one signaling motif (usually zeta subunit of the T-Cell Receptor) [26]. The historical development and composition of CARs are discussed at length in the following paper: Savoldo, B., et al., CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. J Clin Invest. 121(5): p. 1822-6[26]. Based upon these principles, we performed a dose-escalation trial of anti-CD19 CAR T cells (2nd generation) after a pre-CAR lymphodepleting regimen.

1.2.3 Development of an Anti-CD19 CAR for Clinical Adoptive T Cell Transfer

CD19 was chosen as the target because it is universally expressed on pediatric pre-B ALL cells[27,28], and the only normal cells that express CD19 are B cells and perhaps follicular dendritic cells[29]. Importantly, CD19 is not expressed on pluripotent hematopoietic stem cells[29]. While destruction of normal B cells is a drawback to targeting CD19, clinical experience suggests that destruction of normal B cells is a tolerable toxicity of leukemia directed therapy. When patients receive the anti-CD20 monoclonal antibody rituximab, the number of normal B cells is severely depressed for several months[30], yet patients that receive chemotherapy plus rituximab do not have an increased rate of infections when compared to patients that receive chemotherapy alone[31]. Similarly, patients with congenital absence of B cells have prolonged survival and acceptable quality of life as a result of intravenous infusions of IgG[18].

The anti-CD19 CAR used in this study consists of three main components: the variable regions of the anti-CD19 monoclonal antibody FMC63[32], the signaling domain of the CD28 costimulatory molecule, and the signaling domain of the CD3 zeta chain[33]. This receptor is referred to as FMC63-28. The DNA encoding this receptor was cloned into the MSGV1 retroviral vector backbone[34] to form the plasmid shown in Figure 1A. This plasmid is referred to as MSGV1-FMC63-28. For the remainder of this document the FMC63-28 receptor will be referred to as the anti-CD19 CAR.

Figure 1

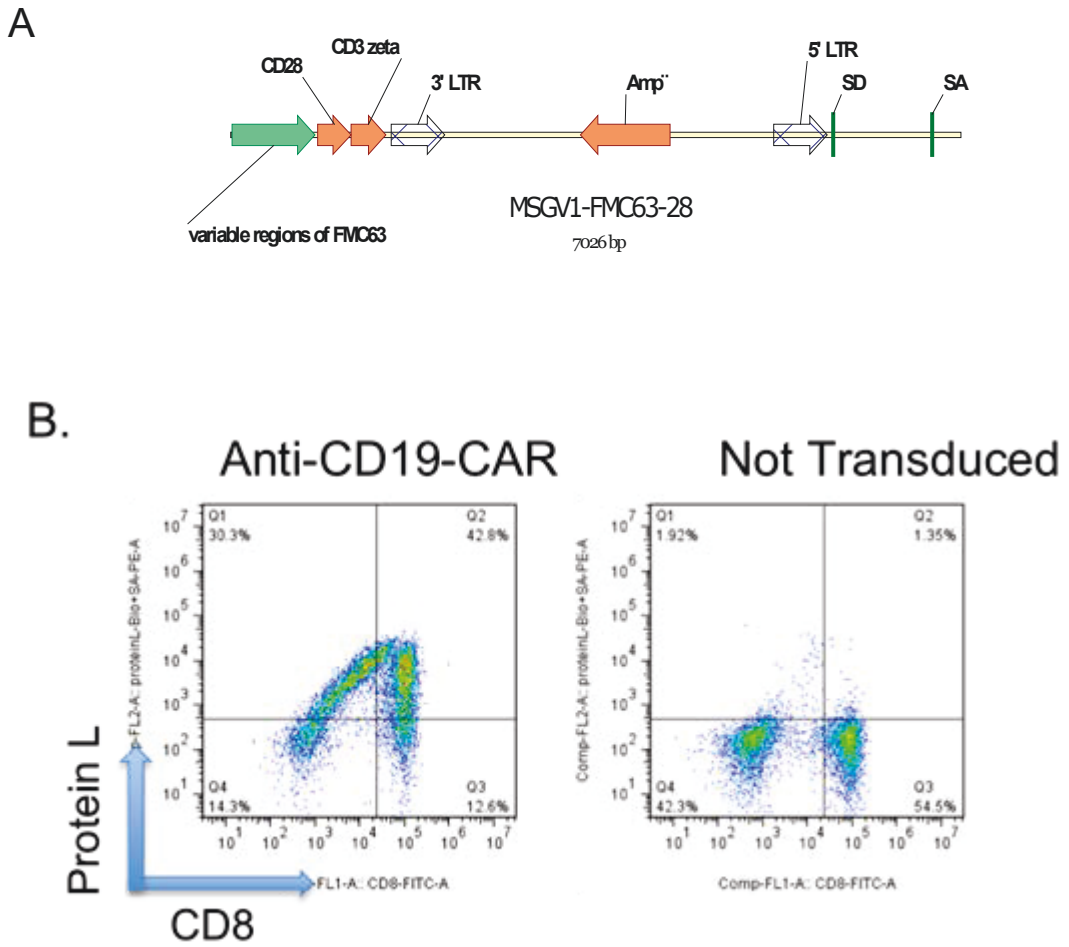

Figure 1. **Normal PBMC can be transduced with an anti-CD19 CAR.** A. Linearized plasmid map of the MSGV1-FMC63-28 gamma-retroviral vector that encodes the FMC63-28 anti-CD19 chimeric antigen receptor. B. Expression of the anti-CD19 chimeric receptor can be detected on the surface of transduced T cells by staining with biotinylated Protein L. Plots are gated on live CD3+ lymphocytes.

Preliminary experiments using transiently produced retroviral supernatant for transduction showed that the FMC63-28 receptor functioned well in vitro; therefore, a stable producer cell clone that produces gibbon ape leukemia virus (GALV) pseudotyped retrovirus encoding the FMC63-28 anti-CD19 CAR was generated. The supernatant from this clone was used for the experiments described in this document.

In addition to the extensive in vitro experiments already performed[34] we confirmed the suitability of the anti-CD19 CAR for use in a clinical trial targeting pediatric B-cell malignancies. Normal peripheral blood mononuclear cells (PBMC) were activated using artificial antigen presenting cells (aAPC) loaded with the anti-CD3 monoclonal antibody OKT3 and in the presence of IL-2 to activate T cells and induce their proliferation. After 48 hours of stimulation, the T cells were exposed to gamma-retrovirus encoding the anti-CD19 CAR that was spin-loaded onto a retronectin-coated plate, then cultured overnight with a second transduction performed on Day 2. Transduction efficiency was determined using biotinylated Protein L. Protein L is a recombinant

protein that interacts with the constant region of kappa chains in scFv (present in anti-CD19-CAR) or Fab fragments. Following transduction, the cultured cells were almost entirely $CD3^+$ T cells, with transduction efficiencies of 30-70% as measured by CAR expression. An example of staining of anti-CD19-transduced T cells with biotinylated Protein L is shown in Figure 1B.

CD19-CAR transduced T cells demonstrate cytotoxicity against CD19 expressing ALL cell lines *in vitro* but not CD19 negative controls with an average of 50-60% cytotoxicity at effector-to-target ratios as low as 2.5:1 (Figure 2A). Anti-CD19-CAR T cells also produce significant levels of interferon-γ (IFNγ), tumor necrosis factor-α (TNFα) and IL-2 specifically in response to CD19-expressing target cell lines (Figure 2B-D).

In a pilot experiment, immunodeficient NOG (NOD/Shi-*scid*/IL-2Rγ^{null}) mice were injected via tail vein with one million NALM6-GL cells (human ALL stably expressing GFP and luciferase) four days prior to injection of either anti-CD19-CAR T cells or stimulated but untransduced T cells (Mock). At the time of injection of T cells, mice already had bioluminescent evidence of leukemia (Figure 3A). Surprisingly, only forty hours after T cell injection the mice that received anti-CD19-CAR T cells nearly cleared their leukemia. By Day 7, all but one of the treated mice were free of leukemia while the mice that received Mock T cells continued to progress in their disease burden (Figure 3B) and required sacrifice by Day 17.

Figure 2

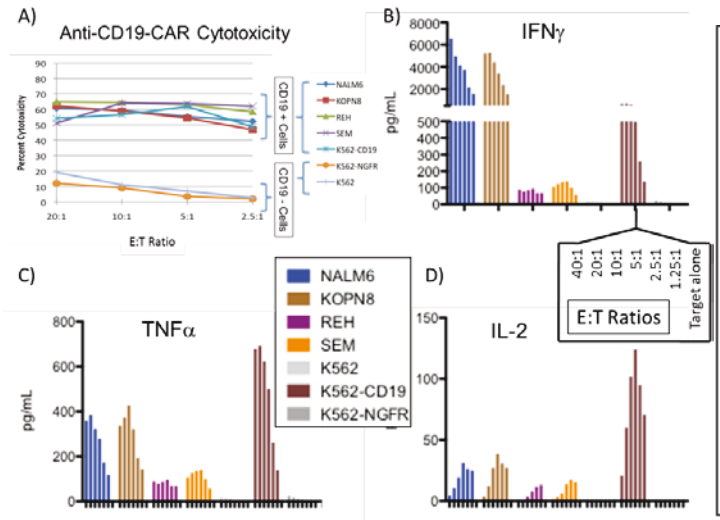

Figure 2. Anti-CD19-CAR T Cells effectively kill ALL. A) Anti-CD19-CAR T cells can kill CD19+ ALL targets but do not kill CD19 – cells. Effector-to-target ratios are plotted against percent cytotoxicity. Anti-CD19-CAR T cells produce significant amounts of INFγ (B), TNFα (C), and IL-2 (D) when encountering CD19+ ALL targets but not CD19 – targets. Each group of columns represent one target with decreasing E:T ratios. Positive control included K562 cells stably transfected with CD19 (K562-CD19), while negative controls included both K562 cells and K562 cells stably transfected with nerve growth factor receptor (K562-NGFR).

Figure 3

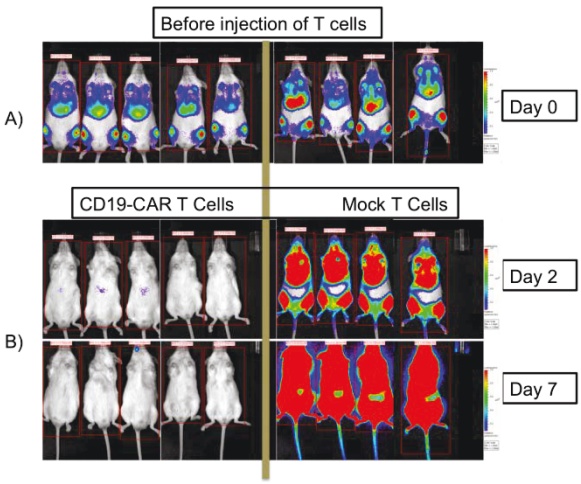

Figure 3. CD19-CAR T cells treat mice with ALL. NOG mice were injected with NALM6-GL on Day -4 and imaged on Day 0 (A). CD19-CAR or Mock T cells were injected on Day 0. By Day 2, nearly all evidence of leukemia had been cleared from the treated mice (B). On Day 7 only one mouse had evidence of ALL.

Figure 4

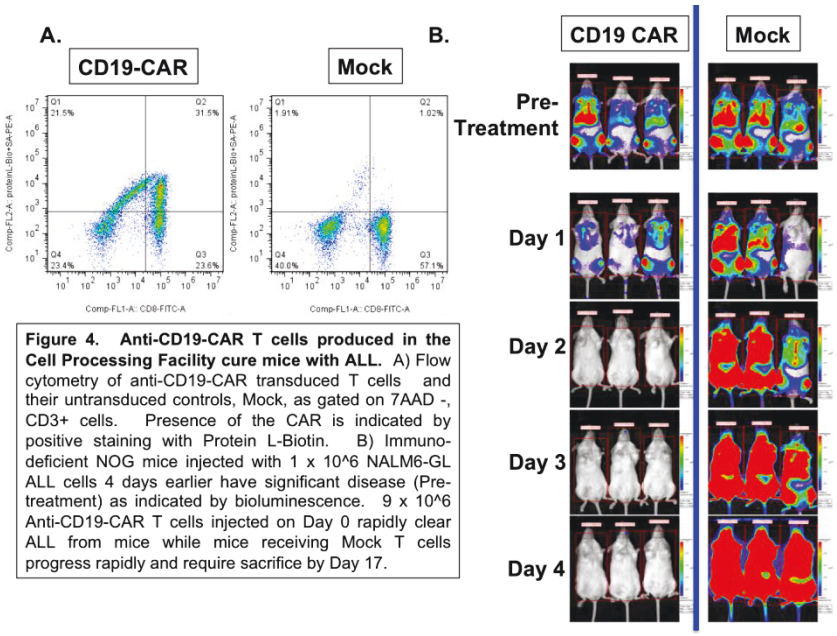

Figure 4. Anti-CD19-CAR T cells produced in the Cell Processing Facility cure mice with ALL. A) Flow cytometry of anti-CD19-CAR transduced T cells and their untransduced controls, Mock, as gated on 7AAD -, CD3+ cells. Presence of the CAR is indicated by positive staining with Protein L-Biotin. B) Immuno-deficient NOG mice injected with 1 x 10^6 NALM6-GL ALL cells 4 days earlier have significant disease (Pre-treatment) as indicated by bioluminescence. 9 x 10^6 Anti-CD19-CAR T cells injected on Day 0 rapidly clear ALL from mice while mice receiving Mock T cells progress rapidly and require sacrifice by Day 17.

We have replicated this data using anti-CD3/anti-CD28 beads in lieu of aAPC for T cell stimulation as this is the approach we will use to expand the anti-CD19-CAR cells in this trial. PBMC will be collected, CD3+ cells will be enriched using anti-CD3/anti-CD28 beads cultured in the presence of IL-2 for 48 hours, then transduced as previously described. Anti-CD19-CAR T cells produced in this manner have equivalent transduction efficiencies as indicated by interaction with Protein L (Figure 4A). Immunodeficient NOG mice were again injected with one million NALM6-GL cells four days prior to injection of anti-CD19-CAR T cells generated using beads. Control mice received equivalent numbers of untransduced T cells (Mock) that had been stimulated with beads. All mice treated with anti-CD19-CAR T cells cleared all bioluminescent evidence of ALL within 48 hours (Figure 4B). This indicates that anti-CD19-CAR T cells are a potent therapeutic agent against human ALL and strongly supports pursuit of such therapy in human clinical trials targeting pediatric ALL.

1.2.4 Other Anti-CD19 CAR Clinical Trials

Several other groups have constructed anti-CD19 CARs[35]. Jensen and coworkers completed a clinical trial of adoptive transfer of T cells transfected with a plasmid encoding an anti-CD19

chimeric receptor[36]. The T cells expressing the anti-CD19 receptor did not persist *in vivo* and did not cause objective remissions of lymphoma. Our proposed clinical trial differs from the completed trial of Jensen and coworkers in many ways. The most important difference is that our proposed trial will use a retroviral vector rather than plasmid transfection to transfer an anti-CD19 receptor to T cells, thus resulting in integration and persistent (rather than transient) CAR expression. Another potentially important difference is that the CAR we propose to utilize in our clinical trial contains the signaling component of CD28, whereas the receptor construct used by Jensen and coworkers did not include a CD28 moiety.

Researchers at MSKCC conducted a clinical trial of adoptive transfer of T cells that have been transduced with retroviruses encoding an anti-CD19 receptor (NCI Clinical Trials PDQ, trial MSKCC trial #09-114, Principal Investigator: R. Brentjens). This trial enrolled only adult ALL patients. In this trial, CAR-transduced T cells are transferred to patients without preparative chemotherapy prior to cell transfer. They have since modified their protocol to include a preparative regimen and are enrolling both children and adults with ALL (NCT01860937). Our trial differs from the trial of Brentjens and coworkers in several ways. First, we will treat a pediatric population with B-cell malignancies and will not exclude patients who have already undergone an allogeneic stem cell transplant (which comprises the majority of pediatric patients with refractory ALL). Secondly, we will include a lymphodepleting preparative regimen prior to cell infusion, which should allow for improved engraftment and function of infused T cells. Third, since we have demonstrated clearance of mild CNS leukemia in 2 patients with a single infusion of CAR T cells, we will allow enrollment of patients with any level of CNS leukemia and regardless of bone marrow relapse status. Finally, we will incorporate our recently modified CRS grading and treatment system[37] in an effort to reduce the incidence of Grade 4 CRS.

CD19 CAR Trials Currently Proposed or Underway				
Trial Location	Cell Dose/kg	Cytokine	Costim	Notes
NCI SB	~5 x 10^6	High Dose IL-2	CD28	High toxicity, cell dose and IL2 dose lowered
NCI SB	1 x 10^6 2.5 x 10^6	None	CD28	Reduced intensity preparative regimen
NCI ETIB	0.5-10 x 10^6	None	CD28	Relapse after Allo-SCT using donor as source for T cells; No preparative regimen
Baylor COM	1 x 10^6 5 x 10^6 10 x 10^6	None	50% CD28, 50% none	Children included
Baylor COM	0.3-2.4 x 10^6	None but may get ipilimumab	CD28	Relapse after Allo-SCT, children included
Baylor COM	3.75 x 10^6 11.2 x 10^6 33 x 10^6	None	CD28	Donor multi-virus CTL transduced post-SCT, children included
University College London	1.6 x 10^6	None	None	Donor EBV CTL post-SCT followed by vaccination w/EBV-LCL; children included
MSKCC	3 x 10^6	None	CD28	Donor EBV CTL post-SCT

	1×10^7 3×10^7			
MSKCC	1×10^6 3×10^6	None	CD28	MRD patients get higher dose of cells; dose is "T cells"; dealer's choice preparative regimen
MSKCC	5×10^6 1×10^7 2×10^7	None	CD28	Cells given after myeloablative allo-SCT; adults only
University of Pennsylvania	1.5×10^9 to 5×10^9 CD3+ cells (Flat dosing) in 4 split doses	None	4-1BB	dealer's choice preparative regimen
University of Pennsylvania	1-5 x 10^8 CD3+ cells	None	4-1BB	Phase II; Unclear preparative regimen; Adults only
Children's Hospital of Philadelphia	1×10^7 1×10^8 CD3+ cells (Flat dosing)	None	4-1BB	Phase I; Children included; dealer's choice preparative regimen
Seattle Children's Hospital	Unknown	None	4-1BB	Dealer's choice preparative regimen; Children included

Table 1. Clinical trials using anti-CD19-CAR strategies that are proposed or currently enrolling.

At Baylor College of Medicine, Dotti, Brenner, and coworkers are conducting three clinical trials involving anti-CD19 CAR. First, two types of anti-CD19-CAR-transduced T cells, one with both the CD3-zeta and CD28 signaling moieties and one with only CD3-zeta, are administered to adult patients with indolent B cell malignancies (NCI Clinical Trials PDQ, trial NCT00586391) in a 1:1 ratio at three dose levels. The aim of the trial is to evaluate the importance of the CD28 moiety on T cell persistence and function. Recent results from this and other trials indicate that CARs with only CD3-zeta do not have significant clinical activity or persistence *in vivo* beyond a few days[26]. Our proposed trial differs from this trial in that our anti-CD19-CAR will exclusively have both CD3-zeta and CD28. In addition, the use of anti-CD3/-CD28 beads to expand our CD19-CAR cells is not being performed at Baylor.

A second trial at Baylor involves EBV-specific T cells are first pulsed with dendritic cells and peptide for CMV and adenovirus to generate tri-viral specific T cells, which are then transduced with anti-CD19-CAR (NCI Clinical Trials PDQ, trial #BCM- H-23627). Tri-viral anti-CD19-CAR T cells are then administered to patients with pre-B ALL, CLL, or B-cell lymphomas undergoing allogeneic hematopoietic stem cell transplantation. Generation of cells using this method takes several months. Our proposed trial differs from this trial in that we will produce cells in a few days' time thereby limiting the possibility of enrolled patients dying before cells are prepared. Also, we will include patients who have previously received an allogeneic transplant but obtain cells for transduction directly from the patient rather than the original donor. Tolerized donor derived cells obtained directly from patients who have no GVH and are off immunosuppression have less chance of generating severe GVH upon reinfusion as compared to host-naïve T cells obtained directly from the donor. Finally, our trial will incorporate a lymphodepleting preparatory regimen whereas that of Bollard and coworkers does not.

The third trial at Baylor treats children after allogeneic SCT who have relapsed with leukemia using anti-CD19 CAR that contains the CD3-zeta and CD28 signaling moieties. Patients on this trial may also get the anti-CTLA4 monoclonal antibody, ipilimumab, in an effort to improve CAR T cell persistence and activity after infusion. Our protocol is open to all children regardless of bone marrow transplant status and will not use ipilimumab since we have seen dramatic complete responses in many patients without additional T cell support.

Gress and colleagues with ETIB, NCI are enrolling adults who have previously had an allogeneic stem cell transplant for a B-cell malignancy and are now recurrent or refractory (NCI Clinical Trials PDQ, trial #10-C-0054). In this trial, T cells are obtained from the patient's donor, activated with anti-CD3 (OKT3) in the presence of IL-2, and then transduced with anti-CD19-CAR. No preparative regimen is used. As is often seen in patients who receive donor lymphocyte infusions (DLI), these cells may induce significant GVH. The key difference between this and our study is that for patients who have had an allogeneic transplant we will obtain tolerized T cells directly from those patients who have no active GVH off immunosuppression. This may result in less risk for severe GVH. Further, to improve the function of CAR T cells we will administer a preparative regimen.

Carl June and coworkers at the University of Pennsylvania have opened a Phase I clinical trial of anti-CD19-CAR transduced T cells in adults with CD19+ B-cell malignancies (NCI Clinical Trials PDQ, trial #UPCC 04409). They are evaluating two different anti-CD19-CARs delivered using a lentiviral vector: one with both CD3-zeta and the costimulatory molecule 4-1BB (CART-19-zeta:41BB) and one with only CD3-zeta (CART-19-zeta). The first group of patients who received only CART-19-zeta:41BB have tolerated it well and some have had significant clinical responses[38,39]. The intended dose range was 1 x 10^9 to 1 x 10^{10} CD3+ T cells (not on a /kg basis), but they have had significant difficulty in growing T cells ex vivo as most of their patients had prior treatment with fludarabine for CLL. However, one patient who received only 2 x 10^5 CART-19-zeta:41BB cells/kg had a complete response. This provides support for potential clinical activity at the dose levels we have proposed in the present study. The use of CD28 rather than 4-1BB as costimulation for T cells, the use of a non-ablative preparative regimen prior to anti-CD19-CAR T cell infusion, and including pediatric patients are key differences between June's study and ours.

Interestingly, one patient experienced grade 3 tumor lysis syndrome with symptoms of chills, low grade fever and fatigue starting 14 days after the first cell infusion, progressing to rigors, diaphoresis, anorexia, nausea and diarrhea. Laboratory values were consistent with tumor lysis syndrome with hyperuricemia, elevated LDH and elevated creatinine levels. The patient also had transient grade 1 thrombocytopenia from day 19 through day 26 and grade 1 or 2 neutropenia from day 17 through day 33. Other signs and symptoms probably related to the study treatment included grade 1-2 elevations in alkaline phosphatase and transaminases, grade 2 hypogammaglobulinemia, and constitutional symptoms, including those noted above as well as myalgias and headache.

June has recently opened a Phase II clinical trial of CART-19-zeta;41BB but this is restricted to adults and the preparative regimen used, if any, is unclear. His group has not published pediatric phase I results.

In collaboration with Dr. June, Stephen Grupp and colleagues are enrolling children with ALL on a Phase I trial of CART-10-zeta;41BB. Two case reports demonstrated complete responses and significant cytokine storm[40]. No other results have been published but dosing is based on total T cells rather than CAR T cell dose and patients receive dealer's choice preparative regimen.

Persis Amrolia and colleagues at the Great Ormond Street Hospital for Children in London have opened an anti-CD19-CAR trial for pediatric patients with pre-B ALL who are undergoing allogeneic stem cell transplantation from an EBV seropositive donor (NCI Clinical Trials PDQ, trial #UCL/09/0050). Enrolled patients will receive 2 x $10^8/m^2$ (approx. 1.6 x 10^6/kg) donor derived, EBV-specific T cells modified to express an anti-CD19-CAR that contains only CD3-zeta after transplantation. Patients will then receive 3 doses of irradiated donor-derived EBV-lymphoblastoid cells from the cell line used to generate the CTL as a vaccine. Our proposed protocol differs significantly from Amrolia and coworkers in that our anti-CD19-CAR contains both CD3-zeta and CD28, will not be used in conjunction with allogeneic stem cell transplantation, and will not be EBV-specific. The latter feature of our trial allows for relatively rapid production of the cells while not necessarily sacrificing long-term persistence of transduced T cells *in vivo*.

Rosenberg and colleagues are conducting a clinical trial of autologous CD19-CAR T cells in the NIH Clinical Center (#09-C-0082) that employs high dose and low dose IL-2 and is limited to adult patients with B-cell lymphoma and CLL[41]. While the CAR cells are identical to those used in this trial, our method for growing the CAR-transduced T cells (anti-CD3/CD28 beads), the absence of IL-2 therapy and the enrollment of children differs from this trial. Eight patients in the Rosenberg study have been treated, two of whom have been treated twice. Six of seven evaluable patients treated had responses of their advanced, progressive B-cell malignancies. Cells containing the anti-CD19 CAR gene were detected in all patients post adoptive therapy, while four of the eight had elevated serum inflammatory cytokine levels (IFN gamma and tumor necrosis factor alpha [TNFα]). Toxicities included grade 3 and 4 myelosuppression, elevated liver function tests, hypermagnesemia, hypocalcemia, hypophosphatemia, hypernatremia, hyperuricemia, hyperbilirubinemia, hypotension, fatigue, febrile neutropenia, sepsis, hypoxia, dyspnea, pleural effusion, acute renal failure, and capillary leak syndrome, Additional toxicities included: reversible grade 4 somnolence (2 patients) and grade 4 creatinine (1 patient), and reversible grade 2 left ventricular dysfunction and reversible grade 1 pulmonary hypotension (1 patient). Three patients on this study were intubated for 7, 9 and 10 days, respectively. One patient underwent dialysis. One patient died 18 days after CAR-transduced T cell infusion with culture-proven influenza A, nonbacterial thrombotic endocarditis, and cerebral infarction, which were deemed unrelated to the anti-CD19 CAR-transduced cells. Results[41] demonstrated that:

- Elevated IFN-gamma and TNF-alpha levels in a subset of patients occurred during the first 10 days after cell infusion;
- In 3 of the 4 patients with prominent elevations of serum inflammatory cytokines, the elevations occurred 4 or more days after the last dose of exogenous IL-2 administration, suggesting their toxicities were not attributable to the IL-2, but rather to serum inflammatory cytokine levels caused by the anti-CD19 CAR transduced cell infusions; and

- The severity of the toxicity experienced by patients in this trial did not correlate with the number of IL-2 doses administered, although it may have contributed to the severity, as demonstrated in a mouse model[42].

Rosenberg, et al[39] conclude that anti-CD19 CAR transduced T cells can eradicate CD19+ cells in humans and that the cytokine-associated toxicities that have occurred with these cells may not be related to exogenous IL-2 administration.

Again, our study will differ significantly from the Rosenberg trial by not including aldesleukin in the regimen and should therefore result in fewer or less severe side effects. However, in light of these recent findings we will closely monitor patients and attempt to correlate toxicities observed with serum cytokine levels, although this will not be available in real time.

An additional study has been initiated by Kochenderfer (#10-C-0054) using allogeneic T cells genetically modified with the same anti-CD19 CAR retroviral vector in patients with CLL. The first patient experienced dramatic regression. This patient had relapsed after HLA-matched unrelated donor hematopoietic stem cell transplantation, followed by 4 donor lymphocyte infusions (DLIs) and a second stem cell transplant from the original donor, without a response. He received an infusion of $6.2x10^7$ ($1x10^6$ cells/kg) allogeneic anti-CD19-CAR-transduced T cells derived from his unrelated transplant donor five months after the second transplant. He experienced fevers, fatigue, mild hypoxemia and intermittent mild hypotension, with increase in serum magnesium, phosphorous and uric acid, consistent with tumor lysis syndrome. In addition, he experienced a decrease in cardiac left ventricular function, which subsequently improved. The patient's blood B cell count decreased from 286 cells/mcL before the CAR-transduced T cell infusion to 0 cells/mcL 26 days after the cell infusion. CT scans revealed a greater than 50% decrease in the size of multiple lymph nodes after the CAR-transduced T cell infusion, but residual adenopathy was present. Kochenderfer is also treating adults who have relapsed after allogeneic SCT with CAR T cells that are generated from the transplant donor rather then the patient. No preparative regimen is given before cell infusion. Although responses have been small, no reported GVH has occurred to date.

To summarize our responses to date in the 20 patients enrolled, all 20 were infused with CAR T cells. Our feasibility rate was 89% as 2 patients did not meet the assigned dose level but were still infused with cells. One of these received 28,000 CAR-transduced T cells/kg (3% of prescribed dose) and had stable disease. Another received 480,000 CAR+ T cells/kg (16% of prescribed dose), and experienced an MRD-negative CR, then underwent HSCT and remains disease free 13·4 months after CD19-CAR therapy. In an intent-to-treat analysis of all enrolled patients, our therapy produced a 65% Complete Response (CR) rate. Eleven had MRD negative CRs and were therefore considered for allogeneic SCT, which is standard of care for such patients. All but 2 went on to SCT and remain leukemia-free (LFS 75% median f/u 14.5 months). The remaining 2 were determined by their principle oncologist to be medically ineligible for SCT and relapsed with CD19 negative leukemia. Therefore, we determined that our CAR T cell therapy serves as an effective bridge to transplant.

We determined the MTD to be 1 x 10^6 CAR transduced T cells/kg. Three grade 4 CRS have occurred on our protocol during both the dose escalation and dose expansion phases. Specifics are detailed in Section **1.2.5.4**, but briefly all adverse events in these and the remaining patients

were completely reversible. Four patients required use of the anti-IL6 receptor monoclonal antibody, tocilizumab, to blunt the effects of severe CRS. Our experience has led to publication of a consensus paper on the modified grading and treatment of CRS and is incorporated in this protocol to improve safety.

In summary, T cells transduced with gamma-retroviruses encoding the sequence of this CAR can specifically recognize and kill CD19 expressing acute lymphoblastic leukemia cells in vitro and *in vivo* in xenograft models. These anti-CD19-CAR-transduced T cells also produce the cytokines interferon-γ (INFγ), tumor necrosis factor α (TNFα) and IL-2 specifically in response to CD19. In this study, we propose to conduct a clinical trial in which gamma-retroviruses encoding the anti-CD19 CAR will be used to transduce T cells in the Department of Transfusion Medicine Cell Processing Facility from patients with advanced pediatric B cell malignancies. The protocol will enroll patients at the MTD to gain more experience with using this therapy and to decrease the incidence of grade 4 CRS through the use of early intervention using well-defined criteria and by decreasing overall tumor burden in patients with more extensive disease involvement by intensifying the preparative regimen used prior to cell infusion. Subjects will receive a non-ablative, lymphocyte depleting preparatory regimen followed by infusion of anti-CD19-CAR transduced T cells.

1.2.5 Safety Considerations

Several safety concerns regarding the infusion of large numbers of retrovirally modified tumor reactive T-cells should be addressed.

1.2.5.1 Risk of chemotherapy

Toxicities resulting from fludarabine and cyclophosphamide in the doses proposed in the current study are well known. Such a preparative regimen is designed to decrease the number of endogenous T cells, including T regulatory cells, which may otherwise suppress anti-CD19-CAR T cell cytotoxicity, and to induce the production of homeostatic cytokines thereby allowing for better engraftment of the transferred anti-CD19-CAR T cells. The dose limiting toxicity for both fludarabine and cyclophosphamide is myelosuppression, however myelosuppressive effects are expected to be transient using the doses proposed. Other toxicities including fever, nausea, vomiting, stomatitis, diarrhea, anorexia, edema, skin rashes, myalgias, headache, agitation, and fatigue should be easily managed with appropriate supportive care. Sterile hemorrhagic cystitis occurs in about 20% of patients who receive cyclophosphamide. Severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Mesna will be used prophylactically as a uroprotective agent, but is not effective in preventing hemorrhagic cystitis in all patients. Furthermore, hemorrhagic cystitis is unlikely given the relatively low dose of cyclophosphamide administered in this trial. Tumor lysis syndrome (TLS) following fludarabine and cyclophosphamide administration has been observed, especially in patients with advanced bulky disease. Before beginning chemotherapy, patients will be prescribed allopurinol and appropriately hydrated with close monitoring for the development of TLS. Finally, opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed post-fludarabine and cyclophosphamide, especially in heavily pre-treated individuals. Patients will receive appropriate antimicrobial prophylaxis (eg, Bactrim for PCP prophylaxis) during treatment.

Patients with higher disease burdens (Arm 2) will receive a standard salvage regimen in lieu of the fludarabine/cyclophosphamide lymphodepleting regimen. The regimen will consist of

fludarabine, cytarabine, and filgrastim (FLAG) and has been used routinely in the POB. Risks of fludarabine are described above.

1.2.5.2 Risk of Autoimmunity

As discussed above, expansion of tumor reactive cells is a desirable outcome following the infusion of antigen reactive T-cells. Some patients receiving gp100 or MART-1 reactive cells have developed vitiligo, uveitis, hearing loss and rash probably due to destruction of normal melanocytes, though these toxicities have been manageable. In addition, two patients experienced vestibular dysfunction possibly due to the transduced cells. Some of these toxicities can be explained as on-target, off-tissue effects. In a similar fashion, it is likely that anti-CD19-CAR T cells will eliminate most circulating normal B cells since they also express CD19. It is important to note that pluripotent stem cells do not express CD19 and should not be affected by this therapy and that patients who receive the B cell depleting anti-CD20 antibody, rituximab, in conjunction with chemotherapy do not experience additional toxicities over chemotherapy alone. It is conceivable, but not likely, that as leukemic blasts are lysed host antigen presenting cells may present peptides to T cells specific for ALL or that cross-react with other normal host tissues. Termed epitope spread, this phenomenon is actually desired for better immunologic control of disease but may lead to autoimmunity. No confirmed cases of autoimmunity have been encountered on the study to date.

1.2.5.3 Risk of Gene Therapy

Risks of gene therapy include insertional mutagenesis or emergence of replication-competent retrovirus (RCR). While insertional mutagenesis is theoretically possible using retroviral vectors, this has only been observed in the setting of infants treated for X-SCID using retroviral vector-mediated gene transfer into CD34+ bone marrow stem cells. In the case of retroviral vector-mediated gene transfer into mature T-cells, there has been no evidence of long-term toxicities associated with these procedures since the first NCI sponsored gene transfer study in 1989. Despite the fact that clinical data currently available suggest that the introduction of retroviral vectors transduced into mature T-cells is a safe procedure, continued follow-up of all gene therapy patients will be performed as required. The proposed protocol follows all current FDA guidelines regarding testing and follow up of patients receiving gene transduced cells. Similarly, the viral vectors used have been engineered to minimize the risk of emergence of replication competent retrovirus, but patients will be monitored according to Recombinant DNA Advisory Committee guidelines for several years following receipt of this therapy and in the case of the development of second malignancy, all efforts will be made to determine whether replication competent retrovirus has emerged.

1.2.5.4 Risk of Cytokine Storm

Cytokine storm is a safety concern with the use of CAR transduced adoptive cell therapy. The cytokine release syndrome (CRS) is defined clinically by nausea, headache, tachycardia, hypotension, rash and shortness of breath (CTCAE 4.0 definition) but may also result in other organ and neurologic dysfunction. Believed to be caused by the release of cytokines from cells following intense immune activation, IL-6 appears to play a major role. A rapid rise in serum cytokine levels (cytokine storm) has been associated with Systemic Inflammatory Response Syndrome (SIRS) potentially leading to multi-organ dysfunction (MOD)[43]. This syndrome has

been described following various forms of immunotherapy, including antibody infusions (e.g. OKT3[44], Rituximab[45], Campath[46], anti-CD28[47]) but also following cellular immunotherapy.

Fatal cytokine storm occurred in one patient in the Surgery Branch, NCI with metastatic adenocarcinoma of the sigmoid colon, receiving anti-Her2-CAR gene engineered lymphocytes and IL-2 after non-myeloablative chemotherapy[24]. This patient received 10^{10} CAR transduced cells, and shortly after infusion experienced shortness of breath, hypotension and required admission to the ICU, vasopressor support and intubation for respiratory failure. She deteriorated despite supportive care and developed liver and kidney failure, expiring within 5 days of receiving the cells. The specific cause of death was determined to be gastrointestinal hemorrhage in the setting of systemic microangiopathic injury, but this was felt to be secondary to the cytokine release syndrome. Current concepts hold that this toxicity was likely primarily related to the dose of cells administered and IL-2 administration after infusion.

A second reported death occurred at Memorial Sloan Kettering Cancer Center in a phase I study in which anti-CD19 CAR T lymphocytes (autologous 19-28z+ T cells) were administered[48]. Three patients with B cell lymphomas received a single dose of 3 X 10^7/kg modified T cells without significant toxicity, with 2 of 3 patients experiencing transient reduction in peripheral lymphadenopathy. The fourth patient with chronic lymphocytic leukemia received cyclophosphamide (1500 mg/m^2) prior to cell infusion, and six hours later experienced fever, hypotension, dyspnea, and renal failure. Thirty hours later, the patient required intubation and expired soon after when withdrawal of support was requested. Immunohistochemistry (IHC) studies revealed significant numbers of 19-28z+ T cells in the bone marrow, spleen, liver and lymph nodes. Serum cytokine analyses in patient 4 were elevated compared to the first 3 patients enrolled. Intense analysis determined the outcome was in large part mediated by cyclophosphamide induced 'cytokine storm' in a setting of preexisting subclinical infection, leading to a sepsis-like scenario and which likely enhanced the modified T cell in vivo activation. The investigators feel that these findings underscore the potency of adoptively transferred genetically modified T cells.

Two additional deaths in adults were reported at Memorial Sloan Kettering Cancer Center in April 2014. Specifics have not yet been published in the medical literature but initial indications are that one had intractable seizures and the other developed cardiotoxicity both at least possibly related to CAR T cells. We have not seen any seizure activity in any of our patients, though this will be a risk especially in patients with CNS leukemia. We will therefore prophylactically start CNS leukemia patients on an anti-epileptic medication.

One patient on our trial had cardiac arrest driven by cytokine storm and was successfully resuscitated. This patient was hypotensive but stable on multiple vasopressors for several days before acutely decompensating. Immediate changes to this protocol were implemented at that time aimed at better monitoring and intervening earlier in severe CRS. The current version incorporates a new CRS grading system that tolerates low-dose vasopressor support but calls for anti-cytokine therapy(ies) such as tocilizumab or corticosteroids for patients requiring more aggressive intervention or grade 3 organ dysfunction.

We have found that all patients who had grade 3 or 4 CRS have had complete responses and that all of these (except 1 who had grade 3 CRS driven by dysphasia and not hypotension) had high

leukemia burdens. Therefore, some degree of CRS may be tolerated in the interest of achieving a CR.

Based on these reports, serum cytokine levels, including IFN-γ, IL-2, IL-6, GM-CSF and TNFα, will be assayed serially (0, 1, 12, 24, 48, 72, 96, 120 hours, then daily until day 14 and on day 28 and in the case of symptoms consistent with cytokine storm, levels will be monitored) following infusion of anti-CD19-CAR T cells. The results of the assays will not be available in real time but if toxicity is observed following infusion, review of the cytokine levels will be undertaken to assess risk to subsequent patients. Cytokine levels will also be evaluated retrospectively for correlation with anti-tumor effects. In addition, subjects will be monitored closely after infusion of anti-CD19 CAR T cells, and if discharged prior to day 14, be required to return to clinic daily for evaluation. After day 14, subjects will return to clinic at least twice a week for up to 28 days for evaluation. Investigators will maintain a low threshold for re-admission to the hospital and cytokine storm will be assessed and managed according to **Appendix F**.

In relation to the cytokine storm, recent reports from previous studies using anti-CD 19 CAR T cells have documented transient neurologic symptoms including cognitive confusion, visual hallucinations, and lack of responsiveness to commands. Moreover, prior research indicates that cytokine inflammation is associated with poorer executive functioning and memory in people with cancer[49,50]. For these reasons, this study will evaluate the cognitive status of patients prior to receiving their CD19 infusion and again 21 to 28 days after the infusion to assess possible changes related to this treatment. We also will evaluate cognitive functioning again for the participants who return to the NIH at 3 months post-infusion to assess later cognitive effects.

1.2.5.5 Risk of Cardiac Dysfunction

Cardiovascular dysfunction is a significant and yet incompletely defined aspect of CRS. The hemodynamic abnormalities associated with CRS can range from tachycardia to life-threatening hypotension. A recent trial of anti-CD19 CAR T cells documented shock requiring vasoactive infusion in 13.6% of participants[51]. Since the current treatment of CRS involves tocilizumab and corticosteroids, which can be cytotoxic to the infused therapeutic T cells, it is important to define the cardiovascular dysfunction associated with CRS in an effort guide therapy and ultimately define patients at high risk for cardiac sequela. Thus, the one of the secondary objectives is to define anti-CD19 CAR T cell immunotherapy related cardiac dysfunction. We will evaluate traditional cardiac biomarkers pre- and post-infusion of anti-CD19 CAR T cells in the study population. In addition, we will evaluate echocardiograms performed pre- and post-infusion to directly assess both systolic and diastolic function using 2-D and strain imaging. Strain echocardiography is a sensitive technique for myocardial function assessment and is well validated in detecting subclinical chemotherapy mediated cardiac toxicity[52]. Strain echocardiography is utilized to monitor patients undergoing chemotherapy since changes in myocardial strain during therapy predict progression to overt heart failure[52]. We will retrospectively combine cardiac biomarkers, 2-D and strain echocardiography with clinical parameters in an effort to define anti-CD19 CAR T cell mediated CRS cardiac dysfunction and evaluate if certain variables can be utilized to predict the severity of the syndrome in affected patients.

1.3 EXPLORATORY ANALYSIS

1.3.1 Use of Adaptive sequencing for MRD monitoring

The persistence of minimal residual disease is a poor prognostic factor in pediatric patients with acute lymphoblastic leukemia. Recent use of high-throughput sequencing (HTS) has shown that MRD testing using this technique is more sensitive at detecting low levels of leukemia in patient samples, as compared to the gold standards of multi-channel flow cytometry (MFC) or allele-specific oligonucleotide (ASO) PCR amplification[53]. Flow cytometry typically permits detection of recurrent or persistent disease with sensitivity on the order of 1 cell in 104, while PCR can detect disease on the order of 1 cell in 105. Various groups have tested new methods of MRD monitoring using next generation sequencing (NGS) to determine if this provides a more precise way of detecting low levels of leukemia (on the order of 1 cell in 106) that could be used predictively for relapse[54-56]. Adding this testing to our current CD2219 protocol can yield information on very low levels of MRD in our patients, and help us retrospectively analyze data acquired for these patients after CAR therapy. As many of the patients treated on this study proceeded to transplant, it would be useful to know what depth of remission could be attained with use of CAR therapy as a bridge to transplant. This information is extremely important to the field of CAR therapy and transplantation. These studies will be performed using samples already collected.

1.4 PROTOCOL SUMMARY AND RATIONALE

The primary goal of this trial was to evaluate the safety and feasibility of administering anti-CD19 CAR T cells after a nonmyeloablative conditioning regimen in children and young adults who have or have not undergone prior allogeneic SCT. During the dose escalation phase, no DLTs were encountered at dose level 1 in either the post-HSCT or no-HSCT cohorts. Two DLTs were encountered during the dose escalation phase at dose level 2. Subject #7 had Grade 4 CRS and Subject #10 had Grade 3 neurologic toxicity manifested as dyphasia in the setting of high fever and chills but remained hemodynamically stable. The symptoms resolved within 12 hours with only supportive care.

The MTD was therefore defined as 1 x 10^6 CAR+ T cells/kg (dose level 1) and that dose was expanded. Two additional grade 3 and two additional grade 4 CRS were encountered in the subsequent 10 patients enrolled in the dose expansion phase.

The feasibility was established in this patient population. All patients underwent apheresis and T-cell collection without difficulty. Cell therapy products met the criteria for release prior to administration in 18 of the 20 patients. Two subjects had low cell counts, which did not meet release criteria.

Secondary aims include determining the persistence of infused cells and evaluating in a preliminary fashion, the clinical activity of this regimen.

1.5 PROTOCOL STATUS SUMMARY AND AMENDMENT JUSTIFICATION

1.5.1 Protocol Status

In total 53 subjects were enrolled (51 with ALL, 2 with DLBCL) on 12-C-0112. This study has since closed to enrollment but remains open for data analysis. The overall response rate for those with ALL was 62% (n=31). One of the two patients with DLBCL had an FDG-PET response but it did not meet criteria for PR due to insufficient shrinkage of tumor size. Thirty-one patients (62%) have had complete responses (CR) and 28 of these were minimal residual disease (MRD) negative CRs. Twenty-two of these patients have since gone on to receive a potentially curative

hematopoietic stem cell transplantation. As previously noted cytokine release syndrome (CRS) was the dose limiting toxicity (DLT) establishing MTD.

1.5.2 Rationale for Amendment F

Based upon the severity of CRS observed in patient #14, additional monitoring and early CRS intervention was discussed with the FDA. The early treatment for CRS will include specific clinical signs and symptoms that will trigger administration of anti-IL6R moAb therapy (tocilizumab) and/or other pharmacologic therapies including etanercept (Enbrel) or IV steroids.

1.5.3 Rationale for Amendment G

Based on the relationship observed between the incidence of Grade 3-4 CRS and patients with high-disease burden, additional investigation is warranted to determine if reduction of disease burden prior to administration of the anti-CD19 CAR will decrease the incidence of severe CRS, while using an updated assessment/treatment algorithm for CRS. During the phase 1 dose escalation and expansion, of the first 20 patients enrolled with ALL, eight (8) patients had ≥ 50% bone marrow blasts. Excluding one patient who received less cells than dose level 1 (< 1 x 10^6 transduced T cells/kg), 4 out of the 7 patients treated experienced a clinical response AND had grade 3 or 4 CRS. The remaining 3 patients did not have severe CRS and did not have a clinical response. Of the three patients who experienced a grade 4 CRS seen to date, all occurred in patients with ≥ 50% bone marrow blasts.

It is also important to note that when the toxicity and response was correlated with the patients who had peripheral blood blasts during eligibility screening, 15/18 patients with ALL had blasts in peripheral blood detected by flow cytometry. This measure of peripheral blood blasts was therefore not a good indicator of who would experience severe CRS. Rather, four (4) patients had blasts in peripheral blood detected on routine CBC. Three of these did not have severe CRS or respond. The one that did respond (#14) had cardiac arrest. Further, of the 5 non-responders who were infused with adequate cell dose, 3 had blasts on CBC at eligibility screening. Therefore, for purposes of this study, high-burden disease (Arm 2) will be defined as M3 bone marrow blasts and/or presence of peripheral blood blasts on routine CBC.

1.5.4 Rationale for Amendment H

No significant liver toxicities related to CAR T cells have been seen to date. Currently, eligibility requirements dictate that liver transaminases (ALT, AST) be ≤ 5 X ULN. Several patients have leukemia involvement of their liver resulting in elevated transaminases. In cases where transaminitis is attributed to disease involvement, patients with Grade 3 or less ALT, AST should NOT be excluded from enrolling. As an added safety measure, we have now required that ALT and AST be ≤ 10 X ULN prior to infusing CAR T cells.

Three patients have received second infusions of CAR T cells after having an MRD+ response to the first infusion or relapsed after attaining an MRD negative remission, and none of these have responded to the second infusion and none had toxicity. All 3 patients were treated with the lymphodepleting regimen (fludarabine/cyclophosphamide) prior to their second cell infusion. In some, anti-CD19 CAR T cell immune responses have developed which may limit the efficacy of a second dose. This amendment allows for more flexibility in choosing the chemotherapy regimen prior to second cell infusions to include FLAG and etoposide/ifosfamide regimens already included in the protocol (Arm 2). These are standard of care regimens for ALL that does

not increase the risk of the CAR T cells. In fact, we found that disease burden directly correlates with severity of CRS. Since these patients seeking second treatment will have responded to the first infusion, and therefore have lower disease burdens, their risk of severe CRS should be lower as well.

1.5.5 Rationale for Amendment P

Cardiovascular dysfunction associated with CAR infusion and CRS has not been well studied. Cardiology studies including echocardiograms, and laboratory investigations will be retrospectively analyzed in an effort to define anti-CD19 CAR T cell mediated CRS cardiac dysfunction and evaluate if certain variables can be utilized to predict the severity of the syndrome in affected patients. Additionally, MRD assessments using sequencing was shown to be advantageous in the pre-transplant setting[56] thus we will incorporate this as an exploratory objective to retrospectively compare this method of detection as compared to gold standard flow cytometry in patients who achieved MRD negativity by flow cytometry.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

Under no circumstances are subjects who enroll once in this study permitted to be reenrolled for a second time (e.g., into a later cohort). Study subjects who have relapsed or treatment refractory ALL or NHL may enroll as defined by the following inclusion and exclusion criteria.

2.1.1 Inclusion Criteria

2.1.1.1 Patient must have a CD19-expressing B cell ALL or lymphoma and must have relapsed or refractory disease after at least one standard chemotherapy and one salvage regimen. In view of the PI and the primary oncologist, there must be no available alternative curative therapies and subjects must be either ineligible for allogeneic stem cell transplant (SCT), have refused SCT, or have disease activity that prohibits SCT at this time.

2.1.1.2 CD19 expression must be detected on greater than 15% of the malignant cells by immunohistochemistry or greater than 30% by flow cytometry in a CLIA approved test in the Laboratory of Pathology, CCR, NCI, NIH or from the referring institution or reference laboratory. The choice of whether to use flow cytometry or immunohistochemistry will be determined by what is the most easily available tissue sample in each patient. In general immunohistochemistry will be used for lymph node biopsies, flow cytometry will be used for peripheral blood and bone marrow samples.

2.1.1.3 Patients must have measurable or evaluable disease at the time of enrollment, which may include any evidence of disease including minimal residual disease detected by flow cytometry, cytogenetics, or polymerase chain reaction (PCR) analysis.

2.1.1.4 Greater than or equal to 1 year of age (and at least 15 kg) and less than or equal to 30 years of age.

2.1.1.5 Adequate absolute CD3 count estimated to be required to obtain target cell dose based on discussion with DTM apheresis and Cell Processing Section, DTM.

2.1.1.6 Subjects with the following CNS status are eligible only in the absence of neurologic

symptoms suggestive of CNS leukemia, such as cranial nerve palsy

- CNS 1, defined as absence of blasts in cerebral spinal fluid (CSF) on cytospin preparation, regardless of the number of WBCs;
- CNS 2, defined as presence of < 5/uL WBCs in CSF and cytospin positive for blasts, or > 5/uL WBCs but negative by Steinherz/Bleyer algorithm
- CNS3 with marrow disease who has failed salvage systemic and intensive IT chemotherapy (and therefore not eligible for radiation)
- Patients with isolated CNS relapse will be eligible if they have previously been treated with cranial radiation (at least 1800 cGy)

2.1.1.7 Ability to give informed consent. For subjects <18 years old their legal guardian must give informed consent. Pediatric subjects will be included in age appropriate discussion and verbal assent will be obtained for those ≥ 12 years of age, when appropriate.

2.1.1.8 Clinical performance status: Patients > 10 years of age: Karnofsky ≥ 50%; Patients ≤ 10 years of age: Lansky scale ≥ 50% (see **Appendix A** for conversion). Subjects who are unable to walk because of paralysis, but who are upright in a wheelchair will be considered ambulatory for the purpose of calculating the performance score.

2.1.1.9 Patients of child-bearing or child-fathering potential must be willing to practice birth control from the time of enrollment on this study and for four months after receiving the preparative regimen.

2.1.1.10 Females of child-bearing potential must have a negative pregnancy test because of the potentially dangerous effects on the fetus.

2.1.1.11 Cardiac function: Left ventricular ejection fraction ≥ 40% by MUGA or cardiac MRI, or fractional shortening ≥28% by ECHO or left ventricular ejection fraction ≥50% by ECHO.

2.1.1.12 Patients with history of allogeneic stem cell transplantation are eligible if at least 100 days post-transplant, if there is no evidence of active GVHD (See **Appendix C**) and no longer taking immunosuppressive agents for at least 30 days prior to enrollment.

2.1.2 Exclusion Criteria

Subjects meeting any of the following criteria are not eligible for participation in the study.

2.1.2.1 Recurrent or refractory ALL limited to isolated testicular disease.

2.1.2.2 Hepatic function: Inadequate liver function defined as total bilirubin > 2x upper limit of normal (ULN) (except in the case of subjects with documented Gilbert's disease > 3x ULN) or transaminase (ALT and AST) > 20x ULN based on age- and laboratory specific normal ranges;

2.1.2.3 Renal function: Greater than age-adjusted normal serum creatinine (see Table below) and a creatinine clearance < 60 mL/min/1.73 m^2.

Age (Years)	Maximum Serum Creatinine (mg/dL)

≤5	0.8
5 < age ≤ 10	1.0
>10	1.2

2.1.2.4 Hematologic function

- Absolute neutrophil count (ANC) < 750/µL, or platelet count < 50,000/µL, if these cytopenias are not judged by the investigator to be due to underlying disease (i.e. potentially reversible with anti-neoplastic therapy);
- A subject will not be excluded because of pancytopenia ≥ Grade 3 if it is due to disease, based on the results of bone marrow studies.

2.1.2.5 Hyperleukocytosis (≥ 50,000 blasts/µL) or rapidly progressive disease that in the estimation of the investigator and sponsor would compromise ability to complete study therapy;

2.1.2.6 Pregnant or breast-feeding females;

2.1.2.7 Recent prior therapy:

- Systemic chemotherapy ≤ 2 weeks (6 weeks for nitrosoureas) or radiation therapy ≤ 3 weeks prior to apheresis;

  Exceptions:

  a. There is no time restriction in regard to prior intrathecal chemotherapy provided there is complete recovery from any acute toxic effects of such;
  b. Subjects receiving hydroxyurea may be enrolled provided there has been no increase in dose for at least 2 weeks prior to starting apheresis (see Section **4.1**);
  c. Patients who relapse while receiving standard ALL maintenance chemotherapy will not be required to have a waiting period before entry onto this study provided they meet all other eligibility criteria;
  d. Subjects receiving steroid therapy at physiologic replacement doses only are allowed provided there has been no increase in dose for at least 2 weeks prior to starting apheresis;
  e. For radiation therapy: Radiation therapy must have been completed at least 3 weeks prior to enrollment, with the exception that there is no time restriction if the volume of bone marrow treated is less than 10% and also the subject has measurable/evaluable disease outside the radiation port.

2.1.2.8 Other anti-neoplastic investigational agents currently or within 30 days prior to apheresis (i.e. start of protocol therapy);

2.1.2.9 Subjects must have recovered from the acute side effects of their prior therapy, such that eligibility criteria are met. Cytopenias deemed to be disease-related and not therapy-related are exempt from this exclusion.

2.1.2.10 HIV/HBV/HCV Infection:

a. Seropositive for HIV antibody. (Patients with HIV are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy in the future should study results indicate effectiveness.)

b. Seropositive for hepatitis C or positive for Hepatitis B surface antigen (HbsAG).

2.1.2.11 Monoclonal antibody therapy administered within 30 days of the agent prior to apheresis;

2.1.2.12 Uncontrolled, symptomatic, intercurrent illness including but not limited to infection, congestive heart failure, unstable angina pectoris, cardiac arrhythmia, psychiatric illness, or social situations that would limit compliance with study requirements or in the opinion of the PI would pose an unacceptable risk to the subject;

2.1.2.13 Second malignancy other than in situ carcinoma of the cervix, unless the tumor was treated with curative intent at least two years previously and subject is in remission;

2.1.2.14 History of severe, immediate hypersensitivity reaction attributed to compounds of similar chemical or biologic composition to any agents used in study or in the manufacturing of the cells (i.e. gentamicin).

2.1.3 Recruitment Strategies

The following recruitment strategies will be employed to elicit potential candidates for this trial:

1. Listed on clinical trials.gov;
2. Listed in PDQ;
3. In addition, patients from POB clinic who are eligible for participation will be offered participation in this study

Prior to distribution of any recruitment materials, such materials will be submitted to the NCI IRB for review.

2.2 SCREENING EVALUATION

Within 2 weeks (except as specified otherwise) prior to enrollment:

2.2.1 Clinical evaluation

2.2.1.1 Complete physical examination, including vital signs, noting in detail the exact size and location of any lesions that exist.

2.2.1.2 Height, weight

2.2.1.3 Performance status determination

2.2.2 Laboratory studies

2.2.2.1 Evaluation for HIV seropositivity to consist of ELISA and, if positive, confirmation by Western blot within 4 weeks. The investigator, in the event of a positive finding, will make appropriate counseling available.

2.2.2.2 Evaluation for Hepatitis B surface antigen (HbsAG) and anti-HCV antibodies, within 4 weeks.

2.2.2.3 Creatinine Clearance: A measured 24 hour urine creatinine clearance test may be performed if the serum creatinine is elevated, and the measured value will be recorded in the CRF and may be used to qualify the subject for study participation.

2.2.2.4 β-HCG pregnancy test on all women of child-bearing potential (within one week)

2.2.2.5 Peripheral blood CD3 count.

2.2.2.6 PT/PTT

2.2.2.7 General Tests: The following will be obtained during the screening process:

2.2.2.8 Chem 20: Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid; CRP

2.2.2.9 CBC with differential and platelet count

2.2.2.10 Urinalysis and culture, if indicated

2.2.2.11 CD19 staining of malignant cells by immunohistochemistry or flow cytometry (testing is permitted to be conducted at any time since most recent disease presentation).

2.2.2.12 Additional Tests: The PI may order additional tests in some subjects if needed to fully assess clinical status and obtain baseline results. Examples of such tests include: fibrinogen, haptoglobin, cholesterol, triglyceride, immunoglobulin levels, and viral serology or PCR for cytomegalovirus (CMV), herpes simplex virus (HSV), varicella zoster virus (VZV), and Epstein-Barr virus (EBV).

2.2.3 Disease Evaluation (method determined by patients' type and location of disease):

2.2.3.1 Imaging Studies such as Chest CT and appropriate imaging methods of any sites relevant to the subject's disease (e.g., PET/CT).

2.2.3.2 Bone marrow aspirate, including flow cytometry, and biopsy

2.2.3.3 Spinal fluid for cell count and cytospin

2.2.4 Other Tests

2.2.4.1 ECG

2.2.4.2 Echocardiogram, Cardiac MRI,or MUGA

2.3 REGISTRATION PROCEDURES

Screening: Authorized staff will register patients signing the screening consent for analysis of CD19 expression with the Central Registration Office (CRO) (301-402-1732) by completing the screening portion of the eligibility checklist.

Treatment Enrollment Procedures: Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration

Office, CRO staff will call Department of Transfusion Medicine (DTM) Cell Processing Service (CPS) to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

2.4 TREATMENT ASSIGNMENT PROCEDURES

2.4.1 Cohorts

Number	Name	Description
1	Cohort 1	Patients without high-burden disease or patients for whom chemotherapy toxicity is a concern will receive standard preparative regimen.
2	Cohort 2	Patients with high-burden disease who receive standard chemotherapy to reduce burden, (defined as patients with ALL who have M3 bone marrow blasts and/or presence of peripheral blood blasts on routine CBC, or patients with lymphoma).

2.4.2 Arms

Number	Name	Description
1	Arm 1	Lymphodepleting regimen of Fludarabine and Cyclophosphamide.
2	Arm 2	Intensive standard of care chemotherapy, in lieu of the lymphodepleting chemotherapy regimen, to decrease tumor burden in preparation for the administration of the CAR T cells.

2.4.3 Arm Assignment

Patients in Cohort 1 will be directly assigned to Arm 1.

Patients in Cohort 2 will be directly assigned to Arm 2.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a phase I safety/feasibility study in pediatric and young adult patients with CD19-expressing B cell ALL or lymphoma who have relapsed or refractory disease after at least one standard chemotherapy and one salvage regimen. All patients enrolled on this trial have been deemed incurable by standard therapies. Autologous PBMC will be obtained by leukapheresis and transduced with anti-CD19-CAR retroviral vector.

Patients will be enrolled into one of two arms:

Arm 1: Patients without high-burden of disease or patients requiring the cyclophosphamide/fludarabine preparative regimen for safety concerns;

Arm 2: Patients with high-burden of disease (defined as patients with ALL who have M3 bone marrow blasts and/or presence of peripheral blood blasts on routine CBC, or patients with lymphoma).

All eligible patients will undergo apheresis and anti-CD19 CAR T cells will be manufactured from fresh or frozen PBMCs. PBMCs previously collected by DTM and cryopreserved may be used so long as the subject has not had a hematopoietic stem cell transplant between the time of PBMC collection and enrollment on this protocol. PBMCs collected by an extramural institution may not be used.

Patients in Arm 1 will receive the lymphodepleting chemotherapy regimen outlined in Sections **3.4** and **3.9.1**.

Patients in Arm 2 will be treated with intensive standard of care chemotherapy, in lieu of the lymphodepleting chemotherapy regimen, to decrease tumor burden in preparation for the administration of the CAR T cells. Choice of chemotherapy will be individualized since patients will have previously received a variety of treatment regimens and may have received different lifetime anthracycline doses or may have experienced significant toxicities with certain agents, i.e. asparaginase. Only standard of care chemotherapy agents will be used. Preferred regimens are given in Section **3.9** but patients may be treated with another standard regimen not listed if these are determined to pose an unacceptable medical risk to the patient. No investigational therapy will be administered for disease control. At least 2 days must pass between the last dose of chemotherapy (excluding IT chemotherapy) and administration of the anti-CD19 CAR T cells.

CAR T cells will be cryopreserved on day 7 of culture unless the target dose has not been reached or to accommodate scheduling purposes. In this case, the PI after discussion with CPS will determine if the culture is likely to expand further given more culture time. If not, cells will be cryopreserved despite the dose level not being met and will be administered to the patient on schedule. If cells are deemed likely to expand further, the culture time may be extended up to 14 days total. All patients will receive anti-CD19 CAR T cells no sooner than 10 days after culture initiation (at least 2 days after that last dose of chemotherapy). Patients will be evaluated after treatment for toxicity, antitumor effects and for persistence and functionality of transduced T cells. Toxicity assessment of neurologic symptoms will include cognitive confusion, visual hallucinations, and lack of responsiveness to commands. In addition, this study will evaluate the cognitive status of patients prior to receiving their CD19 infusion and again after the infusion to assess possible changes related to this treatment.

3.2 APHERESIS FOR CELL ACQUISITION

Patients will undergo a 2-5 blood volume apheresis, as estimated by recipient weight and target cell harvest dose in the Department of Transfusion Medicine (DTM). A target cell number extrapolated based upon the expected expansion and planned cell dose of transduced T cells/kg will be collected. Citrate anticoagulant will be used in subjects greater than 18 kg. For subjects less than 18 kg, low-dose heparin may be added. Prophylactic intravenous CaCl2 and MgSO4 infusions may be administered by the DTM physician per standard operating procedure. Bilateral

peripheral venous access will be used whenever possible. Alternatively, a temporary central venous catheter may be placed for collection and supportive care should significant toxicity occur. The CVL will be inserted by Critical Care or Interventional Radiology staff with the assistance of anesthesiology as indicated. For patients weighing between 15 and 25 kg, red blood cell priming may be used to prevent dilutional anemia and increase the collection efficiency. The decision will be made on a case-by-case basis after discussion between the primary investigator, pediatric medical care, and DTM teams, and the patient's parent.

3.3 CELL PROCESSING

Fresh or cryopreserved PBMCs will be prepared on approximately Day -11. CD3+ cells will be enriched using anti-CD3/anti-CD28 beads and cultured in the presence of IL-2 in order to stimulate T-cell growth. After two days of stimulation T cells will be transduced by exposure to supernatant containing the anti-CD19 CAR retroviral vector in bags coated with RetroNectin® (Takara Biomedicals, Japan) to facilitate the interaction between the viral particles and the activated cells. Expansion of these transduced cells will be supported with IL-2. CD19-CAR transduction efficiency will be determined by FACS analysis for the CAR using the anti-idiotype monoclonal antibody. Successful CAR gene transfer for each transduced PBL population will be defined as >15% CAR positive cells.

In some patients, the peripheral blood might be contaminated with large numbers of CD19-expressing malignant cells. However, the anti-CD3/anti-CD28 magnetic beads will provide a selection step for the non-leukemic T cell population. Further, ALL blasts are not likely to survive ex vivo under these culture conditions and culture duration and will not be expanded by the anti-CD3/CD28 stimulation. This was evident in the results of the validation run using cells from a patient with CLL. In previous testing performed for this study, the CD19 expressing malignant cell results was 0.

The release criteria will be based upon analyses for each dose of anti-CD19 CAR retroviral-transduced autologous PBL and will include:

Test	Method	Criteria
Cell viability[1]	Trypan blue exclusion	≥60%
Cell number[1]	Cell counter	within 20% of planned dose level
% CAR+ cells[2]	Anti-idiotype flow cytometry	≥15%
% CD19 positive cells[1]	Flow cytometry	<5%
Endotoxin[1]	Gel Clot	≤ 5 EU/mL
Mycoplasma[2]	Mycoplasma test	Negative
RCR[2]	RCR-PCR	Negative
RCR[5]	S+L- Assay	Negative
Sterility testing[4]	gram stain, culture	Negative

[1]Performed on sample from final product immediately prior to infusion if given fresh or immediately prior to cryopreservation, results available at the time of infusion.

[2]Performed on a sample collected between Day –4 and Day –1. Results are available at time of infusion.

[4]Gram stain is performed on final product prior to infusion and is available at the time of infusion, cultures will be sent from Day –2 product at the time of infusion if given fresh or at time of cryopreservation, and results will be in process at the time of infusion, therefore they may not be definitive.

[5]Sample collected from the final product prior to infusion. Results will not be available before cells are infused into the patient.

Any prepared cells not required for infusion or for research or regulatory purposes will be cryopreserved by standard DTM techniques and will be made available should the subject be eligible for a second infusion as outlined in Section **3.11**.

3.4 ANTI-NEOPLASTIC THERAPY

Patients must have evaluable disease at the time of protocol enrollment.

Given the correlation in the original 20 patients treated on this study between the incidence of severe CRS and amount of disease burden, patients with high-burden disease (Arm 2: M3 bone marrow blasts and/or presence of peripheral blood blasts on routine CBC) will receive standard of care chemotherapy as described in Section **3.9**.

Patients in Arm 1 will receive fludarabine/cyclophosphamide preparative regimen on Days -4 through -2.

3.5 IMPACT OF INTERCURRENT ILLNESS AFTER ENROLLMENT

Should a subject develop a serious or life-threatening condition or infection after enrollment but before anti-CD19-CAR T cells are administered, the subject will be treated for their condition as clinically indicated and administration of anti-CD19-CAR T cells will be delayed. Cells will be maintained in culture for up to a total culture time of 14 days while attempts are made to correct the condition. Cells may be infused within this period only if the PI determines that administration of the cells does not pose any additional risk to the subject. Otherwise, the cells will be cryopreserved and, if clinically indicated, the subject may receive them up to 21 days from the planned infusion date, and be considered evaluable. If the patient is able to receive cells within 21 days of the planned original dose on Day 0, administration should follow the procedure as outlined in Section **3.10** and the patient will remain evaluable for toxicity and efficacy. Alternatively, if > 21 days has transpired prior to subject recovery, the subject has the option of receiving the cells on at a later point once the condition has resolved, but these patients will be replaced on study and not considered evaluable for toxicity or response endpoints. Clinical response in these patients will be analyzed and may be reported descriptively.

3.6 TREATMENT LIMITING TOXICITY

Adverse events that are considered disease-related (not suspected of relationship to anti-CD19-CAR T cells) will not be considered treatment-limiting toxicities. Only those AEs suspected to be related to pre-infusion chemotherapy and/or anti-CD19-CAR T cells (any component of the treatment regimen) will be used in the definition of TLT. Toxicities occurring after initiation of the chemotherapy preparative regimen but prior to anti-CD19 CAR cell infusion, will primarily be attributable to the chemotherapy administration or disease, if not extraneous causes. After cell infusion, toxicities will be evaluated for temporal and causal relationship to chemotherapy versus cell infusion. Some symptoms may overlap and attribution will not be clearly definable, in which case, toxicities will be attributed as possibly related to both chemotherapy and cell infusion.

Toxicities will be attributed to the T cells if: 1) they were NOT present before T cell infusion; OR 2) they increase in Grade in temporal association with the T cell infusion; AND 3) they are not clearly explained by other factors.

Patients who are fully assessable are those that have completed the chemotherapy regimen and received the anti-CD19 CAR T cell infusion. Chemotherapy-related toxicities experienced by patients who are unable to receive anti-CD19 CAR T cells (See Section **8.3** for the definition of inevaluable patients) will be considered in the definition of TLT.

See Section **7.1** Causality for definitions of 'suspected'. The definition of TLT in these studies uses NCI's Common Terminology Criteria for Adverse Events (CTCAEv4.0).

3.6.1 Definition of TLT

Adverse events that are at least possibly related to the treatment regimen (chemotherapy and/or anti-CD19-CAR T cells) and are ≥ Grade 3 in severity will be considered TLTs with the following additional criteria or exceptions:

3.6.1.1 Hematological Toxicity:

- Subjects with normal, Grade 1 or Grade 2 Hematologic Parameters at baseline (independent of transfusion) and cytopenias NOT due to Bone Marrow Involvement by Disease
    - Any Grade 4 hematological toxicity (with the exception of lymphopenia) lasting greater than 30 days will be considered a TLT. Lymphocyte count and subsets will not be considered in the definition of TLT.
- Subjects with abnormal blood counts (Grades 1 through 4) at baseline due to bone marrow involvement by disease
    - These subjects will be considered non-evaluable for hematologic TLT but evaluable for all other aspects of the study.

3.6.1.2 Non-hematological Toxicity:

- Any Grade 3 or greater, non-hematological toxicity will be considered a TLT with the following **exceptions**:
    a. Tumor lysis syndrome, including associated abnormalities (eg, electrolytes, uric acid, renal function)
    b. Grade 3 low electrolyte levels that are correctable and asymptomatic. Grade 3 hypoalbuminemia. Such individuals should receive supplementation as indicated.
    c. Hypocalcemia toxicity grade should be assigned based on the calcium level corrected for degree of hypoalbuminemia according to the following formula: For every albumin decrease of 1 gm/dL a total calcium increase of 0.2 mmol/L is to be made;
    d. Subjects on anticoagulant therapy or with pre-existing coagulopathy with abnormal coagulation parameters.

e. Grade 3 transaminase, alkaline phosphatase, bilirubin or other liver function test elevation, provided there is resolution to ≤ grade 2 within 14 days.

f. Grade 3 or 4 fever;

g. Grade 3 or 4 infection or neutropenic fever unless subjects have normal blood counts at baseline, and infection is not considered likely related to the chemotherapy regimen and relationship to anti-CD19-CAR T cells is suspected (Note: Grade 4 infection uncontrolled for > 7 days will be considered TLT.);

h. Toxicities occurring within 24 hours post cell infusion related to cell infusion, (including Grade 3 and 4 allergic reaction) that are reversible to a grade 2 or less within 8 hours with up to two doses of acetaminophen 15 mg/kg/dose (to a max adult dose of 650 mg) or up to two doses of diphenhydramine 1 mg/kg up (to a max adult dose of 50 mg).

i. Grade 3 or 4 transient (< 72 hours) serum hepatic enzyme abnormalities.

j. Grade 3 nausea and/or anorexia.

k. Grade 3 cytokine release syndrome (**Appendix F**) will not constitute TLT.

- A Grade 2 non-hematologic toxicity of > 30 days duration will constitute a TLT (with the exceptions noted above in Section **3.6.1.2**).
- In patients with history of prior SCT, any histologically proven acute GVHD grade 2 or higher (See **Appendix C**) within 30 days of receiving the anti-CD19 CAR cells will be considered TLT.

3.6.2 Maximal Tolerated Dose (MTD) (**COMPLETED 6-14**)

The MTD is a dose level immediately below the level at which the enrollment is stopped for a stratum due to a DLT, as explained specifically below:

- If **more than one subject** in the first three subjects included in a dose level experience DLT as defined above, MTD will have been exceeded.
- If DLT develops in **one** of the 3 subjects included in a cohort, the cohort will be then expanded up to six:

  If 2 or more of these 6 included patients develop DLT, the MTD will have been exceeded.

  If no additional subject develops a DLT, the MTD will not have been exceeded and the next dose level can be administered after the four week safety assessment period of the last patient at this dose level.

If MTD is exceeded at any dose level, three subjects will be added to the immediate lower dose level, unless it has been previously expanded to six. If less than 2 of 6 subjects develop DLT at that level, it will be defined as the MTD.

3.7 DOSE ESCALATION (COMPLETED 6-14)

Cell dose will be body weight-based. In patients with BMI equal to or less than 30, the dose of anti-CD19-transduced T cells for each cohort will be based on doses calculated using actual body

weight. Patients with BMI greater than 30 will have doses calculated using ideal body weight (IBW) plus 50% of the difference between actual weight and IBW (see **Appendix D** for calculation).

There will be a Phase I dose-escalation design in two strata with three dose cohorts. One stratum will enroll subjects who have previously undergone allogeneic stem cell transplant (SCT), and the other stratum will include subjects who have not undergone SCT. The number of anti-CD19-CAR transduced T cells transferred for each dose level cohort will be:

Dose Escalation Schedule	
Dose Level	**Dose of anti-CD19-CAR transduced T cells**
Cohort Level 1	1×10^6 transduced T cells/kg (± 20%)
Cohort Level 2	3×10^6 transduced T cells/kg (± 20%)
Cohort Level 3	1×10^7 transduced T cells/kg (± 20%)

Each dose cohort will initially include a minimum of 3 patients. Patients per stratum will initially be enrolled sequentially. A two week (14 days) safety assessment period will follow regimen completion (defined as infusion of anti-CD19 CAR cells) of the first patient in each cohort. Subsequent patients in that cohort may be enrolled after a one week (7 day) safety assessment period. Four weeks must elapse after completion of cell infusion in the final patient in a cohort to allow for safety assessment before accruing patients to the next dose cohort level. Therefore, enrollment will not proceed to a higher dose level until all patients have been treated in the prior cohort and the last patient treated on the completed cohort has been observed for at least 4 weeks after infusion of anti-CD19 CAR cells.

Toxicity data collected from the prior SCT stratum dose cohorts may be used to make dose escalation decisions in the non-SCT dose cohorts. For example, if 3 patients in the prior SCT stratum complete a cohort without DLT, the next patient enrolled in either strata may proceed to the next dose cohort. But if 3 patients in the NON-SCT stratum complete a cohort without DLT, the prior SCT stratum must continue enrollment until 3 patients complete therapy without DLT prior to dose escalation.

If cell growth limitations preclude administration of the number of cells targeted for the assigned cohort level, the patient will receive as many cells as possible and the patient will be enrolled in the appropriate cohort for the number of cells infused, allowing for an additional three patients to be enrolled per cohort due to cell growth limitations. If a DLT occurs in an additional patient entered at a lower dose due to cell growth limitations, accrual will continue at the previously planned dose level for subsequent patients. If a minimum of 1x105/kg anti-CD19-CAR-transduced T cells cannot be obtained for infusion, the patient will still receive the cell infusion, as the effective dose of cells is not well defined, and the patient will be evaluable for feasibility but not analyzed for MTD, although toxicities will be assessed and reported separately. If the third dose level in each stratum is completed without DLT, an MTD may not be determined. This will be considered the 'highest cell dose' studied, and will be the dose level that will be studied further in the expansion cohort. Alternatively, if no toxicity or clinical activity is observed after

completion of the 3rd dose cohort, consideration may be given to adding additional dose cohorts in a protocol amendment.

Dose escalation will follow the rules outlined in the Table below.

Number of Patients with DLT at a Given Dose Level	**Escalation Decision Rule**
0 out of 3	Enter up to 3 patients at the next dose level. If 0 out of 3 occurs in the prior SCT stratum, both strata may dose escalate. If 0 out of 3 occurs in the NON-SCT stratum, enrollment must continue in the prior SCT stratum until 0 out of 3 (or 1 out of 6) patients demonstrate DLT.
$\geq$ 2	Dose escalation to that stratum will be stopped. The other stratum may continue to dose escalate independently. This dose level will be declared the maximally administered dose (highest dose administered) for that stratum. Up to three (3) additional patients from that stratum will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter up to 3 more patients from this stratum at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Up to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
$\leq$1 out of 6 at highest dose level below the maximally administered dose	This is the MTD and is generally the recommended phase 2 dose. At least 6 patients must be entered at this dose level.

3.8 DOSE EXPANSION COHORT (COMPELTED 6-14)

To gain further experience with the safety, feasibility and clinical activity of the anti-CD19 CAR T cells in this patient population, the MTD (or highest cell dose studied) will be expanded to a total of 15 patients (i.e., 9 additional patients at the MTD). If the MTD or highest cell dose studied is the same for both strata (prior SCT vs. NO prior SCT) one expansion cohort will be studied and efforts will be made to include a balance of patients with prior SCT, without prior

SCT, ≤ 12 years of age and > 12 years of age. If different MTDs are observed per stratum, a total of 12 patients per stratum will be enrolled at the MTD, attempting to balance enrollment between patients ≤ 12 years of age and > 12 years of age.

3.9 DRUG ADMINISTRATION

3.9.1 Arm 1 Chemotherapy

Each patient in Arm 1 will receive the lymphodepleting regimen as follows: (This may be given as an inpatient or outpatient as necessary).

Drug	**Dose**	**Supportive Care**	**Chemo Days**
Fludarabine	25 mg/m^2 per day IV infusion in 50mL of 0.9% sodium chloride over 30 minutes± 10 min, daily for 3 days.		1,2,3
Cyclophosphamide	900 mg/m^2 per day IV infusion over 60 minutes ± 10 min, once on Day 3 after fludarabine	IV pre-hydration 2 hours ± 30 min prior to cyclophosphamide and for at least 8 hours after with 0.45% sodium chloride and 5% dextrose (or other composition appropriate for the clinical situation) at a rate of at least 90 mL/m^2/hour. Hydration after cyclophosphamide will include Mesna as described below. Urine specific gravity ≤1.010 prior to infusion.	3

3.9.1.1 Mesna

Mesna will be administered at a dose of 540 mg/m^2 per day by continuous IV infusion on day -2. The first 180 mg/m^2 will be mixed with the cyclophosphamide in an appropriate amount of fluid based on the hydration rate. Immediately upon completion of this initial infusion, mesna at a dose of 360 mg/m^2 in 0.45% sodium chloride and 5% dextrose (or other composition appropriate for the clinical situation) will be infused over 8 hours after completion of the cyclophosphamide.

3.9.1.2 Hydration with Cyclophosphamide

IV hydration will be initiated 2 hours ± 30 min prior to cyclophosphamide using 0.45% sodium chloride with 5% dextrose (or other composition appropriate for the clinical situation). Hydration

may be temporarily interrupted to give fludarabine or cyclophosphamide but should continue until at least 8 hours after the cyclophosphamide dose has been completed.

3.9.1.3 Adjusting hydration/electrolytes during preparative regimen.

During chemotherapy, IV furosemide (0.5 - 1 mg/kg/dose to a maximum of 20 mg per dose) may be administered as needed to maintain normal urine output and fluid balance. All patients should receive at least maintenance fluids (1440mL/m2/day), orally and/or intravenously unless medically contraindicated. Patients deemed at high risk for tumor lysis will receive additional hydration; see Section **4.3.1** for fluid hydration guidelines. Hydration will be adjusted to maintain fluid balance, urine output and urine specific gravity. In addition, serum electrolyte levels will be monitored every 12 hours and IV fluid content including potassium chloride supplementation will be adjusted to maintain normal serum electrolyte levels.

3.9.1.4 Anti-emetics

Routine anti-emetic prophylaxis and treatment should be employed. Corticosteroids may not be used (except for physiologic replacement as outlined in Section **2.1.2.8**, or in IT chemotherapy as outlined in Section **4.1.1**).

3.9.2 Arm 2 Chemotherapy

Standard of care chemotherapy will be administered to patients as inpatient and will be used prior to cell infusion in patients in Arm 2 to reduce the disease burden while depleting endogenous lymphocytes in preparation for cell infusion. The choice of chemotherapy agents will be determined by the research team based on the patient's prior therapies, e.g. total lifetime anthracycline doses, responses to prior regimens, and toxicities from prior regimens. Only FDA approved chemotherapy agents will be used. Efforts will be made to treat patients with one of the two regimens outlined below. At least 2 days must pass after the last dose of chemotherapy prior to cell infusion in Arm 2. Supportive care medications will be administered as per standard of care requirements for the agents administered.

Standard chemotherapy will be given for 1 cycle (5 days for the two regimens outlined below), and cells will be administered as soon as possible, but no sooner than 2 days after the last dose of chemotherapy.

3.9.2.1 FLAG Chemotherapy

Drug	**Dose**	**Supportive Care**	**Days**
Fludarabine	25 mg/m^2/d IV over 30 min± 10 minutes	IV pre-hydration 1 hour prior to fludarabine daily for 5 days with 0.9% NaCl and KCl 10 meq/liter at a rate of 90 ml/m^2/hour to a maximum rate of 100 ml/hour. Following fludarabine infusion, restart fluid at a rate of 90 ml/m^2/hour to a maximum rate of 100 ml/hour continue until	1, 2, 3, 4, 5 (Hour 0-1)

		cytarabine infusion begins.	
Cytarabine (Ara-C)	2000 mg/m^2/d IV over 4 hrs± 30 minutes, 4 hrs after fludarabine begins	a. Corticosteroid ophthalmic drops 2 drops to each eye every 6 hours starting prior to first dose and until 24 hours after the last dose of cytarabine completed. b. Begin cytarabine doses 3.5 hours after completion of the preceding fludarabine. c. Infuse in 250 ml of D5W. d. IV post-hydration x 4 hours daily for 5 days with 0.9% NaCl and KCl 10 meq/liter at a rate of 90 ml/m2/hour to a maximum rate of 100 ml/hour. e. In the event of signs of CNS toxicity, cytarabine infusion will be interrupted and the M.D. notified. No further cytarabine will be administered if there is CNS toxicity (any grade) deemed related to cytarabine.	1, 2, 3, 4, 5 (Hour 5-9)
Filgrastim	5 µg/kg/dose subcutaneously daily starting one day prior to starting FLAG	Administer daily until ANC>1000 on 2 consecutive days	0 and continue until criterion is met

IV hydration composition may be modified to compensate for electrolyte abnormalities or for risk of significant tumor lysis syndrome.

3.9.2.2 Etoposide/Ifosfamide Regimen

Prior to chemotherapy, patients should receive appropriate hydration so that urine flow is 2 ml/kg/hr for at least one void or one hour prior to initiation of chemotherapy.

Drug	**Dose**	**Supportive Care**	**Days**
Etoposide	100 mg/m^2/dose IV in		1, 2, 3, 4, 5

	D5W or NS IV over 1 hour± 10 min daily X 5 days		(Hour 0-1)
Ifosfamide	1,800 mg/m^2/dose and Mesna 360 mg/m2/dose IV in 100 mL D_5W over 1 hour ± 10 min daily X 5 days	Recommended hydration to be maintained at 125 ml/m^2/hr during days of ifosfamide administration.	1, 2, 3, 4, 5 (Hour 1-2)
Mesna	360 mg/m^2/dose IV over 15 minutes ± 5 min immediately after ifosfamide infusion, then every 3 hours for 6 additional doses PO or IV over 15 ± 5 min minutes daily on days 1 through 5 (total of 35 doses).	Adequate oral hydration (minimum 500 mL PO q 4-6 hr) must be maintained.	1, 2, 3, 4, 5 (Hour 2-20)

IV hydration composition may be modified to compensate for electrolyte abnormalities or for risk of significant tumor lysis syndrome.

3.9.2.3 Treatment Modifications

Hematuria

For microscopic hematuria with <50 RBC/HPF in urine, continue cyclophosphamide or ifosfamide at full dose. For microscopic hematuria with >50 RBC/HPF initiate IV hydration at 2-times maintenance rate and initiate bladder irrigation with normal saline 100-150 ml/m2/hr prior to cyclophosphamide/ifosfamide; initiate bladder irrigation with subsequent courses. For gross hematuria discontinue cyclophosphamide and ifosfamide until the hematuria has resolved and then follow the guidelines for microscopic hematuria with >50 RBC/HPF (hydration and bladder irrigation). If the gross hematuria recurs or does not resolve after 2 weeks, discontinue all use of cyclophosphamide and ifosfamide.

3.10 ANTI-CD19 CAR T CELL INFUSION – DAY 0

(may be delayed up to Day 21 for clinical issues or cell growth limitations.)

3.10.1 Cell Infusion Criteria

Subjects must meet the following criteria in order for cells to be infused (based on labs obtained within 24 hrs of cell infusion):

3.10.1.1 Anti-CD19-CAR T cells must have met release criteria (Section **3.3**)

3.10.1.2 Subject has no evidence of hemodynamic instability

3.10.1.3 Subject has not developed symptoms concerning for new, systemic infection or condition that in the opinion of the PI may pose an unacceptable risk to the subject

3.10.1.4 There is no evidence of uncontrolled, significant tumor lysis syndrome prior to cell infusion

3.10.1.5 At least 2 days have elapsed since the last dose of chemotherapy in Arm 2.

3.10.1.6 Liver transaminase (ALT and AST) ≤ 10 X ULN based on age- and laboratory specific normal ranges

If these criteria are not met, measures will be taken to resolve the underlying condition(s) and if successful cells may be infused up to 21 days following the time of the planned infusion, or attempts to infuse at a later time may be made per Section **3.5**.

3.10.2 Inpatient and outpatient care post-cell infusion

Patients will be admitted for cell infusion to NIH in accordance with CC and nursing policies. Patients will remain hospitalized until stable after cell infusion. Once discharged, patients must remain outpatient in close proximity to the NIH for daily evaluations until Day 14, and then twice weekly evaluations until Day 28.

3.10.3 Premedications

Patients will receive the following medications 30-60 minutes prior to cell infusion:

- Diphenhydramine 0.5 - 1 mg/kg/dose (maximum 50 mg/dose) PO or IV over 10-15 minutes;
- Acetaminophen 15 mg/kg/dose (maximum 650 mg/dose) PO.

3.10.4 Cell Administration

Cells will be administered at a dose of 1 X 10^6 anti-CD19 CAR T cells/kg (+/- 20%). Cells are delivered to the patient care unit by a staff member from the DTM CPS. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), an identification of the product and documentation of administration are entered in the patient's chart, as is done for blood banking protocols.

The cells are to be infused intravenously (IV) over 10-20 minutes ± 10 min via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.

3.10.5 Required monitoring during cell infusion:

3.10.5.1 Monitoring will include vital signs (blood pressure, heart rate, respiratory rate) prior to infusion, every 15 minutes ± 5 min for 1 hour after the start of infusion, every 30 minutes ± 10 min for the second hour and then hourly ± 10 min for an additional 2 hours or until stable, and more frequently if clinically indicated. Temperature will be evaluated at least hourly.

3.10.5.2 Supplemental oxygen will be available at the bedside.

3.10.5.3 If an allergic or other acute reaction occurs, studies appropriate for investigation of a transfusion reaction will be performed (urinalysis, CBC, Coomb's test). Acute reactions will be treated according to institutional standards of care.

3.11 OPTION FOR ADDITIONAL DOSE(S) OF ANTI-CD19 CAR T CELLS

On the day cells are cryopreserved, up to 6 aliquots sufficient for additional dosing will be cryopreserved for potential future use by the Subject. The remaining cells will be cryopreserved and used for research. The second and any subsequent doses will be administered at dose 1 X 10^6 anti-CD19 CAR T cells/kg (+/- 20%). If the subject experienced treatment limiting toxicities (TLT) from prior dose, the second and any subsequent doses will be a half-log less at 3 X 10^5 anti-CD19 CAR T cells/kg (+/- 20%). Subjects will have the option for additional infusions of anti-CD19-CAR T cells (including a preparative chemotherapy regimen selected based on available standard chemotherapy regimens (prioritizing FLAG and Etoposide/ifosfamide), toxicity assessment and research blood sampling) if the following criteria are met:

3.11.1 Eligibility Criteria for subsequent cell infusions in subjects who did not experience TLT after first infusion:

3.11.1.1 Response to previous infusion: Subjects who had a PR, or SD with clinical benefit may elect to receive another infusion of cells. Subjects that initially had a CR may only receive a second dose if evaluable disease recurs. Clinical benefit is indicated by an improvement in the subject's health status (e.g., decreased transfusion requirement, improved cytopenias, decrease in number of blasts not sufficient to reach a PR, improved performance status or quality of life, etc.).

3.11.1.2 At least 28 days have passed since the previous cell infusion.

3.11.1.3 Any toxicity regardless of causality to the cell infusion after the previous anti-CD19 CAR cell infusion must resolve to a grade 2 or less or return to baseline levels.

3.11.1.4 Subjects must meet all the initial eligibility criteria as outlined in Section **2**,

3.11.1.5 An adequate number of cryopreserved cells must be available:

3.11.2 Eligibility Criteria for subsequent cell infusions in subjects who experienced TLT after the first infusion:

3.11.2.1 Subsequent doses will be reduced to a lower dose level.

3.11.2.2 Disease burden must be reduced according to the following:

1. Improvement from M3 to M2 or less marrow classification, or
2. Improvement from M2 to M1 or less marrow classification, or
3. For patients beginning with M1 marrow, a decrease of at least 50% in their disease as determined by flow cytometry (MRD).

3.11.2.3 Response to previous infusion: Subjects who had a PR, or SD with clinical benefit may elect to receive another infusion of cells. Subjects that initially had a CR may only receive additional dose if evaluable disease recurs. Clinical benefit is indicated by an improvement in the subject's health status (e.g., decreased transfusion requirement, improved cytopenias, decrease in number of blasts not sufficient to reach a PR, improved performance status or quality of life, etc.).

3.11.2.4 At least 28 days have passed since the previous cell infusion.

3.11.2.5 Any toxicity, regardless of causality to the cell infusion after the anti-CD19 CAR cell

infusion must resolve to a grade 2 or less, or return to baseline levels.

3.11.2.6 Subjects must meet all the initial eligibility criteria as outlined in Section **2**.

3.11.2.7 An adequate number of cryopreserved cells must be available.

3.11.3 All patients receiving additional doses will undergo chemotherapy prior to cell infusion based on disease burden as described in Section **3.9**.

3.11.4 On day 0, the cellular product will be thawed and administered IV immediately at a rate of approximately 10-15 ml/min or as tolerated based on volume status and/or DMSO toxicity. Do not exceed 20 ml of product per kg body weight (if 10% DMSO) or 40 ml of product per kg body weight (if 5% DMSO) so that per 24-hour period total DMSO administered is less than 1 mL/kg/day.

3.11.5 Any subject who receives subsequent doses of anti-CD19 CAR T cells will be observed for 28 day post cell infusion and post-infusion monitoring will be the same as for the 1st infusion and all toxicities, including secondary reactions, will be recorded and reported.

3.12 On-Study Evaluation

3.12.1 Prior to chemotherapy regimen (within 24 hours unless otherwise specified)

3.12.1.1 Complete physical examination, including, weight and vital signs

3.12.1.2 Chem 20: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid)

3.12.1.3 Thyroid panel (within 14 days)

3.12.1.4 C-reactive protein (CRP)

3.12.1.5 CBC with differential and platelet count

3.12.1.6 PT/PTT (within 14 days)

3.12.1.7 Disease staging (within 14 days)

- Flow cytometry of peripheral blood for CD19 determination, includes site density, absolute blast count
- All patients will have lumbar puncture for examination of cerebral spinal fluid (at time of screening evaluation).

3.12.1.8 β-HCG pregnancy test on all women of child-bearing potential (within 72 hours of starting chemotherapy)

3.12.1.9 Ophthalmologic Exams: For subjects with a history of prior allogeneic stem cell transplantation, ophthalmologic examinations (slit-lamp and fundoscopic) will be performed prior to apheresis and 4 weeks after cell infusion. These examinations will be performed for safety monitoring and will not be used to disqualify the subject for study participation unless active GvH is identified.

3.12.1.10 Patients ≥ 12 years of age: ECOG 0, 1, or 2; patients < 12 years of age: Lansky

scale ≥ 60% (see **Appendix A**)

3.12.1.11 Baseline Cognitive Evaluation (within 30 days prior to Day 0): A brief battery of cognitive tests evaluating memory, attention, processing speed, and executive functions (less than one hour to complete) will be administered to participants prior to anti-CD19-CAR T cell infusion (see **Appendix G**). A parent/adult observer also will complete a brief symptom checklist assessing the severity and duration of any neurologic symptoms over the past week (less than 15 minutes to complete).

3.12.2 During chemotherapy (Arm 2)

Evaluations conducted during standard of care chemotherapy in Arm 2 will be according to the package insert recommendations or best clinical practice procedures associated with the chemotherapy regimen administered. These evaluations will be individualized to the patients receiving chemotherapy in Arm 2 but at a minimum will include once or twice per week:

3.12.2.1 Physical exam and vital signs

3.12.2.2 Chem 20: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LDH, Uric Acid, CRP)

3.12.2.3 CBC with differential and platelet count.

3.12.3 Prior to cell infusion (within 24 hours unless otherwise noted)

3.12.3.1 Complete physical examination, including performance status, weight and vital signs

3.12.3.2 Chem 20: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid, CRP)

3.12.3.3 CBC with differential and platelet count

3.12.3.4 Urinalysis

3.12.3.5 Serum Cytokine Levels (pre-infusion)

3.12.3.6 Detection of RCR by PCR and persistence of anti-CD19-CAR T cells (Section **5.1.3** and **Appendix E**) as a baseline before cell infusion (within 72 hours of cell infusion)

3.12.4 During and After Cell Infusion:

3.12.4.1 Vital signs will be monitored as per Section **3.10.5**, and then routinely (every 4 hours) unless otherwise clinically indicated.

3.12.4.2 Serum Cytokine Levels: 1, 12 and 24 hours following infusion (See **Appendix E**).

3.12.5 Monitoring Day 1 to Day 28 (including Day 28)

3.12.5.1 Daily history and physical exam with vital signs and performance status through Day 14, then at least twice weekly until Day 28.

3.12.5.2 Serum cytokine levels Days 1(24hr ±4 hours), 2 (48 hr ±4 hours), 3 (72 hr ±4 hours),

then daily on days 4-14 and Day 28 (± 3 days) following cell infusion. Levels should also be obtained daily if clinical symptoms of cytokine release syndrome develop.

3.12.5.3 Twice weekly laboratory analysis: CBC with differential, LDH, SGPT (ALT), SGOT (AST), alkaline phosphatase, bilirubin (total and direct), BUN, creatinine, electrolytes, calcium, magnesium, phosphorus, uric acid, albumin, CRP, and urinalysis

3.12.5.4 Post-Infusion Neurologic Symptom Checklist (Day 14 +/- 1 day) (**Appendix H**): A parent/adult observer for each participant will be asked to complete a brief symptom checklist to assess the severity and duration of any neurologic symptoms during the past week (about 2 minutes to complete).

3.12.5.5 Research Testing

- Persistence/Expansion of anti-CD19-CAR T cells:
  - i. Peripheral blood for flow cytometry [POB Panel] or TBNK in 3 mL PTT to be sent to NCI Flow Cytometry Lab at baseline and on Days 1, 7, 14, and 28 ± 3 days. If there is a response, repeat monthly for 2-3 months, then every 3-6 months as needed.
  - ii. Peripheral blood for flow cytometry for CAR persistence at D-1, Day -2, or Day -3and on Days 1, 7, 14 and 28 ± 3 days in 3 mL PTT: to be sent Dr. Stetler-Stevenson's Lab. If there is a response, repeat monthly for 2-3 months, then every 3-6 months as needed. CD 64 will be collected from the serum cytokine specimens according to **Appendix E**.
  - iii. CSF (3-8 mL in spinal fluid collection tube) for flow cytometry each time intrathecal chemotherapy is administered after cell infusion (maximum of 5 doses) to be sent to Dr. Stetler-Stevenson's Lab. IT chemotherapy will be administered in an isovolumetric manner (See Section **4.1**); therefore, CSF removed for this purpose will be utilized to determine if Anti-CD19-CAR T cells are present in the CSF.
- Cardiology Studies
  - i. Echocardiograms were performed at baseline, during CRS, and at 1-month restaging
  - ii. Cardiology studies, troponin, BNP, and CK/CK-MB were obtained at baseline, during cytokine release syndrome, and at 1-month restaging.

3.12.6 Analysis of Disease: Day 28+/-4 (method determined by patients' type and location of disease)

3.12.6.1 Imaging Studies such as Chest CT and appropriate imaging methods of any sites relevant to the subject's disease (e.g., PET/CT).

3.12.6.2 Bone marrow aspirate and biopsy with flow cytometry for B cell leukemia/lymphoma markers will be performed in all patients: Day 28+/-4

3.12.6.3 In ALL patients and in lymphoma patients with pre-treatment peripheral blood involvement with lymphoma, flow cytometry: Day 24-32.

3.12.6.4 In addition, an LP will be performed for staging with the option to administer intrathecal chemotherapy. Intrathecal therapy will NOT be administered until at least Day 24 (i.e., during the first re-staging evaluation performed at Day 28 +/- 4) unless the

patient is suspected to have progressive disease and IT chemotherapy is needed to reduce the risk of seeding the CSF with leukemia from the diagnostic procedure. Subsequent intrathecal therapy will be given no more frequently than monthly unless CNS leukemia involvement is confirmed. Further, intrathecal chemotherapy will NOT be administered in any patient who develops CNS toxicity related to CAR T cells until at least 2 weeks after complete resolution of neurologic toxicity. In cases where CNS toxicity is due to progression of CNS leukemia IT chemotherapy may be administered. CSF will be evaluated by flow cytometry for disease and CAR T cell presence.

3.12.6.5 Post-Infusion Cognitive Evaluation (Day 21 - 28): A brief battery of cognitive tests evaluating memory, attention, processing speed, and executive functions (less than one hour) will be re-administered to patients after the anti-CD19-CAR T cell infusion (see **Appendix G**) between Day 21 and Day 28. In addition, a parent/adult observer for each participant will complete a post-infusion neurologic symptom checklist (about 2 minutes to complete).

3.12.7 Evaluations AFTER Day 28

After Day 28, if the patient is clinically stable, they may be discharged to the care of their primary oncologist and return to NIH as determined by the following protocol evaluations or changes to their clinical condition, at the discretion of the PI.

All protocol designated disease response evaluations will be performed at NIH, unless otherwise specified. After Day 28, patients will be evaluated (+/- two week) at 2, 3, 6, 9, and (+/- 4 weeks) 12 months after cell infusion and then, every 6 months during the second year, and then annually, except as noted below, or as clinically indicated.

At a minimum, evaluations will include:

3.12.7.1 History and Physical examination including vital signs

3.12.7.2 Laboratory Assessment: Chem 20: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid), complete blood count, IgG levels (every 3-6 weeks).

3.12.7.3 Toxicity assessment, including evaluation of visual symptoms and if changes have occurred from baseline, i.e. changes in visual acuity, an ophthalmologic consult will be performed.

3.12.7.4 Disease Evaluation (based on etiology and location of disease):

- Scans and x-rays: Chest CT and appropriate imaging of any sites relevant to the subject's disease. When applicable, PET scans will be performed 3 and 6 months after cell infusion and then as clinically indicated.
- Bone marrow aspirate and biopsy with flow cytometry for B cell leukemia/lymphoma markers will be performed in all patients.

- Flow cytometry for CD19 and CD22 in ALL patients and in lymphoma patients with pre-treatment peripheral blood involvement with lymphoma. Repeat evaluations will be performed as indicated above until off study criteria are met.
- In addition, a diagnostic LP with the option for administration of intrathecal chemotherapy (per Section **4.1**) will be performed at each restaging, and as clinically indicated. CSF will be evaluated by flow cytometry for disease and CAR T cell presence.

3.12.7.5 Post-Infusion Follow-up Cognitive Evaluation (3 months +/- 4 weeks): A brief battery of cognitive tests evaluating memory, attention, processing speed, and executive functions (less than one hour to complete) will be re-administered to patients if they return for a 3-month visit (+/- 4 weeks) after the anti-CD219-CAR T cell infusion (see **Appendix G**). In addition, a parent/observer for each participant will complete a post-infusion neurologic symptom checklist (less than 15 minutes to complete).

3.12.7.6 Persistence/Expansion of Anti-CD19-CAR T cells:

i. PCR: 1-3 mL of blood in a PAXGene or a EDTA tube to be sent to NCI/Frederick until negative by flow cytometry

ii. Peripheral blood for flow cytometry: to be sent to NIH Clinical Pathology Flow Cytometry Lab until negative by flow cytometry.

iii. CSF (3-8 mL in flow media) for flow cytometry each time a diagnostic LP is performed to be sent to (NCI Flow Cytometry ab) NIH Clinical Pathology Flow Cytometry Lab if performed at the NIH CC and schedule permits.

3.12.7.7 Detection of RCR per Section **5.1.4**.

3.12.7.8 Long-term follow up of patients receiving gene transfer (on this study or on a long-term follow-up protocol):

Physical examinations with medical history will be performed and documented annually for 5 years following cell infusion to evaluate long-term safety on this protocol or on 15-C-0028: Follow-Up Evaluation for Gene-Therapy Related Delayed Adverse Events after Participation in Pediatric Oncology Branch Clinical Trials. This long term follow up may be performed by the local health care provider who will submit documentation of H&P, with the necessary blood samples for RCR to POB NIH. After 5 years, health status data will be obtained from surviving patients via telephone contact or mailed questionnaires annually and blood will be requested to be sent to NIH annually for 10 more years for storage in the event of a new cancer, wherein RCR studies will be performed. The total follow up period for retroviral vectors is 15 years.

3.12.8 Off Treatment Follow up Evaluations AFTER Day 28

Subjects will be followed per the following

- Off treatment all studies will be done as per day 28 (+/-4).
- If no response and no ongoing toxicity (i.e. progressive disease) then no further clinical follow up required except for ongoing toxicity follow up and evaluations contained in Sections **3.12.6** and **3.12.7**, which will be collected for 15 years.

- If response, results and documentation for physical examinations, vital signs, CBC with diff, Chem 20, and IGG levels, will be collected. Bone marrows/lumbar punctures will be done at the primary oncologist's discretion and documentation collected. All will be collected until relapse or bone marrow transplantation or other therapeutic interventions are initiated. The evaluations in Sections **3.12.6** and **3.12.7** will be collected for 15 years.

3.12.9 Follow up Off-Study

Once a patient develops PD or goes on to other therapies, patients will be followed for Gene therapy follow up **ONLY** as per Section **5.1.3**, on this protocol or on 15-C-0028: *Follow-Up Evaluation for Gene-Therapy Related Delayed Adverse Events after Participation in Pediatric Oncology Branch Clinical Trials* protocol per FDA regulations.

3.12.9.1 Gene therapy follow up (See Section **5.1.3** and **Appendix E**)

a. Persistence/Expansion of Anti-CD19-CAR T cells

b. Detection of RCL

c. Long-term follow up for toxicity assessment

3.13 Study Calendar

See **Appendix B**: Study Calendar

3.14 Protocol Accrual Suspension

The study will be halted pending discussions with the FDA and NCI IRB if any of the following conditions are met throughout the study implementation:

a. Development of EBV lymphoma or polyclonal lymphoproliferative disease (PLPD).

b. Grade 4 cytokine release syndrome in **more than** 2 subjects in the protocol (both cohorts).

c. Any Grade 5 event at least possibly related to the research product.

3.15 Criteria for Removal from Protocol Therapy and Off Study Criteria

3.15.1 Criteria for removal from protocol therapy

Patients will be taken off treatment (and followed until off-study criteria are met) for the following:

- Treatment limiting toxicity (TLT) due to cell infusion [unless criteria for subsequent infusions are met]. The definition of TLT is in Section **3.6.1**.
- Grade 3 autoimmunity that involves vital organs (heart, kidneys, brain, eye, liver, colon, adrenal gland, lungs).
- Grade 3 or 4 toxicity due to cell infusion (reaction to cellular product or infusion reaction) that is not reversible to a grade 2 or less within 8 hours with acetaminophen and/or diphenhydramine as outlined in Section **3.6**, the patient will not have the option of receiving a second infusion of cells.

- General or specific changes in the patient's condition render the patient unacceptable for further treatment on this study in the judgment of the investigator.
- Progressive disease (unless criteria are met for additional doses of cells)

3.15.2 Off-Study Criteria

- Patient withdrawal from protocol (in which case the reason will be documented, if possible)
- Death
- Conclusion of the 15 years of follow up, or subject enrolls in long-term follow up protocol of patients receiving gene transfer.

3.15.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off-study. A Participant Status Update Form from the website (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

Concomitant medications to control side effects of therapy will be given. Patients who require transfusions will receive irradiated blood products. Serotonin antagonists may be administered for nausea and vomiting as prophylaxis prior to chemotherapy and as needed. Additional antiemetics will be administered as needed for nausea and vomiting uncontrolled by serotonin antagonists. Antibiotic coverage for antimicrobial prophylaxis and central venous catheters may be provided at the discretion of the investigator. If patients require steroid therapy beyond physiologic replacement and for purposes other than to control CRS they will be taken off treatment.

4.1 CONCURRENT THERAPY FOR EXTRAMEDULLARY LEUKEMIA OR CNS LYMPHOMA

Patients with CNS leukemia or lymphoma are allowed. Concurrent craniospinal radiation will not be allowed. Concurrent therapy or prophylaxis for CNS leukemia or lymphoma consisting of standard intrathecal chemotherapy will be allowed as clinically indicated in patients with ALL or pediatric NHL at times of restaging spinal taps. Intrathecal chemotherapy will NOT be given until at least Day 24 (i.e., during the first re-staging evaluation performed at Day 28 +/- 4) unless the patient is suspected to have progressive disease and IT chemotherapy is needed to reduce the risk of seeding the CSF with leukemia from the diagnostic procedure. Subsequent intrathecal therapy will be given no more frequently than monthly unless CNS leukemia involvement is confirmed. Further, intrathecal chemotherapy will NOT be administered in any patient who develops CNS toxicity related to CAR T cells until at least 2 weeks after complete resolution of neurologic toxicity.

4.1.1 Regimen

The standard intrathecal chemotherapy regimen used to prevent or manage CNS relapse will be employed. Individual agents may be eliminated from the standard triple intrathecal (TIT)-chemotherapy regimen based on clinical contraindications.

4.1.2 Dose

TIT-chemotherapy will be dosed by age as follows:

Age (years)	MTX (mg)	HDC (mg)	ARA-C (mg)	Volume (ml)
<1	7.5	7.5	15	5
1	8	8	16	6
2	10	10	20	7
3-8	12	12	24	8
≥9	15	15	30	10

MTX – methotrexate; HDC – Hydrocortisone; ARA-C – cytarabine

4.1.3 Administration

Delivery should be isovolumetric (ml CSF out = ml drug in) via lumbar puncture (LP) in the lateral decubitus position. Patients should remain in prone or Trendelburg position for 30 minutes post LP to facilitate drug circulation throughout the CNS.

Leucovorin will be given as 10 mg/m2/dose (rounded up to the nearest 5 mg increment to a maximum dose of 15 mg) PO or IV x 2 doses, 24 hours and 30 hours after intrathecal methotrexate.

4.2 INFECTION PROPHYLAXIS

Dosing of pediatric patients for infection prophylaxis will be in accordance with NIH Clinical Center Blood & Marrow Transplant Consortium guidelines (http://intranet.cc.nih.gov/bmt/clinicalcare/guidelines.shtml).

Special Statement about IVIG: Since anti-CD19-CAR T cells have been demonstrated in other studies to also eliminate normal B cells, serum IgG levels will be monitored before cell infusion and every 3-6 weeks after infusion. If IgG < 500, IVIG will be administered at a dose of 500 mg/kg with the appropriate pre-medications and per institutional guidelines unless clinically contraindicated.

4.3 PROPHYLAXIS AND TREATMENT OF TUMOR LYSIS SYNDROME IN CHILDREN

Pediatric patients at greatest risk are those with high disease burden (e.g. bulky disease) and high cell turnover (e.g. elevated uric acid, LDH).

4.3.1 Regimen

Subjects deemed to be at high risk of tumor lysis syndrome should begin allopurinol at a dose of approximately 100 mg/m^2/dose po TID (maximum dose 200 mg TID). This should be started at

least 8 hours prior to the first dose of the preparative regimen and continued until disease burden is reduced (e.g. peripheral blasts clear) or it is apparent that no tumor lysis has developed.

Additional suggested monitoring and supportive care for subjects deemed to be at high risk of tumor lysis syndrome:

- Hydration: Starting at least 6 hours prior to initiating preparative regimen, and continuing for at least 24 hours after cell infusion, IV fluids should be administered at a rate of 90-120 ml/m2/hour (1.5 – 2 times maintenance) to maintain urine specific gravity <1.010 and normal urine output. Potassium should be avoided. Then IV+PO should continue at 90/m2/hour (1.5 times maintenance) until disease burden is reduced (e.g. peripheral blasts clear) or it is apparent that no tumor lysis has developed after at least 72 hours
- For subjects judged to be at high risk for uric acid nephropathy, consider the use of rasburicase rather than allopurinol.

For subjects with renal insufficiency, consult Nephrology.

4.3.2 Blood Product Support

Using daily CBC's as a guide, the patient will receive platelets and packed red blood cells (PRBC's) as needed. Attempts will be made to keep Hb >8.0 gm/dl, and plts >10,000/mm3. All blood products with the exception of the lymphocyte product will be irradiated. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBC's and decrease the risk of CMV infection.

4.3.3 Hydroxyurea

Patients receiving hydroxyurea are eligible providing the dose has been stable for at least two weeks prior to starting apheresis. Hydroxyurea must be discontinued at least 24 hours prior to the start of the first dose of fludarabine.

4.3.4 Treatment of Cytokine Storm

Cytokine storm should be monitored closely according to the algorithm detailed in **Appendix F**. If treatment is indicated according to the algorithm it should be initiated with tocilizumb (anti-IL6R mAb) with other measures including corticosteroids or other immunosuppressive mAbs initiated at the discretion of the Principal Investigator and/or his designee.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

5.1.1 Post cell infusion evaluations for T-cell persistence:

Samples will be drawn as outlined in **Appendix E** and Section **3.12** for evaluation of CAR-T cell persistence.

5.1.2 Immunological Testing

When appropriate, apheresis will be performed or the maximum amount of blood allowed to be drawn in a single day based on the patient's weight will be obtained and PBMC will be isolated through whole blood by purification using centrifugation on a Ficoll cushion. Aliquots of these PBMC will be:

a. Cryopreserved for immunological monitoring of cell function,

b. Subjected to DNA and RNA extraction for PCR analysis of vector copy number estimation

c. Lymphocytes will be tested directly and following in vitro culture. Direct immunological monitoring will consist of quantifying CD3+ T cells that express the anti-CD19 CAR by FACS analysis using the anti-idiotype antibody to the CAR. Site density of the CD19 antigen will be quantified on any blasts present using flow cytometry techniques.

5.1.3 Monitoring Gene Therapy Trials: Persistence and RCR

a. Immunological monitoring using both anti-idiotype antibody staining to detect CARs on the surface of T cells and CD19-specific cytokine production to detect functional anti-CD19 T cells will be used to quantitate persistence of T cells in the blood. Persistence will be determined at about Day 1, 7, 14, and 28 after cell infusion, then monthly (+/- 7 days) for 2-3 additional months, and then every 3-6 months thereafter until no CAR T cells are detected. In addition, CAR-transduced T cell might be quantitated by using specific PCR assays capable of detecting the unique DNA sequence for each retroviral vector engineered T-cell.

b. Patients' blood samples will be obtained and undergo analysis for detection of RCR by PCR prior to cell infusion (at baseline) and at 3, 6, and 12 months post cell administration. Blood samples will be archived annually thereafter if all previous testing has been negative with a brief clinical history. If a patient dies or develops neoplasms during this trial, efforts will be made to assay a biopsy sample for RCR. If any post-treatment samples are positive, further analysis of the RCR and more extensive patient follow-up will be undertaken, in consultation with the FDA. RCR PCR assays detect the GaLV envelope gene and are performed under contract by the Indiana University Vector Production Facility. The results of these tests are maintained by the contractor performing the RCR tests and by the Pediatric Oncology Branch research team.

c. Due to the nature of these studies, it is possible that expansion of specific T-cell clones will be observed as tumor reactive T-cells proliferate in response to tumor antigens. Therefore, care will be taken to track T-cell persistence both immunologically and molecularly. Blood samples (5-10 mL) for persistence of CAR transduced cells will be obtained prior to cell infusion (baseline), at 1, 7, 14, and 28 days (+/- 4 days), then so long as a response continues to be demonstrated, every month (+/- 7 days) for 2-3 months then every 3-6 months until off-study criteria are met. If any patient has more than 5% persistence of CAR gene transduced cells at month 6 (by anti-idiotype antibody staining) the previously archived samples will be subjected to techniques that would allow the identification of clonality of persisting CAR gene transduced cells. Such techniques may include T cell cloning or LAM-PCR. If a predominant or monoclonal T cell clone derived from CAR gene transduced cells is identified during the follow-up, the integration site and sequence will be identified and subsequently analyzed against human genome database to determine whether the sequences are associated with any known human cancers. If a predominant integration site is observed, the T cell cloning or LAM-PCR test will be used at an interval of no more than three months after the first observation to see if the clone persists or is transient. In all instances where monoclonality is persistent

and particularly in instances where there is expansion of the clone, regardless of whether or not the sequence is known to be associated with a known human cancer, the subject should be monitored closely for signs of malignancy, so that treatment, if available, may be initiated early.

5.1.4 Minimal Residual Disease (Leukemic Cells)

Patients who are anticipated to be MRD negative by flow cytometry, may have one additional sample collected at the time of disease evaluation (5 mL of blood or 2 mL of bone marrow in EDTA tube) to evaluate MRD. Samples will be sent immediately to the laboratory where DNA will be extracted and sent to Adaptive Biotechnology Corporation in Seattle where next generation DNA sequencing will be used to identify residual leukemia. Clinical parameters considered when selecting patients for sample submission include clinical course (i.e. presence of cytokine storm), decrease in circulating blasts (if any) or B-cells, or presence of CAR T cells by flow cytometry on periodic measurements of blood (as described in Immunologic Testing).

As of Amendment P, all patients who attained flow cytometric MRD negativity following CAR infusion will have their samples sent (de-identified and only if available) to Adaptive to evaluate for MRD using next generation DNA sequencing to identify residual leukemia.

5.1.5 Blood and Tissue Specimen Banking

Blood and tissue specimens from consenting participants collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if the risks of the new questions were covered in the consent document. If new risks are associated with the research (e.g., analysis of germ line genetic mutations) the principal investigator must amend the protocol and obtain informed consent from all research subjects. Any new use of samples will require prospective IRB review and approval.

5.1.6 Minimal Residual Disease Detection

DNA samples from blood and/or bone marrow will be stripped of identifiers and coded, and may be sent to Adaptive Biotechnology Corporation in Seattle where next generation DNA sequencing will be used to identify residual leukemia.

5.1.6.1 T-cell Analysis

Samples of blood and/or bone marrow stripped of identifiers and coded, may be sent to Adaptive Biotechnology Corporation in Seattle. T cells will be analyzed for clonality by sequencing the T cell receptors.

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

Blood and tissue collected during the course of this study will follow the handling procedures established by the Central Repository for NCI/Frederick. Blood and tissue processed by the Department of Transfusion Medicine (DTM), Clinical Center will comply with the Standard Operating Procedures of DTM. All samples (blood or tissue) are tracked by distinct identification labels that include a unique patient identifier and date of specimen collection. All cryopreserved samples are tracked for freezer location and storage criteria. All samples are stored in monitored freezers/refrigerators either in the Central Repository for NCI/Frederick or in the investigator's

laboratory at specified temperatures with alarm systems in place. All samples (blood or tissue) are documented in a secure central computer database with identification and storage location, with computer backup according to established standards for the Central Repository for NCI/Frederick.

At the completion of this protocol, samples will be stored for future use under a repository protocol. Any new use of samples will require an IRB approved protocol or approval of an exemption from IRB review. Otherwise, specimens will be disposed of in accordance with the environmental protection laws, regulations and guidelines of the Federal Government and the State of Maryland.

Any new uses of samples collected during the course of this trial must be reviewed and approved by the NCI IRB. Any loss or unintentional destruction of the samples will be reported to the IRB.

5.2.1 Pediatric Research Samples

Research blood sample aliquot size will be minimized for patients < 18 years of age and the total amount restricted to a maximum of 3 ml/kg per draw and 7 ml/kg per 6-week period (maximum 450 ml). In the event that blood draws are limited due to patient size, research studies will be performed in order of priority as listed in **Appendix E**. Small volume apheresis collections will not be included in this calculation since such is performed isovolumetrically with minimal red cell loss.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

6.2 RESPONSE CRITERIA

Note that response criteria depend on the tumor type described below:

For the purposes of this study, patients should be re-evaluated for response as outlined in Section **3.12.6**. In addition to a baseline scan, confirmatory scans should also be obtained 4 weeks following initial documentation of objective response.

6.2.1 Response Criteria Lymphoma (Adapted from: Cheson BD, et al: Revised response criteria for malignant lymphoma. J Clin Oncol 2007; 25 :579-86)[57]

6.2.1.1 Complete Response (CR): CR requires all of the following:

Complete disappearance of all detectable clinical evidence of disease and disease-related symptoms if present before therapy.

1. Typically, FDG-avid lymphoma (large cell, mantle cell and follicular lymphomas are all typically FDG-avid): in patients with no pretreatment PET scan or when the PET scan was positive before therapy, a post-treatment residual mass of any size is permitted as long as it is PET negative.
2. Variably FDG-avid lymphomas/FDG avidity unknown: in patients without a pretreatment PET scan, or if a pretreatment PET scan was negative, all lymph nodes and nodal masses must have regressed to normal size (≤ 1.5 cm in greatest diameter if > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their long axis and more than 1 cm in their short axis before treatment must have decreased to ≤ 1.0 cm in their short axis after treatment.
3. The spleen and/or liver, if considered to be enlarged before therapy on basis of physical exam or CT scan, must be normal size on CT scan and not be palpable on physical examination and nodules thought to represent lymphoma must no longer be present.
4. A bone marrow aspirate and biopsy is performed only when the patient had bone marrow involvement with lymphoma prior to therapy or if new abnormalities in the peripheral blood counts or blood smear cause clinical suspicion of bone marrow involvement with lymphoma after treatment. The bone marrow aspirate and biopsy must show no evidence of disease by morphology or if indeterminate by morphology it must be negative by immunohistochemistry. The biopsy core sample must be a minimum of 20 mm in length.

6.2.1.2 Partial Response (PR): PR requires all of the following:

1. ≥ 50% decrease in sum of the product of the diameters (SPD) of up to 6 of the largest dominant nodes or nodal masses. Dominant nodes or nodal masses should be clearly measurable in at least 2 perpendicular dimensions, should be from different regions of the body if possible and should include mediastinal and retroperitoneal nodes if possible.
2. No increase in size of nodes, liver or spleen and no new sites of disease.
3. If multiple splenic and hepatic nodules are present, they must regress by ≥ 50% in the SPD. There must be a > 50% decrease in the greatest transverse diameter for single nodules.
4. Bone marrow is irrelevant for determination of a PR. If patient has persistent bone marrow involvement and otherwise meets criteria for CR the patient will be considered a PR.
5. Typically FDG-avid lymphoma: for patients with no pretreatment PET scan or if the PET scan was positive before therapy, the post-treatment PET scan should be positive in at least one previously involved site. Note: in patients with follicular lymphoma or mantle-cell lymphoma, a PET scan is only indicated in patients with one or at most two residual masses that have regressed by 50% on CT scan.

6.2.1.3 Progressive Disease (PD): Defined by at least one of the following:

1. ≥ 50% increase from nadir in the sum of the products of at least two lymph nodes, or if a single node is involved at least a 50% increase in the product of the diameters of this one node.
2. Appearance of a new lesion greater than 1.5 cm in any axis even if other lesions are decreasing in size
3. Greater than or equal to a 50% increase in size of splenic or hepatic nodules
4. At least a 50% increase in the longest diameter of any single previously identified node more than 1 cm in its short axis.
5. Lesions should be PET positive in typically FDG-avid lymphomas unless the lesion is too small to be detected by PET (<1.5 cm in its long axis by CT)

6.2.1.4 Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD. PET should be positive in typically FDG-avid lymphomas.

Flow cytometric, molecular or cytogenetic studies will not be used to determine response.

6.2.2 Response Criteria for ALL

*Modified from: Cheson BD, et al. Revised recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia[58].

Bone Marrow Classification

	% blasts (at least 200 cells counted)
M1	<5%
M2	5 - 25%
M3	> 25%

6.2.2.1 Complete Response (CR)

1. M1 marrow, absence of peripheral blasts (morphologic), absence of extramedullary sites of disease, peripheral blood neutrophil count > 1,000/□L and platelet count > 100,000/□L. This parameter will be the requisite criterion for CR. The following additional parameters will be reported as exploratory findings.
2. Morphologic CR with incomplete blood count recovery (CRi): Above CR criteria without specified blood counts.
3. Cytogenetic CR (CRcyto): In addition to above CR criteria, reversion to normal karyotype for those with previously detected cytogenetic abnormality.
4. Molecular CR (CRmolec): In addition to above CRc criteria, normalization of previously detected molecular cytogenetic abnormality.

6.2.2.2 Partial Response (PR)

1. M2 marrow and a decrease in the percentage of marrow blasts by at least 50%, absence of peripheral blasts (morphologic), absence of extramedullary sites of disease.

6.2.2.3 Hematological Activity (HA)

Does not meet the criteria for CR or PR with any of the following:

1. At least a 50% decrease in the percentage of marrow blasts
2. At least a 50% decrease in the absolute peripheral blast count
3. Improvement of the peripheral blood neutrophil count to > 1,000/µL or platelet count to > 100,000/µL

6.2.2.4 Stable Disease (SD)

1. Does not meet the criteria for CR, PR, HA, or PD

6.2.2.5 Progressive Disease (PD)

1. Worse marrow classification (i.e., M status) with at least a 50% increase in the percentage of marrow blasts.

   Or

2. No change in marrow classification (i.e., M status), but a 50% or greater increase in absolute peripheral blast count or extent of extramedullary disease

6.2.2.6 CNS Classification

CNS is the most common site of extramedullary disease in ALL. The following table lists the CNS disease classification. This classification should be used when evaluating the patient's overall response to treatment.

CNS Classification

	CSF Cell Count and Cytology
CNS 1	0 blasts on cytospin
CNS 2	< 5/µl WBCs, cytospin positive for blasts or Traumatic spinal tap with ≥ 10/µL RBCs, WBC ≥ 5/µL, cytospin positive for blasts but negative by Steinherz/Bleyer algorithm
CNS 3	≥ 5/µl WBCs, cytospin positive for blasts or Traumatic spinal tap with ≥ 10/µL RBCs, cytospin positive for blasts, and positive Steinherz/Bleyer algorithm*

*Steinherz/Bleyer algorithm method of evaluating traumatic lumbar punctures:

If the patient has leukemic cells in the peripheral blood and the lumbar puncture is traumatic and contains ≥ 5 WBC/μL and blasts, the following algorithm should be used to distinguish between CNS2 and CNS3 disease:

CSF WBC/RBC > 2X Blood WBC/RBC

6.3 PERSISTENCE CRITERIA FOR ANTI-CD19 CAR TRANSDUCED T CELLS

The absolute number of persisting anti-CD19-CAR-transduced T cells will be calculated by multiplying the absolute peripheral blood lymphocyte (PBL) count by the percentage of PBL that express both CD3 and the anti-CD19 CAR. The percentage of PBL that express both CD3 and the anti-CD19 CAR will be determined by a flow cytometry assay that involves staining with anti-CD3 and anti-idiotype antibody that is specific for the scFv on the extracellular portion of the anti-CD19 CAR.

This analysis will be performed on Days 1, 7, 14, and 28 (+/- 4 days) then, so long as the patient continues to have a response or achieve CR, monthly (+/- 7 days) for 2-3 additional months then every 3-6 months after cell infusion. The 4 week time point is the only primary analysis that will be used for the primary evaluation of the number of CD3+ cells that express the anti-CD19 CAR.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website at (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected” also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.6 Disability

A substantial disruption of a person’s ability to conduct normal life functions.

7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB AND CLINICAL DIRECTOR REPORTING

7.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
   - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
   - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
   - All Grade 5 events regardless of attribution;
   - All Serious Events regardless of attribution.

   NOTE: Grade 1 events are not required to be reported.

7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

7.3 IND Sponsor Reporting Criteria

During the first 30 days after the subject receives investigational agent/intervention, the investigator must immediately report to the sponsor, using the mandatory MedWatch form 3500a or equivalent, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. For serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention, only report those that have an attribution of at least possibly related to the agent/intervention.

Required timing for reporting per the above guideline:

- Deaths (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- Other serious adverse events as well as deaths due to progressive disease must be reported within one business day

Events will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov and to the CCR PI and study coordinator.

7.4 Institutional Biosafety Committee (IBC) Reporting Criteria

7.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of the anti-CD19 CAR transduced cells as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the anti-CD19 CAR transduced cells, but are not fatal or life-threatening, must be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form 3500a.

7.4.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information described below. Alternatively, the IRB continuing review report can be sent to the IBC in lieu of a separate report. Please include the IBC protocol number on the report.

7.4.2.1 Clinical Trial Information

A brief summary of the status of the trial in progress or completed during the previous year. The summary is required to include the following information:

- the title and purpose of the trial
- clinical site
- the Principal Investigator
- clinical protocol identifiers;

- participant population (such as disease indication and general age group, e.g., adult or pediatric);
- the total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons
- the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed,
- if the trial has been completed, a brief description of any study results.

7.4.2.2 Progress Report and Data Analysis

Information obtained during the previous year's clinical and non-clinical investigations, including:

- a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- a summary of all serious adverse events submitted during the past year
- a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death
- a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

7.5 DATA AND SAFETY MONITORING PLAN

7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS and to the Sponsor.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.5.2 Sponsor Monitoring Plan

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subjects' protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by an CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

7.5.3 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NCI-IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 STATISTICAL CONSIDERATIONS

8.1 GENERAL CONSIDERATIONS

All subjects who are enrolled in the study and received the planned dose of anti-CD19 CAR T cells will be included in the statistical summaries and analyses of safety data. All available data will be used and thus, missing data will not be estimated or carried forward in any statistical summary or analyses.

Descriptive summary statistics include the number of subjects, mean, median, percent coefficient of variation (%CV) (if required), standard deviation, and minimum and maximum values (quantitative variables) or the number of subjects and percentages by category (qualitative variables). Changes will be assessed as the difference between the baseline measurement and the appropriate post-cell infusion measurement unless otherwise specified. Baseline is defined as the last non-missing value prior to initiation of preparative regimen except for cytokine analyses where baseline is defined as the last non missing value prior to cell infusion. Individual subject listings of data will also be provided to allow for review of all safety parameters.

8.2 DOSE ESCALATION DESIGN

The cell doses for patients will be weight-based. The Phase I dose-escalation design will consist of three cohorts of a minimum of three patients per cohort as described in Sections **3.6** and **3.7**. **COMPLETED**

In patients with BMI equal to or less than 30, the dose of anti-CD19-transduced T cells for each cohort will be based on doses calculated using actual body weight. Patients with BMI greater than 30, will have doses calculated using ideal body weight dose (IBW) dose plus 50% of the difference between actual weight and IBW (see **Appendix D**).

In order to determine if there are differences in safety or feasibility related to manufacturing or administering anti-CD19 CAR T cells after a nonmyeloablative preparative regimen in children and young adults who have not undergone prior allogeneic stem cell transplant (SCT) versus those that have previously undergone SCT, the enrollment will be stratified for these two groups of subjects. For each stratum of patients, the number of anti-CD19 transduced T cells administered in each cohort will be:

- Cohort 1 1×10^6 transduced T cells/kg (± 20%)
- Cohort 2 3×10^6 transduced T cells/kg (± 20%)
- Cohort 3 1×10^7 transduced T cells/kg (± 20%)

Doses of anti-CD19-CAR transduced T-cells will be administered in a standard 3 + 3 dose escalation design until MTD is determined. After treatment of the first patient in a cohort there will be a two week (14 day) safety assessment prior to enrollment of the 2nd patient in each cohort. Subsequent patients in that cohort may be enrolled after a one week safety assessment period following cell infusion. Patients will be enrolled sequentially; therefore, enrollment will not proceed to a higher dose level until all patients have been treated in the prior cohort and the last patient treated on the completed cohort has been observed for at least 4 weeks. If cell growth limitations preclude administration of the targeted cohort cell dose, the patient will receive as many cells as possible, and be considered part of the lower dose cohort. If a minimum of 1.0 x105 anti-CD19-CAR-transduced T cells per kg cannot be obtained for infusion, the patient will not be treated and if possible a second attempt to produce cells for infusion will be made. As per Section 3.7, toxicity data collected from the SCT stratum dose cohorts may be used to inform dose escalation decisions in the non SCT dose cohorts. For example, if 3 patients in the SCT stratum complete a cohort without DLT, the next patient enrolled in either stratum may proceed to the next dose cohort. But if 3 patients in the NON-SCT stratum complete a cohort without DLT, the SCT stratum must continue enrollment until 3 patients complete therapy without DLT prior to dose escalation. **COMPLETED**

To gain further experience with the safety, feasibility and clinical activity of the anti-CD19 CAR T cells in this patient population, the MTD (or highest cell dose studied) will be expanded to 15 patients. If the MTD or highest cell dose studied is the same for both strata (prior SCT vs. NO prior SCT) one expansion cohort will be studied and efforts will be made to include a balance of patients with prior SCT, without prior SCT, ≤ 12 years of age and > 12 years of age. If different MTDs are observed per stratum, a total of 12 patients per stratum will be enrolled at the MTD, attempting to balance enrollment between patients ≤ 12 years of age and > 12 years of age. **COMPLETED**

8.3 SAFETY AND FEASIBILITY OF HIGH-DISEASE BURDEN VS. NON-HIGH-DISEASE BURDEN

Given the impressive response to this therapy, Amendment G allows us to explore further the study findings. Based on the relationship observed between the incidence of Grade 3-4 CRS and patients with high-disease burden, additional investigation is warranted to determine if reduction of disease burden prior to administration of the anti-CD19 CAR will decrease the incidence of severe CRS, while using an updated assessment/treatment algorithm for CRS. Amendment G will allow us to evaluate a safe option for bringing this therapy to patients with high-burden of disease. For purposes of this study, high-burden disease will be defined as M3 bone marrow blasts and/or presence of peripheral blood blasts on routine CBC. We will therefore explore the safety and feasibility of this therapy in two groups of patients:

1. Arm 1 = Patients without high-burden disease, or who due to safety concerns require the cyclophosphamide/fludarabine preparative regimen
2. Arm 2 = Patients with high-burden disease

Thirty patients are to be enrolled in arm 1 and 30 patients in arm 2 in order to have sufficient patients to establish a reasonably precise confidence bound on the safety of the regimen overall as well as the feasibility of delivering treatment to patients in arm 2.

8.3.1 Safety

All 60 patients on arms 1 and 2 will be considered together for a safety evaluation. Combining arms 1 and 2, if there are 0-1 patients in the first 10 who have a grade 4 CRS, then accrual would continue as planned per arm. However, if there are 2 patients within the first 10 who experience a grade 4 CRS then no further patients may be accrued. If accrual beyond the initial 10 is permitted, then this will continue to be the case provided that the cumulative percentage of patients with grade 4 CRS on both arms combined is below 20%; realizing 20% or more with grade 4 CRS at any time would require ending accrual and amending the study to reconsider toxicity definitions (since they are newly implemented and not fully vetted) or revise the treatment regimen. The upper one sided 90% CI on 2/10 is 45.0% which would indicate possible consistency with a very high rate of grade 4 CRS. Provided that these interim toxicity limits are not met, accrual will be permitted until 60 total patients are enrolled, 30 per arm and the overall fraction with grade 4 CRS will be determined. As illustrations, if 6/60 have grade 4 CRS, the upper one-sided 90% CI bound on this fraction is 16.9% and if the fraction ends up being as high as 20% at the end of 60 patients, e.g. 12/60 with grade 4 CRS, the upper 90% one-sided confidence bound is 28.2%.

8.3.2 Arm 2 Feasibility

In addition to safety, establishing feasibility of administering the anti-CD19 CAR T cells within 21 days of the planned infusion is an important goal of this study. Feasibility will be assessed only in Arm 2 and defined by the successful manufacturing, expansion, and administration (within 21 days of the planned infusion date) of the anti-CD19 CAR T cells to satisfy the dose level and meet the requirements of the COA. Unless there is excessive toxicity, enroll 30 evaluable patients on arm 2 over a period of approximately 3 years. If there are 22 or more patients among 30 who are able to fully receive therapy, then the probability this would be the case is 87.1% if the true rate of feasibility were 80% while it is only 9.4% if the true rate of feasibility were 60%. Thus, obtaining 22 or more patients who are able to receive treatment out

of 30 would demonstrate a feasibility rate more consistent with 80% or greater as opposed to 60% or less, and thus this would be a reasonable goal.

8.4 ACCRUAL TARGET AND STUDY DURATION

Twenty subjects were enrolled to achieve MTD and expanded cohorts in the initial objectives of this study. To complete Arm 1 and 2 primary objectives added in Amendment G, up to 30 subjects will be enrolled in each arm. The study will allow for a small number of inevaluable patients of 5 in each arm (patients enrolled but who cannot receive cells, either due to physical deterioration or withdrawal of consent during cell growth, or inability to grow adequate cells). Thus the maximum accrual will be 90 patients (20 previously treated + 30 in each arm [60 total] + 5 inevaluable patients in each arm [10 total]. Up to a total of 180 patients may be screened to allow for enrollment of up to 90 patients eligible to proceed to treatment.

8.5 FEASIBILITY

In addition to safety, establishing feasibility of the creation of anti-CD19 CAR T cells within 21 days of the planned infusion for patients (who have had prior SCT or not) is an important goal of this study. Feasibility will be defined by the successful manufacturing and expansion of the anti-CD19 CAR T cells to satisfy the targeted dose level and meet the requirements of the COA. If after the first 6 patients per stratum have been enrolled, more than 3 are unable to have adequate anti-CD19 CAR T cells produced (that meet COA for infusion), accrual to that stratum in the study will be terminated, since the upper 90% one-sided confidence interval about 3/6 is 79.9%; thus, it would be unlikely that the true feasibility rate is 80% or greater, which would be desirable. Each stratum will be evaluated independently for feasibility. **COMPLETED**

8.6 SECONDARY ENDPOINTS

8.6.1 Efficacy Analyses

The best overall response will be assessed at end of study, based upon the disease assessments recorded during the study visits, and reported by dose cohort in terms of confirmed CR/PR, unconfirmed CR/PR, Hematological Activity (ALL only), SD, or PD. The rate of relapse from CR prior to end of study will also be summarized by dose cohort only for the subjects who achieve confirmed CR during the study. The overall objective response rate (CR + PR) will be summarized by dose cohort. The duration of objective response will be summarized for those subjects who achieve objective response (CR + PR). In subjects who have a confirmed response, duration of response will be calculated from the first date of documented response until progression. Unconfirmed CR or PR is defined as the first documentation of response. Overall survival (OS) and progression-free survival (PFS) will be assessed by dose cohort. Progression-free survival (PFS) will be measured from the start of the preparative regimen until the documentation of disease progression or death due to any cause, whichever occurs first. Overall survival (OS) will be determined as the time from the start of the preparative regimen until death.

8.6.2 Persistence of CD19-CAR T Cell Analyses

Peripheral blood, bone marrow aspirate, and CSF will be collected and separately analyzed for the presence of anti-CD19 CAR T cells. The percentage of all CD3+ cells in a sample that are positive by flow cytometry for anti-CD19 CAR containing T cells will be analyzed and reported as time from T cell infusion.

8.6.3 Cytokines

Endogenously produced IFNg, TNFa, GM-CSF, IL-6 and IL-2 will be measured by ELISA and reported as change from baseline. The baseline will be defined as the cytokine level just prior to cell infusion. Cytokine levels will also be analyzed in the context of clinical response.

8.6.4 Monitoring for CD19+ Cells

Peripheral blood, bone marrow aspirates, and CSF will be collected and analyzed for the presence of CD19+ cells by flow cytometry. Any CD19+ cells will be analyzed to determine whether these are normal B cells or leukemia blasts. The percentage of CD19+ normal B cells and CD19+ leukemia blasts relative to total cells analyzed in the sample will be reported and analyzed in relation to time from cell infusion and compared to baseline. Baseline percentage of CD19+ normal B cells and CD19+ leukemia blasts will be defined as the result obtained from the same sample type (e.g. peripheral blood, CSF, or aspirate) prior to cell infusion.

8.6.5 Toxicity in patients with CNS disease

Patients enrolled in Arm 1 or Arm 2 after approval of Amendment G who have CNS disease will be pooled and analyzed for descriptive reporting of efficacy and toxicity. This data may be used in an exploratory fashion to develop new questions related to use of and indications for anti-CD19 CAR gene engineered T cells.

8.6.6 Analyses of Cognitive Testing Data

To analyze the data for the secondary cognitive objective, we will use paired t-tests to examine the change in scores between the baseline and post-infusion evaluation to determine if the change is significantly different from zero. For the exploratory cognitive objectives, we will use analysis of variance with repeated measures to examine change in scores between the baseline and the follow-up evaluations. In addition, correlations or analyses of variance will be computed to examine the relationship between cognitive scores and cytokine levels.

8.6.7 Analyses of Cardiology Studies

The effect of CAR cell therapy on cardiac function will be compared with baseline data. Changes in cardiac function will be correlated with levels of inflammatory markers and biomarkers. Further analysis depends on the type of data obtained.

Data will be analyzed using SAS 9.4 (Cary, NC). Data will be expressed as mean (standard deviation). In all analyses, the conventional α-level of 0.05 will be used for significance testing.

9 HUMAN SUBJECTS PROTECTIONS

9.1 RATIONALE FOR SUBJECT SELECTION

The patients to be entered in this protocol have B cell malignancies which are refractory to standard therapy, and limited life expectancies. Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore gender and ethnic aspects

of clinical research on the other hand. If differences in outcome that correlate to gender or to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

9.2 PARTICIPATION OF CHILDREN

The age range of patients eligible for this trial is greater than 1 year of age but less than or equal to 30 years of age. Physicians, nurses, and multidisciplinary support teams of the POB, NCI and Clinical Center will provide patient care. The staff of the POB has expertise in the management of children with complex oncologic disorders and complications of therapy. Full pediatric support and subspecialty services are available at the NIH Clinical Center.

9.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The experimental treatment has a chance to provide clinical benefit though this is unknown. The risks in the investigational portion of this treatment are detailed in Section **1.2.5**. The goal of this study is to improve upon the number of patients who may benefit from adoptive cell therapy by using patients' own transduced T-cells without the need to identify anti-tumor T-cells uniquely from each patient as was required in many cancer therapy protocols. The success of this effort cannot be predicted at this time. Because all patients in this protocol have B cell malignancies without curative options and limited life expectancies the potential benefit is thought to outweigh the potential risks. It is anticipated that this study will provide scientific information relevant to tumor immunotherapy. Consequently, participation of children involves greater than minimal risk, but presents the prospect for direct benefit for patients who are minors (45CFR 46.405).

9.3.1 Risks of Anti-CD19 CAR T Cells

The risk profile of anti-CD19 CAR cells in children is not fully known at this time. DLT on the dose-escalation phase of our study was CRS. In one clinical trial in adults in the Surgery Branch, NCI two events of reversible grade 4 somnolence and one of grade 4 keratinize were attributed to the anti-CD19 CAR-transduced cells. One each event of grade 2 left ventricular dysfunction and grade 1 pulmonary hypotension were attributed to the anti-CD19 CAR-transduced cells and IL-2, and both of these toxicities resolved. Three patients on this study were intubated and one patient underwent dialysis, for which the attribution is unknown. Aldesleukin will not be used in the present study.

Section **1.2.5** discusses the potential risks of this investigational therapy, including risk of chemotherapy, risk of autoimmunity, risk of the gene therapy component, and risk of cytokine storm. Transient, reversible mild effects have been observed with the administration of fresh cells, including chills, fever, rigors, diaphoresis, anorexia, nausea, diarrhea, headache and myalgias. As described in Section **1.2.4** recently reported toxicities associated with similar studies using anti-CD19 CAR T cells included grade 3 tumor lysis syndrome, and reversible laboratory abnormalities, thrombocytopenia, neutropenia, and hypogammaglobulinemia.

9.3.2 Risk of Apheresis

Apheresis is a safe procedure that is routinely performed in healthy children and adults. Participants will be closely monitored and procedures to minimize risks and prevent side effects are incorporated into all aspects of the protocol. The POB, DTM, and NIH CC have broad

expertise to adequately manage side effects, including in pediatric apheresis subjects. The potential risks of apheresis in this trial are as follows:

a. The most common side effects of apheresis are pain and bruising at IV sites. A central venous catheter may be required. Possible side effects include pain, bleeding, bruising, infection, thrombosis, vascular perforation, and risks associated with the sedation used for placement.

b. During apheresis, mild side effects from citrate anticoagulant are common and include chills, numbness and tingling ("pins and needles"), anxiety, muscle cramps, and nausea. More serious side effects due to citrate-induced hypocalcemia are uncommon and include low blood pressure, seizures, weakness, and tetany. Citrate reactions rapidly resolve when the collection is slowed down or stopped. Prophylactic IV CaCl2 and MgSO4 infusions may be administered to donors deemed to be at high risk of citrate toxicity. Risks of parenteral calcium and magnesium include extravasation necrosis and cardiovascular effects including bradycardia and blood pressure changes. However, side effects are unlikely given the low rate of infusion and use of large bore catheters for apheresis.

c. Transient mild thrombocytopenia is common after apheresis, but bleeding is unlikely.

d. Dilutional anemia occurs during apheresis, but this is unlikely to be clinically significant.

e. Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

9.3.3 Risks of the chemotherapy regimens and supportive therapies

The chemotherapy agents and supportive medications used in this study are FDA approved agents with well-known toxicity profiles. Refer to Section **11** for summary of toxicities.

9.4 RISKS/BENEFITS ANALYSIS

Patients will be monitored frequently as both inpatients and outpatients and side effects will be treated promptly. These measures will help to mitigate the potential risks observed with the preparative chemotherapy regimen and the cell administration. The enrollment profile and dose escalation rules will help to ensure that patients are not exposed to unacceptable risk as a safe dose level is determined.

Patients on this study may directly benefit from participation. It is anticipated that this study will provide scientific information relevant to tumor immunotherapy. Consequently, participation involves greater than minimal risk, but presents the prospect for direct benefit for patients who are minors (45CFR 46.405).

9.5 CONSENT AND ASSENT PROCESS AND DOCUMENTATION

Pediatric patients who meet the thorough screening for protocol eligibility will have apheresis conducted to obtain PBMC on this study once the protocol consent is signed. The investigational nature and research objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and potential benefits, and alternative therapies will be carefully explained to the patient's parents/guardians, who are asked to review it and to ask questions prior to agreeing to participate in this protocol. The patient and his/her parent(s) or guardian are

reassured that participation on trial is entirely voluntary and that they can withdraw or decide against treatment at any time without adverse consequences. The investigators are requesting a waiver from the IRB to allow only one parent to sign the informed consent to enter a child on the protocol. Because many patients must travel to the NIH from long distances at substantial expense, requiring both parents to be present for the consent process could be a financial hardship for many families. When necessary, the protocol will be reviewed by telephone with the other parent and their approval will be verified. When guardianship status of the child is uncertain, a social worker will be asked to investigate and, if necessary, seek documentation of custody status. The research nurse, principal investigator, associate investigator, or clinical associate is responsible for obtaining written consent from the patient. Where deemed appropriate by the clinician and the child's parents or guardian, the child will also be included in all discussions about the trial and verbal assent will be obtained. The parent or guardian will sign the designated line on the informed consent attesting to the fact that the child has given assent.

9.5.1 Screening Sample Consent

The screening consent will initially be used to test samples for CD19 expression, prior to complete eligibility screening and enrollment. Telephone consent may be employed in order to screen outside samples from prospective subjects for CD19 expression. In such cases, a protocol investigator will review the Screening Consent form by telephone. The consent/assent signatures will be witnessed and a copy will be faxed and the original sent by mail to the PI. Prospective subjects who consent to send such samples for outside testing will NOT be registered with the NCI Central Registration Office unless they are subsequently enrolled on protocol. Subjects and their referring medical team will be notified of the results and records will be maintained with the protocol research files. The Principal Investigator, Associate Investigator, or their designee will be available to answer all questions from prospective patients or parents/guardians.

The attached informed consent documents contain all elements required for consent.

9.5.2 Consent for minors when they reach the age of majority

When a pediatric subject reaches age 18, continued participation (including ongoing interactions with the subject or continued analysis of identifiable data) requires consenting of the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained. We request a waiver of informed consent for those individuals who became lost to follow up during their participation in the research study or who, prior to the approval of Amendment P were taken off study prior to reaching the age of majority.

Requirements for Waiver of Consent consistent with 45 CFR 46.116 (d):

(1) The research involves no more than minimal risk to the subjects.
    a. Analysis of samples and data from this study involves no additional risks to subjects.
(2) The waiver or alteration will not adversely affect the rights and welfare of the subjects.
    a. Retention of these samples or data does not affect the welfare of subjects.
(3) The research could not practicably be carried out without the waiver or alteration.
    a. Considering the length of time between the minor's last contact with the research team and their age of majority, it will likely be very difficult to locate

them again. A significant reduction in the number of samples analyzed is likely to impact the quality of the research.

(4) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.

9.5.3 Informed consent of non-English speaking subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2), and 21 CFR 50.27 (b) (2)). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long-translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process for non-English speaking subjects and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

10 PHARMACEUTICAL INFORMATION

10.1 RETROVIRAL VECTOR CONTAINING THE ANTI-CD19 CAR GENE

Cells will be administered intravenously through a free-flowing IV at dose 1 X 10^6 anti-CD19 CAR T cells/kg (+/- 20%) as described in Section **3.10.4**.

The retroviral vector supernatant (PG13-CD19-H3) encoding a chimeric antigen receptor (CAR) directed against the B cell antigen, CD19, was prepared and preserved following cGMP conditions in the Surgery Branch Vector Production Facility (SBVPF). The retroviral vector utilizes the MSGV1 retroviral vector backbone and consists of 7026 bps including the 5' LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor sites, FMC63-based (anti-CD19 FMC63-28) CAR protein containing a signal peptide (human GM-CSF receptor), FMC63 light chain variable region (FMC63 VL), linker peptide, FMC63 heavy chain variable region (FMC63 VH), CD28 (hinge, transmembrane and cytoplasmic region), and TCR zeta (cytoplasmic region), followed by the murine stem cell virus 3'LTR. The physical titer will be determined by RNA dot blot according to sponsor certificate.

The supernate will be stored at SBPVF upon the completion of production at –80° C or shipped on dry ice and stored at Cryonix, Rockville, MD. Both storage facilities are equipped with around-the-clock temperature monitoring. Upon request, supernatant will be delivered on dry ice to be used in in vitro transductions of PBL. There will be no re-use of the same unit of supernate

for different patients. Retroviral titer has been shown to be stable after immediate thawing and immediate administration (coating the tissue culture wells previously coated with Retronectin). Handling of the vector should follow the guidelines of Biosafety Level-2 (BSL-2). The specific guidelines for Biosafety Level-2 (BSL-2) can be viewed at http://bmbl.od.nih.gov/sect3bsl2.htm.

10.2 FLUDARABINE

10.2.1 Description

(Please refer to package insert for complete product Information) Fludarabine phosphate is a synthetic purine nucleoside that differs from physiologic nucleosides in that the sugar moiety is arabinose instead of ribose or deoxyribose. Fludarabine is a purine antagonist antimetabolite.

10.2.2 How Supplied

It will be purchased by the NIH Clinical Pharmacy Department from commercial sources. Fludarabine is supplied in a 50 mg vial as a fludarabine phosphate powder in the form of a white, lyophilized solid cake.

10.2.3 Stability

Following reconstitution with 2 mL of sterile water for injection to a concentration of 25 mg/ml, the solution has a pH of 7.7. The fludarabine powder is stable for at least 18 months at 2-80C; when reconstituted, fludarabine is stable for at least 16 days at room temperature. Because no preservative is present, reconstituted fludarabine will typically be administered within 8 hours. Specialized references should be consulted for specific compatibility information. Fludarabine is dephosphorylated in serum, transported intracellularly and converted to the nucleotide fludarabine triphosphate; this 2-fluoro-ara-ATP molecule is thought to be required for the drug's cytotoxic effects. Fludarabine inhibits DNA polymerase, ribnucleotide reductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.

10.2.4 Storage

Intact vials should be stored refrigerated (2-8°C).

10.2.5 Administration

Fludarabine is administered as an IV infusion in an appropriate solution over 30 minutes. To prevent undue toxicity the dose will be based on BSA (25 mg/m^2/dose)

10.2.6 Toxicities

At doses of 25 mg/m^2/day for 3 days, the primary side effect is myelosuppression; however, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Serious opportunistic infections have occurred in CLL patients treated with fludarabine. Hemolytic anemia has been reported after one or more courses of fludarabine with or without a prior history of a positive Coomb's test; fatal hemolytic anemia has been reported. In addition, bone marrow fibrosis has been observed after fludarabine therapy. Other common adverse effects include malaise, fever, chills, fatigue, anorexia, nausea and vomiting, and weakness. Irreversible and potentially fatal central nervous system toxicity in the form of progressive encephalopathy, blindness, and coma is only rarely observed at the currently administered doses of fludarabine. More common neurologic side effects at the current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesia, visual or hearing disturbances, and sleep disorders.

Adverse respiratory effects of fludarabine include cough, dyspnea, allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of CLL.

10.3 CYCLOPHOSPHAMIDE

(Refer to FDA-approved package insert for complete product information)

Description:

Cyclophosphamide is a nitrogen mustard-derivative alkylating agent. Following conversion to active metabolites in the liver, cyclophosphamide functions as an alkyating agent; the drug also possesses potent immunosuppressive activity. The serum half-life after IV administration ranges from 3-12 hours; the drug and/or its metabolites can be detected in the serum for up to 72 hours after administration.

10.3.1 How Supplied

Cyclophosphamide will be obtained from commercially available sources by the Clinical Center Pharmacy Department.

10.3.2 Stability

Following reconstitution as directed with sterile water for injection, cyclophosphamide is stable for 24 hours at room temperature or 6 days when kept at 2-80C.

10.3.3 Administration

It will be diluted in an appropriate solution and infused over one hour. The dose will be based on the patient's body weight, at 900 mg/m2/dose after fludarabine infusion on Day -2.

10.3.4 Toxicities

Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea and vomiting, rash and alopecia occur, especially after high-dose cyclophosphamide; diarrhea, hemorrhagic colitis, infertility, and mucosal and oral ulceration have been reported. Sterile hemorrhagic cystitis occurs in about 20% of patients; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Although the incidence of hemorrhagic cystitis associated with cyclophosphamide appears to be lower than that associated with ifosfamide, mesna (sodium 2-mercaptoethanesulfonate) has been used prophylactically as a uroprotective agent in patients receiving cyclophosphamide. Prophylactic mesna is not effective in preventing hemorrhagic cystitis in all patients. Patients who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in patients with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, patients should be watched closely for cyclophosphamide toxicity (due to allopurinal induction of hepatic microsomal enzymes). At high doses, cyclophosphamide can result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain without edema occurs. At high doses, cyclophophamide can result in cardiotoxicity. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from a syndrome of acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first

dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis have occurred following IV administration. Mesna (sodium 2-mercaptoethanesulphonate; given by IV injection) is a synthetic sulfhydryl compound that can chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

10.4 MESNA

(Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex, NSC-113891) (Please refer to the FDA-approved package insert for complete product information)

10.4.1 Description

Mesna will be obtained commercially by the Clinical Center Pharmacy Department and is supplied as a 100 mg/ml solution.

10.4.2 Storage

Intact ampoules are stored at room temperature.

10.4.3 Stability

Diluted solutions (1 to 20 mg/mL) are physically and chemically stable for at least 24 hours under refrigeration. Mesna is chemically stable at room temperature for 48-72 hours in D5W, 48-72 hour in D5W/0.45% NaCl, or 24 hours in 0.9% sodium chloride.

10.4.4 Administration

Dilute to concentrations less than or equal to 20 mg mesna/ml fluid in D5W or normal saline and to be administered intravenously as a continuous infusion.

10.4.5 Toxicities

Toxicities include nausea, vomiting and diarrhea.

10.5 IFOSFAMIDE

10.5.1 Description

Ifosfamide for injection is supplied in single-dose vials for constitution and administration by intravenous infusion, each containing 1 gm or 3 gms of sterile ifosfamide. Ifosfamide is a chemotherapeutic agent chemically related to the nitrogen mustards and a synthetic analog of cyclophosphamide.

10.5.2 Supplied

Ifosfamide is commercially available and will be supplied by the Clinical Center Pharmacy Department. Please refer to the package insert for full details.

10.5.3 Storage and stability

Preparation for Intravenous Administration: Injections are prepared for parenteral use by add Sterile Water for Injection, USP or Sterile Bacteriostatic Water for Injection, USP to the vial and shaking to dissolve.

10.5.4 Preparation for Intravenous Administration

Injections are prepared for parenteral use by add Sterile Water for Injection, USP or Sterile Bacteriostatic Water for Injection, USP to the vial and shaking to dissolve.

10.5.5 Toxicities

The dose limiting toxicities are myelosuppression and urotoxicity. Dose fractionation, vigorous hydration and a protector such as mesna can significantly reduce the incidence of hematuriar. Other significant toxicities include alopecia, nausea, vomiting and central nervous toxicities.

10.6 ETOPOSIDE (VEPESID, VP-16)

10.6.1 Availability

Commercially available and will be supplied by the Clinical Center Pharmacy.

10.6.2 Toxicity

Includes nausea, vomiting, stomatitis, diarrhea, neutropenia, thrombocytopenia, and alopecia. Secondary AML has been associated with etoposide. Bradycardia and hypotension are sometimes observed with etoposide administration.

10.7 ACETAMINOPHEN (TYLENOL)

Will be given as a pre-medication. This agent will be provided by the clinical center pharmacy. Please refer to the package insert for complete pharmaceutical information on this product.

10.8 DIPHENHYDRAMINE (BENADRYL)

Will be given as a pre-medication IV over 10-15 minutes This agent will be provided by the Clinical Center Pharmacy. Please refer to the package insert for complete pharmaceutical information on this product.

10.9 INTRATHECAL CHEMOTHERAPY

The following agents will be provided by the Clinical Center Pharmacy. Please refer to the package inserts for complete pharmaceutical information on these products.

10.10 HYDROCOTISONE (CORTEF, SOLU-CORTEF) NCS# 010483

10.10.1 Source and Pharmacology

Synthetic steroid akin to the natural adrenal hormone, cortisol. It binds with steroid receptors on nuclear membrane, impairs cellular mitosis and inhibits protein synthesis. It is phase specific, killing cells primarily during S phase. It has a catabolic effect on proteins and alters the kinetics of peripheral blood leukocytes. It is excreted in the urine and catabolized in the liver.

10.10.2 Toxicity

The following toxicities may occur when hydrocortisone is given intrathecally: Nausea and vomiting, headache, pleocytosis, fever, somnolence, meningismus, learning disability, leukoencephalopathy.

10.10.3 Formulation and Stability

Available as 100mg, 250mg, 500mg, and 1000mg vials for aqueous injection. In powder form, the drug is stable for 2 years at room temperature. After reconstitution, it is stored at room temperature, and should be discarded after 3 days. INTRATHECAL ADMINISTRATION: IT hydrocortisone should be further diluted with physiologic buffered diluents (lactated Ringer's, 0.9% sodium chloride, Elliott's B solution). Do not use Bacteriostatic Water to reconstitute for IT use, use only preservative free solutions.

10.10.4 Guidelines for Administration

Hydrocortisone will be given by intrathecal administration in an age-specified dose and will be mixed with the other agents to the age-specified volume. (Section **4.1**)

10.10.5 Supplier

Commercially Available.

10.11 CYTARABINE (CYTOSINE ARABINOSIDE, ARAC, CYTOSAR) NSC# 063878

10.11.1 Source and Pharmacology

Deoxycytidine analogue which is metabolized to ARA-CTP, a substance which inhibits DNA polymerase. It is S phase specific, and thus affects DNA synthesis. Rapidly catabolized by hepatic cytidine deaminases to AraU.

10.11.2 Toxicity

The following toxicities may occur when cytarabine is given intrathecally: Nausea, vomiting, headache, pleocytosis, arachnoiditis, rash, fever, somnolence, meningismus, convulsions, paresis, myelosuppression, ataxia, learning disability. CNS impairment may not be fully reversible. No further cytarabine will be administered if there is CNS toxicity (any grade) deemed related to cytarabine.

IV Administration: Acute DLT consists of severe leukopenia and thrombocytopenia. Nausea and vomiting may be dose limiting at higher doses. Other adverse reactions include diarrhea, immunosuppression, anorexia, stomatitis, oral ulceration, flu-like syndrome, fever, hepatic dysfunction, and alopecia. At high doses, as in this protocol, keratoconjunctivitis, dermatitis, and central nervous system toxicity (e.g., ataxia, somnolence, coma, dysarthria) may occur. Occasionally, the CNS impairment is not fully reversible. Renal impairment will enhance toxicity. In the event signs of CNS toxicity, the cytarabine will be interrupted and the M.D. notified. In the event of signs of CNS toxicity the cytarabine infusion will be interrupted and the M.D. notified. No further cytarabine will be administered if there is CNS toxicity (any grade) deemed related to cytarabine.

10.11.3 Formulation and Stability

A freeze-dried powder available in 100mg, 500mg, 1g and 2g vials. The unreconstituted form of the drug is stable at room temperature for at least 2 years.

INTRAVENOUS ADMINISTRATION: The manufacturer supplies the drug as unreconstituted lyophilized powder in vials. With each vial, an ampule of bacteriostatic water for injection containing 0.945% benzyl alcohol is provided. To prepare high dose (2 gm/m2) cytarabine intravenous infusions, each vial of drug should be reconstituted with 2 mL (100 mg) or 10 mL (500 mg) of Sterile Water for injection USP (i.e. not the manufacturer-provided diluent) which

contains no preservative. Begin each cytarabine dose 3.5-4 hours after completion of the preceding fludarabine dose.

INTRATHECAL ADMINISTRATION: IT cytarabine should be further diluted with physiologic buffered diluents (lactated Ringer's, 0.9% sodium chloride, Elliott's B solution). Do not use Bacteriostatic Water to reconstitute for IT use, use only preservative free solutions.

10.11.4 Guidelines for Administration

Cytarabine will be given by intrathecal administration in an age-specified dose and will be mixed with the other agents to the age-specified volume (Section **4.1.2**). If emesis occurs it usually occurs 4-6 hours after the intrathecal administration. If the patient has had emesis with prior intrathecal chemotherapy, premedications should be considered.

10.11.5 Supplier

Commercially available. See package insert for further information.

10.12 METHOTREXATE

10.12.1 Source and Pharmacology

Methotrexate is an antimetabolite and antifolate agent with antineoplastic and immunosuppressant activities. Methotrexate binds to and inhibits the enzyme dihydrofolate reductase, resulting in inhibition of purine nucleotide and thymidylate synthesis and, subsequently, inhibition of DNA and RNA syntheses. Methotrexate also exhibits potent immunosuppressant activity although the mechanism(s) of actions is unclear.

10.12.2 Toxicity

The following toxicities may occur when given intrathecally: occasional headache, dizziness, tiredness, blurred vision or loss of balance for a few hours. Up to 15% of children may develop neurological changes including changes in level of consciousness, abnormal movements or confusion, very rarely leucoencephalopathy.

10.12.3 Formulation and Stability

Methotrexate Sodium Injection USP is available for single use only in 20 mg and 1 gram vials, and will be diluted according to TIT in Section **4.1**. Dilutions should be used within 24 hours if kept at room temperature. Unused solution should be discarded after this time in order to avoid risk of microbial contamination. INTRATHECAL ADMINISTRATION: IT methotrexate should be further diluted with physiologic buffered diluents (lactated Ringer's, 0.9% sodium chloride, Elliott's B solution). Do not use Bacteriostatic Water to reconstitute for IT use, use only preservative free solutions.

10.12.4 Preparation

The three agents will be filtered and mixed in sterile preservative free 0.9% sodium chloride immediately (less than 4 hrs) prior to administration. The final concentration should be no greater than 1.5mg/ml for MTX and HDC, and 3 mg/ml for Ara-C.

10.12.5 Guidelines for Administration

Methotrexate will be given by intrathecal administration in an age-specified dose and will be mixed with the other agents to the age-specified volume (Section **4.1.2**). If emesis occurs it usually occurs 4-6 hours after the intrathecal administration. If the patient has had emesis with prior intrathecal chemotherapy, premedications should be considered.

10.12.6 Supplier

Commercially available. See package insert for further information.

10.13 ALLOPURINOL

Allopurinol will be obtained by the Clinical Center Pharmacy Department. It will be used as prophylaxis or treatment of pediatric patients with or at high risk for Tumor Lysis Syndrome. Dosage is approximately 100 mg/m^2/dose po TID (maximum dose 200 mg TID). The most common side effects include hypersensitivity, rash, nausea, vomiting, renal insufficiency, and hepatic dysfunction. Allopurinol should be stopped immediately if rash develops. Consult the package insert for a complete list of all side effects.

10.14 TOCILIZUMAB (ANTI-IL6R MAB)

10.14.1 Description

Tocilizumab is an anti-human IL-6R antibody that binds specifically to cell associated and soluble IL-6 receptors, thus blocking IL-6 signaling. IL-6 is involved in T cell activation, acute phase protein synthesis and stimulation of hematopoietic precursors.

10.14.2 Storage and Stability

Store unused vials refrigerated between 2 and 8 degrees C (36 and 46 degrees F). Do not freeze and protect from light in original package[3]. Diluted tocilizumab solutions may be stored at 2 to 8 degrees C (36 to 46 degrees F) for up to 24 hours. Protect the diluted solution from light. Discard any unused portion remaining in the vial because the product does not contain preservatives[1]. Fully diluted tocilizumab solutions are compatible with polypropylene, polyethylene, and polyvinyl chloride infusion bags, or with polypropylene, polyethylene, and glass infusion bottles[1].

10.14.3 Administration

Allow tocilizumab infusion to reach room temperature prior to infusion. Administer over 60 minutes using an infusion set. Do not administer as an IV push or bolus[1]. Tocilizumab should not be infused with other drugs in the same IV line due to a lack of physical or biocompatibility studies[1].

10.14.4 Dosage

4-12 mg/kg, Single dose should not exceed 800 mg.

10.14.5 Toxicities

The major toxicity associated with Tocilizumab is immunosuppression, the risk of which is further increased when the agent is given with other immunosuppressants including corticosteroids and cytotoxic chemotherapy. The risks and benefits of treatment with tocilizumab should be carefully considered prior to initiating therapy in patients with chronic or

recurrent infection. Patients should be closely monitored for the development of signs and symptoms of infection during and after treatment with tocilizumab, including the possible development of tuberculosis in patients who tested negative for latent tuberculosis infection prior to initiating therapy[3].

10.15 FILGRASTIM (NEUPOGEN)

10.15.1 Description

Filgrastim is a human granulocyte colony-stimulating factor (G-CSF), produced by recombinant DNA technology. Neupogen is a sterile, clear, colorless, preservative-free liquid. See package insert for complete details.

10.15.2 Storage

Filgrastim should be stored at 2 C to 8 C (36 F to 46 F) and should not be frozen.

10.15.3 Administration

Filgrastim is administered intravenously or subcutaneously (under the skin) for 6 to 14 days. Filgrastim vials should not be shaken since the drug may be damaged, and bubbles may form that can prevent some of the drug from being drawn up into the syringe at the time of injection.

Toxicities

The most common side effects are nausea, vomiting, bone pain, fever, fatigue, hair loss, and diarrhea. Headache, weight loss, shortness of breath, mouth sores, and rash also occur. Uric acid, lactate dehydrogenase, and alkaline phosphatase levels may rise and spontaneously return to normal levels

11 APPENDICES

11.1 APPENDIX A -PERFORMANCE STATUS CRITERIA

PERFORMANCE STATUS CRITERIA *Karnofsky and Lansky performance scores are intended to be multiples of 10.*					
ECOG (Zubrod)		**Karnofsky**		**Lansky**	
Score	**Description**	**Score**	**Description**	**Score**	**Description**
0	Fully active, able to carry on all pre-disease performance without restriction.	100%	Normal, no complaints, no evidence of disease.	100%	Fully active, normal.
		90%	Able to carry on normal activity; minor sighs of symptoms of disease.	90%	Minor restrictions in physically strenuous activity.
1	Restricted in physically strenuous activity but ambulatory, able to carry out light or sedentary work, e.g., light housework, office work.	80%	Able to carry on normal activity with effort; some signs or symptoms of disease.	80%	Active, but tires more quickly.
		70%	Cares for self, unable to carry on normal activity or do active work.	70%	Both greater restriction of, and less time spent in, play activities.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.	60%	Requires occasional assistance but is able to care for most of own needs.	60%	Up and around, but minimal active play; keeps busy with quieter activities.
		50%	Requires considerable assistance and frequent medical care.	50%	Gets dressed, but lies around much of the day; no active play; able to participate in quiet play and activities.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	40%	Disabled; requires special care and assistance.	40%	Mostly in bed; participates in quiet activities.
		30%	Severely disabled; hospitalization indicated, although death not imminent.	30%	In bed; needs assistance even for quiet play.
4	Completely disabled. Cannot carry on any self-care. Totally confined to a bed or chair	20%	Very ill; hospitalization necessary; active supportive treatment required.	20%	Often sleeping; play entirely limited to very passive activities.
		10%	Moribund, fatal process progressing rapidly	10%	No play; does not get out of bed
5	Dead	0%	Patient expired	0%	Unresponsive; Dead

11.2 APPENDIX B: STUDY CALENDAR

Procedure	*Screening (within 14 days, unless specified)*	*Day -11*	*Baseline (within 24 hours of starting chemo unless specified)*	*Chemotherapy (see B1, B2 and B3)*		*Prior to Cell infusion (within 24 hrs)*	*Day 0 (Day of cell infusion)*	*During Hospitalization*					*On Treatment 28(±4) days after cell infusion, then 3, 6, 12 mos, then Q 6-12 mos)*	*Off treatment*
								Day 1	*Day 2*	*Day 3*	*Day 4-7*	*Every 2-4 days Until D/C*		
History and PE	X		X			X	X	X	X	X	X	X	**X**[i]	**X**[L]
Vital signs	X		X			X	X[g]	X	X	X	X	X	**X**	
Performance Score	X		X			X	X	X	X	X	X	X	X	**X**
Cognitive Evaluation			X										X[n]	
Neuro symptom checklist												X[m]		
Height and weight	X		*WT only*			*WT only*							*WT only*	*WT only*
Labs														
• CBC with diff and platelets	X		X			X			X		X	X	X	
• PT/aPTT	X		X[e]											
• Chem 20[b]	X		X			X			**X**		X	**X**	X	
• Thyroid Panel			X[e]										X[o]	
• CD19 staining of malignant cells by immunohistochemistry or flow cytometry (anytime)	X													
• Peripheral blood CD3 count	X													
• HIV, HbsAG, anti-HCV, EBV	X[a]													
• IgG level													X[k]	
• Urine analysis, culture if indicated	X					X			X			X		
• β-HCG pregnancy test on all women of child-bearing potential	X[c]		X[f]											
ECG	X												X	X

Procedure	***Screening (within 14 days, unless specified)***	***Day -11***	***Baseline (within 24 hours of starting chemo unless specified)***	***Chemotherapy (see B1, B2 and B3)***		***Prior to Cell infusion (within 24 hrs)***	***Day 0 (Day of cell infusion)***	***During Hospitalization***					***On Treatment 28(±4) days after cell infusion, then 3, 6, 12 mos, then Q 6-12 mos)***	***Off treatment***
								Day 1	***Day 2***	***Day 3***	***Day 4-7***	***Every 2-4 days Until D/C***		
ECHO, cardiac MRI or MUGA	*X*												***X***	*X*
Ophthalmologic Exam			*X*[j]										*X*[j]	
Correlative Research Studies														
• Apheresis		*X*											*X (DAY 28 only)*	*X*
• Research Sampling														
Disease Evaluation														
• CT chest/abdomen/pelvis (or other appropriate imaging)	*X*													
• Lumbar puncture[d]	*X*		*X*[e]										*X*	
• Bone marrow aspirate/biopsy	*X*		*X*[e]										*X*	
Treatment Regimen														
• Anti-CD19-CAR transduced cells							*X*[f]							
Response Evaluation	*X*	*X*	*X*										***X***	*X*
Adverse Events		*X*	→			→	→	→	→	→	→	→	***X*** →	
Concomitant Medications	*X*	*X*	→			→	→	→	→	→	→	→	→	
Research labs	*X*		*X*				*X*	*X*	*X*	*X*	*X*	*X*	***X***	

[a] Within 4 weeks prior to enrollment

[b] Laboratory evaluation to include; Chem 20: Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid); creatinine clearance may be performed if the serum creatinine is elevated.

[c] within 1 week prior to enrollment

[d] for patients with aggressive NHL, ALL, history of leptomeningeal disease, or signs/symptoms of leptomeningeal involvement

[e] Within 14 days of starting chemo preparative regimen

[f] Within 72 hours of starting chemo preparative regimen

[g] Monitor every q15 min X 1 hr, q 30 min for 2nd hour, then q 1 hr for 2 more hrs or until stable, then routinely.

[h] For cell acquisition for product development

[i] Physical examinations annually for 5 years. After 5 years, health status data will be obtained from surviving patients via telephone contact or mailed questionnaires. The long term follow up period for retroviral vectors is 15 years. Persistence of CAR gene transduced cells: prior to cells, 1 week, 4-6 weeks, 3 and 6 months post cell infusion; RCR PCR prior to cell infusion; and at 3, 6, and 12 mos post cell infusion

[j] within 14 days of preparative regimen

[k] every 3-6 weeks post infusion.

[l] cells may be infused up to: Arm 1 = 72 hours following the time of the planned infusion. Otherwise, cells may be frozen and infused up to 7 days following the time of the planned infusion, or attempts to infuse at a later time may be made per Section **3.5**. Arm 2 = 21 days (cryopreserved) following the time of the planned infusion, or attempts to infuse at a later time may be made per Section **3.5**

[m] on or about Day 14 ± 1 day

[n] at 3 month visit only (± 4 weeks)

[o] Thyroid panel only before day 28

[L]Off treatment after day 28.

- Off treatment all studies will be done as per day 28 (+/-4).
- If no response (progressive disease) then no further clinical follow-up required expect for Section **3.12.5**, which will be followed for 15 years.
- If response, results and documentation for physical examinations, vital signs, CBC with diff, Chem 20, and IGG levels, will be collected. Bone marrows/lumbar punctures will be done at the primary oncologist discretion and documentation collected. All will be collected until relapse or bone marrow transplantation. Section **3.12.5**, which will be followed for 15 years.
- If response, results and documentation for physical examinations, vital signs, CBC with diff, Chem 20, and IGG levels, will be collected.

11.2.1 Appendix B 1: Arm 1

Procedure	**Day prior to Cells Infusion**		
	-4, -3	**-2**	**-1**
Fludarabine: 25 mg/m^2/day IV	X	X	REST
Cyclophosphamide: 900 mg/m^2/day IV		X	REST
Mesna 540 mg/m^2/day IV		X	REST

11.2.2 Appendix B 2: Arm 2: FLAG

Procedure	**Day prior to Cells Infusion**		
	Day prior to chemotherapy	-12, -11, -10, -9, -8,	From last day of chemo to Cell Infusion (DAY 0) [at least 2 days]
Fludarabine: 25 mg/m^2/day IV		X	REST
Cytarabine 2000 mg/m^2/day IV		X	REST
Filgrastim µg/kg/dose subcutaneously	X	X	Daily until ANC>1000 on 2 consecutive days

11.2.3 Appendix B 3: Arm 2: Etoposide/ifosfamide

Procedure	**Day prior to Cells Infusion**		
	Day prior to chemotherapy	-12, -11, -10, -9, -8,	From last day of chemo to Cell Infusion (DAY 0) [at least 2 days]
Etoposide 100 mg/m^2/day IV		X	REST

Ifosfamide 1,800 mg/m^2/day and Mesna 360 mg/m^2/dose IV		X	REST
Mesna 360 mg/m^2/dose IV (then PO or IV)		X (after ifosfamide, then every 3 hours X 6 doses)	REST
Physical Exam	X	Twice weekly	Twice weekly
Chem 20:	X	Twice weekly	Twice weekly
CBC with differential	X	Twice weekly	Twice weekly

11.3 APPENDIX C: GRADING AND TREATMENT OF ACUTE GVHD

Acute GVHD

	Skin	Liver	GI Tract	Upper GI	
Stage	**Rash**	**Bilirubin**	**Diarrhea (ml/day)**		
0	No rash	≤ 2 mg/dL	< 500 (<10/kg)		
1	<25%	2.1-3.0 mg/dl	501-1,000 (10-15/kg)	severe nausea/vomiting	
2	25-50%	3.1-6.0 mg/dl	1,001-1,500 (15-20/kg)		
3	>50%	6.1-15 mg/dl	>1,501 (>20/kg)		
4	desquamation	>15 mg/dl	severe pain, ileus		

Glucksberg Grade*

1	1-2		0		0		
2	1-3		1		1		
2_o	0		1		1		
2_s	4		0		0		
3	2-4		2-4 *&/or*		2-4 (1 only >2)		
4	3-4		2-4		2-4		

IBMTR Severity Index*

A	1		0		0		0
B	2	*or*	1-2	*or*	1-2	*or*	1
C	3	*or*	3	*or*	3		
D	4	*or*	4	*or*	4		

***Assigned based on highest score**

Adapted from Rowlings PA et al: IBMTR Severity Index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade. Br J Haematol 97:855,1997; Weisdorf DJ et al: Acute upper gastrointestinal graft-versus-host disease: clinical significance and response to immunosuppressive therapy. Blood 76:624, 1990.

11.4 APPENDIX D: CALCULATION OF WEIGHT FOR CELL DOSE CALCULATION IN MORBIDLY OBESE CANDIDATES

Formulation for deriving the weight to be used in targeting cell doses in morbidly obese cell candidates.

1. **Definition**
   Obesity is defined as a BMI > 30.
   BMI = wgt (kg) / [hgt (M)]2

2. **Calculation of ideal body weight** is performed using the standard, published formula:

   Male: 50 + 2.3(Hgt - 60) where Hgt is in inches, and the result is expressed in kg.
   ex. The ideal weight of a 5'10" male = 50 + 2.3(10) = 73 kg.

   Female: 45.5 + 2.3(Hgt - 60), where height is in inches, and the result is in kg.

3. **Calculation of the "practical weight."**
   Calculate the midway point, halfway between the actual and ideal body weights (ie the average of the two numbers). This is the "practical weight" to be used in calculating the targeted cell dose.

4. **Example**:
   Patient's actual weight = 143 kg.
   Patient' s actual height 173 cm = 69 in
   BMI = 48
   IBW formula = 50 + 2.3(9) = 70.7 kg
   Midway point between 70.0 and 143 = 107 kg.

   The weight we would use in targeting cell dose is 107 kg.

1 dose by weight with adjustment:
- IBW + 50%(Weight-IBW)
- Practical body weight = (IDW + actual BW)/2

2 Formula

IBW (men)

- 52 kg + 1.9 kg/inch above 5 feet
- 50 kg + 2.39 (height in inches – 60)

IBW (women)

- 49 kg + 1.7 kg/inch above 5 feet
- 45.5 kg + 2.39 (height in inches – 60)

11.5 APPENDIX E: RESEARCH SAMPLE REQUIREMENTS*

(the order of the research studies listed below reflect the priority should blood volume for research purposes become limited) Day 0 is the day of cell infusion

Study	Test/Lab Location	Sample Time Points	Volume/type	Tube	Notes
RCR (replication competent retrovirus) **Cell product**	S+L- (product only)/ Indiana University	Prior to cell infusion or cryopreservation of cells	5 x 10^5 cells Cellular product	In cryovial with 1mL freezing media (90% AB serum, 10% DMSO) Frozen at -80 C	Received from CPS Shipped on dry ice priority overnight to: Lisa Duffy Indiana University Vector Production Facility 980 W. Walnut St., R3-C668 Indianapolis, IN 46202 e-mail: ljwoods@iupui.edu phone: 317-274-0323 No Sat or Sun Deliveries
	Rapid PCR analysis (product only) Location: DTM	At least 2-4 days prior to cell infusion	6 x 10^6 cells from both CAR transduced cells AND untransduced control cells	Fresh in culture media in sterile tube To be performed in DTM	Performed in CPS ***Results must be available prior to cell infusion***
Patient Testing RCR (replication competent retrovirus)	High-sensitivity PCR analysis (patient samples)/ Indiana University	1. Prior to cell infusion 2. 3 months(± 1 month) 3. 6 months (± 1 month) 4. 1 year (± 2 month) 5. Archive samples annually thereafter for 5 years (+/- 3 months). All Samples are scheduled from first cell infusion.	3 mL/ peripheral blood	Purple top (EDTA) tube Refrigerated at 4 C PAX DNA tube for archival samples	If all post-therapy RCR-PCR tests are negative in the first year, future samples will be cryopreserved and banked.in Frederick Shipped on ice pack priority overnight to: Lisa Duffy Indiana University Vector Production Facility 980 W. Walnut St., R3-C668 Indianapolis, IN 46202 e-mail: ljwoods@iupui.edu phone: 317-274-0323 Archival samples: Courier to NCI-Frederick repository 301-

					846-5893 to schedule pick-up
Cell product Storage for future research and regulatory studies	Dr. William Kopp SAIC/Frederick	Prior to cell infusion or cryopreservation of cells	10% of cellular product remaining after infusion	In cryovials with 10-50 x10^6 cells in 1mL freezing media each (90% AB serum, 10% DMSO) Frozen at -80 C	Received from CPS Courier to NCI-Frederick repository 301-846-5893 to schedule pick-up
Patient Testing Flow Cytometry: Bone marrow	ALL panel including hematagones/ Stetler-Stevenson Lab 10/6N109 301-402-1716	At time of enrollment and At first disease restaging after each cycle and then at the at PI oncologist discretion in responding patients when available.	5 mL marrow aspirate	10 ml in a heparinized syringe	This test will only be done when a marrow aspirate is clinically indicated or required to monitor disease status Schedule with lab in advance using Microsoft Outlook Calendar and order test in CRIS
CAR T Cell Persistence	POB Panel -T-regs/ TBNK NIH Clinical Pathology Flow Cytometry Lab.	1. Prior to cell infusion 2. Day 1, 7, 14,(+/- 2 days) and 28 (+/- 4 days) 3. If response, repeat at least 3 and 6 months, then every 6-12 months until CAR negative.	6 mL and 3ml peripheral blood	One 6ml and one 3 ml Purple top tube (PTT) at room temperature (1 for flow/1 for CBC)	Schedule with clinical lab 301-496-4879
	Flow Cytometry for CAR T cells and subsets: Stetler-Stevenson Lab 10/6N109 301-402-1716		3 mL peripheral blood or bone marrow	Two 10 ml Green Sodium Heparin Tubes at room temperature	Schedule with Stetler-Stevenson Lab 301-402-1716
DNA for CAR	PCR for CAR promoter from T cell genomic DNA/ Dr. William Kopp SAIC/Frederick	1. Prior to cell infusion 2. Day 1, 7, 14, (+/- 2 days) and 28 (+/- 4 days). If response, repeat at least 3 and 6 months, then every 6-12 months until CAR negative.	3 mL peripheral blood. Day 28 (+/-4) one 3 ml peripheral blood and one 3 ml bone marrow	PAX DNA tube or a EDTA tube sample at room temperature	Samples will be stored and batched Courier to NCI-Frederick repository 301-846-5893 to schedule pick-up
Serum Cytokine Levels (including by not limited to IFN-γ, IL-2, IL-6, and TNFα)	Multiplex cytokine panel Dr. William Kopp SAIC/Frederick	1. Prior to cell infusion (within 24 hours) 2. Day 0 (1 and 12 hours after cells), 1 (24 hrs), 2 (48 hrs), 3 (72 hrs) +/- 4 hours 3. Days 4-14, and 28 (+/- 4	4 mL peripheral blood	For scheduled samples 4 ml Green Sodium heparin tube. Keep refrigerated on patient care unit.. Cytokine storm samples will be refrigerated on patient unit	At any time for patients suspected of developing cytokine storm, additional samples will be collected, stored in the refrigerator sent ASAP for analysis and weekend samples will be spun

		days) 4. If response, repeat at least 3 and 6 months, then every 6-12 months until CAR negative.			and frozen by fellow. Courier to NCI-Frederick repository 301-846-5893 to schedule pick-up
CD64	Flow Cytometry for CD64: Stetler-Stevenson Lab 10/6N109 301-402-1716	1. Prior to cell infusion (Day -2, -1 OR Day 0) 2. Post cell infusion: up to daily for Days 1-14 3. Additional samples may be sent if the patient is febrile	0.5 mL from the Cytokine sample listed above into cryovial.	Mix blood well by inverting several times. Extract 0.5 ml from cytokine sample and place in cyro vial. Place in refrigerator. Sample is viable for 4 hours at room temperature or 48 hours if refrigerated. Send to Flow Cytometry lab (B1B58)	Schedule with Stetler-Stevenson Lab 301-402-1716
Cytogenetics (Cyto)	Dr. Diane Arthur 37/2016 301-435-3711	At PI's discretion.	3-5 ml of bone marrow	Marrow: 0.5 ml preservative-free sodium heparin in capped syringe	Schedule with lab in advance and
Flow Cytometry: CSF	Flow Cytometry for CAR T cells/ Stetler-Stevenson Lab 10/6N109 301-402-1716	At time of enrollment and at each diagnostic (clinically indicated) LP if performed at NIH CC	3-8 mL CSF	Spinal fluid placed immediately in flow media available on 3 NE North Procedure Area.	Delivered immediately to Stetler-Stevenson Lab
Minimal Residual Disease (MRD) for leukemia cells (Real Time PCR)	Evaluation of DNA for MRD of leukemia cells	At time of response evaluation when indicated by PI	5 mL blood or 2 mL bone marrow	EDTA tube; send to POB laboratory for processing	Send DNA to: Adaptive Biotechnologies Corp. 1551 Eastlake Ave E, Suite 200, Seattle WA 98102 ph:206-687-7708 email: hrobins@fhcrc.org **ATTN: clonoSEQ**
Serum Storage	SAIC/Frederick	Prior to chemo, Day -1, 7, 28 (+/-4 days)	10 ml red top tube peripheral blood	Store in refrigerator	Courier to NCI-Frederick repository 301-846-5893 to schedule pick-up
Apheresis (single pass) (Only if the subject has apheresis line.**	SAIC/Frederick	Day 28 (+/-4)	Single pass	Room temperature	Courier to NCI-Frederick repository 301-846-5893 to schedule pick-up

- Sample volume for protocol studies will be restricted. The amount of blood drawn from adult (those 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, in an 8 week period. The amount of blood drawn for research purposes from pediatric patient subjects (those under 18 years of age) will not exceed 5 mL/kg in a single day, and no more than 9.5 mL/kg over an 8 week period.
- **if no apheresis line one time blood draw will be done. 3cc/Kg not to exceed 50 mls. (half in sodium heparin tubes and half in red top tubes. Send to SAIC.

11.6 APPENDIX F: GUIDELINES FOR GRADING AND MANAGEMENT OF SUSPECTED CYTOKINE RELEASE SYNDROME

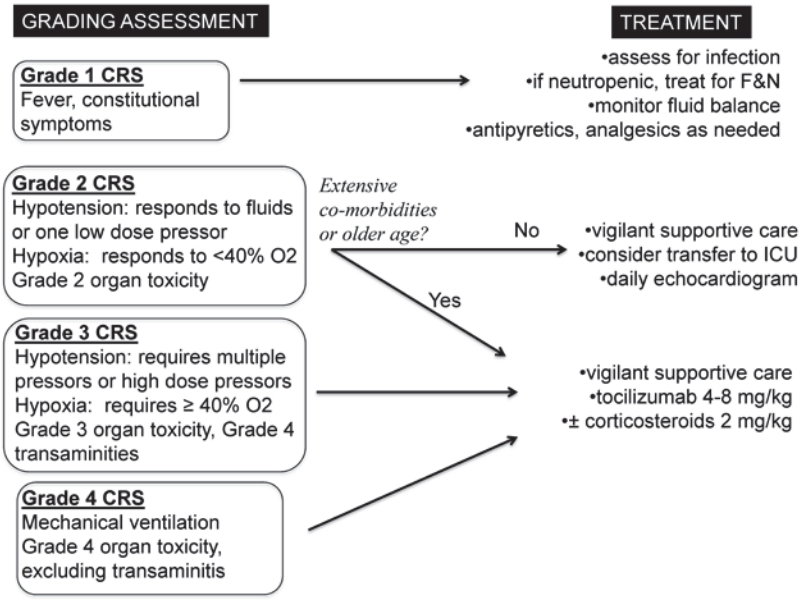

Table 1. Cytokine Release Syndrome Revised Grading System

Grade 1	*Symptoms are not life threatening and require symptomatic treatment only* e.g. fever, nausea, fatigue, headache, myalgias, malaise
Grade 2	*Symptoms require and respond to moderate intervention* -oxygen requirement <40% or -hypotension responsive to fluids or low dose[1] of one vasopressor or -grade 2 organ toxicity
Grade 3	*Symptoms require and respond to aggressive intervention* -oxygen requirement ≥40% or -hypotension requiring high dose[1] or multiple vasopressors or -grade 3 organ toxicity or grade 4 transaminitis
Grade 4	*Life-threatening symptoms* -requirement for ventilator support or -grade 4 organ toxicity (excluding transaminitis)

[1]high dose vasopressor doses shown in Table 2.

Table 2. High-Dose Vasopressors (all doses are required for ≥ 3 hours)

Norepinephrine monotherapy	≥ 20 mcg/kg/min
Dopamine monotherapy	≥ 10 mcg/kg/min
Phenylephrine monotherapy	≥ 200 mcg/kg/min
Epinephrine monotherapy	≥ 10 mcg/kg/min
If on vasopressin	[1]Vasopressin + NE equivalent of ≥ 10mcg/kg/min
If on combination vasopressors (not vasopressin)	[1]Norepinephrine equivalent of ≥ 20 mcg/kg/min

- *[1]VASST Trial Vasopressor Equivalent Equation:*

Norepinephrine equivalent dose = [norepinephrine (mcg/min)] + [dopamine (mcg/kg/min) ÷ 2] + [epinephrine (mcg/min)] + [phenylephrine (mcg/min) ÷10]

11.7 APPENDIX G: NEUROPSYCHOLOGICAL TEST BATTERY

Domains	Measure*	Age range (yrs)	Time (min)
Working Memory	NIH Toolbox List Sorting Test	3.0 - adult	7
Attention	NIH Toolbox Flanker Test	3.0 - adult	4
	Wechsler Cancellation subtest	4 - adult	3
	WRAML Letter Number	5.0 - adult	6
Processing Speed	Wechsler Symbol Search	4 - adult	3
Executive Function	NIH Toolbox Dimensional Change Card Sort Test	3.0 – adult	7
	DKEFS Trailmaking	8 - adult	5
	DKEFS Verbal Fluency (8+ yrs) or McCarthy Verbal Fluency (2.5-7 yrs)	2.5 - adult	6
Background Information	Background Information Form (parent/observer report or adult self-report)	all	(5)
Neurologic Symptoms	Neuro-Symptom Checklist (parent/observer report)	all	(2)

- This full test battery will be administered at baseline and 21-28 days post-infusion for participants 2.5 years and older. The battery also will be administered at 3 months post-infusion if the patient returns to the NIH for a follow-up evaluation. In addition, at Day 14 (+/- 1 day) post-infusion, the Neuro-Symptom Checklist will be administered to a parent/adult observer.
- Other measures may be given in order to test a particular domain more thoroughly if clinically indicated. Also, some measures may not be administered if the participant does not appear to understand the task or becomes frustrated.
- We estimate that this test battery will take less than 1 hour for all participants and less than 45 minutes for children under 6 years of age to complete.

11.8 APPENDIX H: NEURO SYMPTOM CHECKLIST

Domains	Measure*	Age range (yrs)	Time (min)
Working Memory	NIH Toolbox List Sorting Test	3.0 - adult	7
Attention	NIH Toolbox Flanker Test	3.0 - adult	4
	Wechsler Cancellation subtest	4 - adult	3
	WRAML Letter Number	5.0 - adult	6
Processing Speed	Wechsler Symbol Search	4 - adult	3
Executive Function	NIH Toolbox Dimensional Change Card Sort Test	3.0 – adult	7
	DKEFS Trailmaking	8 - adult	5
	DKEFS Verbal Fluency (8+ yrs) or McCarthy Verbal Fluency (2.5-7 yrs)	2.5 - adult	6
Background Information	Background Information Form (parent/observer report or adult self-report)	all	(5)
Neurologic Symptoms	Neuro-Symptom Checklist (parent/observer report)	all	(2)

• This full test battery will be administered at baseline and 21-28 days post-infusion for participants 2.5 years and older. The battery also will be administered at 3 months post-infusion if the patient returns to the NIH for a follow-up evaluation. In addition, at Day 14 (+/- 1 day) post-infusion, the Neuro-Symptom Checklist will be administered to a parent/adult observer.

• Other measures may be given in order to test a particular domain more thoroughly if clinically indicated. Also, some measures may not be administered if the participant does not appear to understand the task or becomes frustrated.

• We estimate that this test battery will take less than 1 hour for all participants and less than 45 minutes for children under 6 years of age to complete.

12 REFERENCES

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