Abbreviated Title: CD19/CD22 T-cell in Peds

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Title: Phase 1 Dose Escalation Study of CD19/CD22 Chimeric Antigen Receptor (CAR) T Cells in Children and Young Adults with Recurrent or Refractory CD19/CD22-expressing B Cell Malignancies

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Drug Name:	Autologous peripheral blood lymphocytes (PBL) cultured with Interleukin-2; Transduced with retroviral (lentiviral) vector (CD19/CD22.BB.z) expressing CD19/CD22 chimeric antigen receptor (CAR); following fludarabine (or pentostatin) and cyclophosphamide	Cyclophosphamide	Fludarabine	Pentostatin
IND Number:	17764			
Sponsor:	Center for Cancer Research, NCI, NIH			
Manufacturer:	Clinical Center Department of Transfusion Medicine (DTM), Center for Cellular Engineering (CCE)	Generic	Generic	Generic

Supplier:	Clinical Center Department of Transfusion Medicine (DTM), Center for Cellular Engineering (CCE)	CC Pharmacy	CC Pharmacy	CC Pharmacy

PRÉCIS

Background:

- Acute lymphoblastic leukemia (ALL) accounts for approximately 25% of childhood cancer. Survival rates have improved, but outcomes for some subgroups, including infants and young adults remain poor, and survival for patients who relapse is <50%, despite allogeneic stem cell transplant following second remission.
- CD19 immune escape has been observed by several groups following CD19-CAR therapy for B-ALL. Investigation of this phenomenon reveals a complex biology responsible for loss or downregulation of CD19 expression observed in these cases.
- Sequential therapy using CD22-CARs to treat CD19 dim/lo escape is associated with rapid development of resistance due to CD22 downregulation. This trial will test whether simultaneous targeting of CD19 and CD22 using a novel bivalent CD19/22-CAR is safe and feasible.

Objectives:

- Assess the safety of administering escalating doses of autologous CD19/CD22-CAR engineered T cells that meet established release specifications in children and young adults with CD19+CD22+ B cell ALL, isolated CNS ALL, or lymphoma following a cyclophosphamide/fludarabine conditioning regimen.

Eligibility:

- Participants between $\geq$ 3 years and $\leq$ 39 years of age, with CD19+/CD22+ B cell ALL, isolated CNS ALL, or lymphoma who have relapsed or have refractory disease after at least one standard chemotherapy regimen and one salvage regimen, with no alternative curative options who meet standard Phase I eligibility criteria.

Design:

- Phase I, 3 + 3 dose escalation design using the following dose levels: -1: 1 x 10^5 transduced T cells/kg (± 20%); 1: 3 x 10^5 transduced T cells/kg (± 20%); 2: 1 x 10^6 transduced T cells/kg; and 3: 3 x 10^6 transduced T cells/kg (± 20%); 4: 1 x 10^7 transduced T cells/kg (± 20%).
- Participants will receive a lymphodepleting preparative regimen of fludarabine (25 mg/m^2/d x 3 on Days -4, -3, -2) and cyclophosphamide (900 mg/m^2/d x 1 on Day -2) followed by infusion of CD19/CD22-CAR T-cells on D0. Participants who are CAR pre-treated (with exception for those with an interval HSCT) will receive increased lymphodepleting preparative regimen of fludarabine (30ˆ mg/m2/d x 4 on Days -5, -4, -3, -2) and cyclophosphamide (600 mg/m2/d x 2 on Days -3, -2) followed by infusion of CD19/CD22-CAR T-cells on D0. If fludarabine is unavailable, pentostatin may be given as an alternative.
- Patients will be evaluated sequentially for toxicity, antitumor effects, CAR expansion and persistence, as well as research correlatives.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

1.1.1.1 Assess the safety of administering escalating doses of autologous CD19/CD22-CAR engineered T cells that meet established release specifications in children and young adults with CD19+CD22+ B cell ALL or lymphoma following a cyclophosphamide/fludarabine conditioning regimen.

1.1.2 Secondary Objectives

1.1.2.1 Determine the feasibility of producing CD19/CD22-CAR T cells meeting the established release criteria.

1.1.2.2 Evaluate the ability of CD19/CD22-CAR T cells to mediate clinical activity in children and young adults with CD19+CD22+ B cell ALL, isolated CNS ALL, or lymphoma.

1.1.3 Exploratory Objectives

1.1.3.1 Evaluate whether participants receiving CD19/CD22-CAR T cells relapse with loss or diminished expression of CD19 and/or CD22.

1.1.3.2 Measure persistence of CD19/CD22-CAR T cells in the blood, bone marrow and CSF, and explore correlations between CD19/CD22-CAR T cell properties and CAR T cell efficacy and persistence.

1.1.3.3 Analyze alterations in early B cell development induced by immune pressure exerted via CD19/CD22-CAR T cells.

1.1.3.4 Assess neurologic and cognitive effects of the anti-CD19/CD22-CAR engineered T cell infusion pre- and post-infusion.

1.1.3.5 Explore the level CD19/CD22 surface expression and CD19/CD22 site density on leukemic blasts and correlate with clinical response to CAR-T cells

1.1.3.6 Explore the role of additional hematologic evaluation to enhance our understanding of the hematologic diathesis that can be seen in association with CAR or cytokine mediated toxicity.

1.1.3.7 Explore whether sequence-based genomic analysis can detect minimal residual disease (MRD) in participants who have persistence of CD19/CAR22+ T cells, and who are MRD negative by flow cytometry.

1.1.3.8 Retrospectively grade Cytokine Release Syndrome (CRS) as per the newly established American Society for Transplantation and Cellular Therapy (ASTCT) Consensus Guidelines that were published in 2019. **COMPLETED**

1.1.3.9 Explore apheresis and CAR T-cell product characteristics that may be associated with clinical outcomes (e.g., CAR T-cell persistence, toxicity and/or efficacy).

1.1.3.10 Explore CAR T-cell expansion by T-cell subsets and immunophenotypic evaluation of markers of T-cell activation and/or exhaustion.

1.1.3.11 Describe the toxicity profile after administration of anti CD19/CD22-CAR T cells in

children and young adults with isolated CNS disease.

1.1.3.12 Describe the toxicity profile, CAR T cell expansion and persistence, overall response rate, and overall survival after reinfusion of anti CD19/CD22 CAR T cells in participants who do not have active disease, but who have early loss of CAR T cells, or loss of B cell aplasia < 6 months post initial infusion.

1.1.3.13 Evaluate the gut microbiome in participants receiving CAR-T therapy and correlate with cytokine release, neurotoxicity, infection, antibiotic use and clinical response.

1.1.3.14 Conduct metabolomic analysis to evaluate how metabolite levels influence efficacy of CAR-T cells, and toxicity in pediatric participants treated with CAR-T cell therapy.

1.1.3.15 Evaluate the efficacy of siltuximab as a treatment for cytokine release syndrome and/or immune effector cell associated neurotoxicity syndrome.

1.1.3.16 Evalaute the impact of non-fludarabine based lymphodepletion on CAR T-cell efficacy, toxicity and CAR T-cell expansion

1.2 Background and Rationale

1.2.1 Acute Lymphoblastic leukemia (ALL) in Children and Young Adults

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy, accounting for approximately 25% of childhood cancer. Disease-free-survival rates have improved during the last 20 years to approximately 90% for children with B-ALL(1), however, outcomes for some subgroups, including infants and young adults remain poor, despite attempts to intensify treatment regimens(2-4). Survival for patients who relapse remains <50%, despite incorporating allogeneic stem cell transplant following second remission. Finally, short-term and long-term toxicity remain substantial(5-9). Novel treatment approaches are needed to improve cure rates and diminish toxicity associated with current standard therapy for B-ALL(6). Outcomes for children and young adults with B cell lymphoma have also improved during the last two decades, although outcomes for patients with B cell lymphoma refractory to frontline therapy remain suboptimal(10, 11).

1.2.2 Chimeric Antigen Receptor (CAR) Therapy

1.2.2.1 CD19-CAR Therapy in B-ALL

CARs are non-native receptors that link an antigen-binding domain to cell signaling domain(s). When expressed in T cells, CARs endow MHC-unrestricted antigen specificity. Most commonly, CARs utilize a single chain variable fragment (scFv) coupled to TCR zeta and CD28 or 4-1BB endodomains. Beginning in 2010, several groups published clinical results using CAR T cells targeting CD19 for B cell malignancies.

1.2.2.1.1 CD19 CAR Therapy

CD19 is a 95 kD transmembrane protein with expression restricted to the B cell lineage. Expression of CD19 is present from the time of immunoglobulin rearrangement through B cell development and maturation until it is lost with terminal plasma cell differentiation(12). It is not expressed on primitive hematopoietic stem cells. CAR T cells targeting CD19 first demonstrated

impressive clinical efficacy in small case series in adults with B cell lymphomas and chronic lymphocytic leukemia(13-15). The first pediatric report followed shortly after and demonstrated striking responses in two children with refractory and relapsed B-ALL(16). The demonstration of exquisite sensitivity of acute B cell leukemias inspired multiple centers to advance translation of CD19 targeting CARs. Despite utilizing varying strategies, clinical trials from multiple institutions have consistently shown dramatic activity of CD19-CAR T cells in patients with relapsed and refractory B-ALL, with complete responses rates of ≥70% across trials(17-20). Kochenderfer et al.(20) demonstrated CAR efficacy in aggressive lymphomas and reported 4 out of 7 complete responses in adults with refractory DLBCL treated with CD19 CAR T cells.

1.2.2.1.2 CD19-CAR T cell dose level

Over 750 patients have now been treated with CD19-CAR T cells for B cell malignancies. As detailed below, numerous clinical and scientific insights, drawn from this emerging experience serve as the basis for the current novel approach to CAR therapy. Responses following CD19-CAR therapy for B-ALL occur rapidly, with maximal response observed within 28 days following infusion of the engineered T cells. The relationship between CAR T cell dose and efficacy is less direct than that of a non-replicating drug, however there does appear to be a threshold dose of CAR T cells required for efficacy and toxicity. In the phase I study of anti-CD19 CAR therapy by Lee et al, the dose level of 1 x 10^6 cells/kg was found to be safe and effective, but the higher dose of 3 x 10^6 cells/kg while effective, was found to exceed the maximum tolerated dose(19). Similarly, the work by Kochenderfer et al., 1-2 x 10^6 cells/kg dose has been safely and effectively employed, while higher doses have been associated with toxicity(20). In contrast, the lower starting cell dose of 3 x 10^5 cells/kg tested in the ongoing phase I study of anti-CD22 CAR T cells at the NCI (PI-Shah) was largely ineffective in the majority of patients treated (1 responder amongst 6), while the 1 x 10^6 cells/kg dose was, as in the anti-CD19 CAR experience, both safe and effective, with the higher cell dose of 3 x 10^6 cells/kg associated with potentially more severe toxicity. In most active clinical studies, the recommended dose of CD19-CAR T cells is 1 x 10^6 cells/kg. The inclusion of endogenous costimulation within CAR constructs permits lower dosing than that required to achieve efficacy when using adoptive T cell methods lacking co-stimulatory signals.

1.2.2.1.2.1 CD19-CAR T cell efficacy

Independent of the infused T cell dose, anti-leukemic effects generally do not occur in the absence of expansion of the CD19-CAR T cells in vivo. Dramatic CD19-CAR T cell expansion can be observed, with >1000-fold expansion occurring within 1-2 weeks in patients with high tumor burden. CAR expansion can be quantified using polymerase chain reaction to measure the number of cells with integrated virus or by flow cytometry, where is it not uncommon to observe that >70% of all circulating T cells transiently express the engineered CD19-CAR receptor.

Expansion of CAR T cells is antigen driven, but is greatly enhanced following treatment with a lymphodepleting preparative regimen. Extensive preclinical work has demonstrated that lymphopenia induces elevations in the availability of cytokines that drive T cell expansion, notably IL-7 and IL-15, and that increased availability of such factors lead to enhanced expansion and improved efficacy(21). Based upon this paradigm, the vast majority of clinical trials using CAR T cell incorporate a pre-infusion preparative regimen, comprising cyclophosphamide and fludarabine, agents known to induce profound lymphocyte depletion.

1.2.2.2 CD19-CAR T Cell Therapy Associated Toxicities

1.2.2.2.1 Cytokine release syndrome (CRS)

Dramatic and rapid expansion of CD19-CAR T cells is often associated with toxicity, including cytokine release syndrome and neurotoxicity. Cytokine release syndrome (CRS) has been the topic of several reports and reviews(17, 22). In brief, CRS comprises a febrile, sepsis-like picture that results from hemodynamic and organ effects of supraphysiologic levels of inflammatory cytokines produced directly or indirectly by the activated T cells. Among the most important of these cytokines are IL-6 and IFN-gamma(17-19). CRS can be safely managed with supportive care and in some cases, immunosuppression using anti-IL6R mAbs therapy and corticosteroids(22). Notably, CRS is limited in patients with low tumor burden with contributions from any existing normal B cell population, and CRS severity correlates with the degree of CAR T cell expansion.

1.2.2.2.2 Neurotoxicity

Neurotoxicity is also observed in a significant minority (20-30% in most series) of patients(17-19) with toxicity ranging from mild to severe. The pathobiology of CD19-CAR associated neurotoxicity is not fully understood, but the clinical syndrome is also associated with increased expansion of CD19-CAR T cells as well as evidence for CD19-CAR T cells in the cerebrospinal fluid. In as much as patients with high tumor burden often have some level of CNS leukemia, it is seen more often in patients with CNS leukemia(19), however, patients without documented CNS leukemia or lymphoma can also manifest the syndrome. Hence, any direct association of CNS disease burden to neurotoxicity severity is unclear. The syndrome typically manifests clinically as confusion, aphasia and/or dysmetria, and occasionally seizures. Radiographic changes are typically not observed and the syndrome typically resolves in 1-2 weeks and appears fully reversible. The prevailing hypothesis regarding the pathophysiology of this syndrome is that it reflects non-specific neurotoxic effects of cytokines and/or activated T cells, rather than a direct on-target effect of CD19-CAR T cells. Indeed, the dose limiting toxicity of IL-6 when administered in a Phase I trial was neurotoxicity and transient aphasia(23). In further support of this hypothesis, the Jensen laboratory at Seattle Children's Hospital has recently developed a rhesus model of neurotoxicity that utilizes a CD20-CAR rather than the CD19-CAR platform and the model appears to model the human syndrome well (ASH presentation, unpublished). This provides further evidence against a direct, on-target effect involving CD19 in brain tissue.

In the summer of 2016, three deaths were reported as a result of severe neurotoxicity and cerebral edema in patients treated with CD19-CAR T cells. Given that more than 750 patients have been treated with these therapeutics, and only one previous death was reported and attributed to neurotoxicity associated with status epilepticus, these events were unexpected and work is underway to understand the basis for this phenomenon. Subsequently, two additional deaths were noted in the same trial utilizing high dose cyclophosphamide (in the absence of fludarabine) and CD19-CAR T cells incorporating a CD28 costimulatory domain. Notably, CAR T cells that incorporate a 4-1BB costimulatory domain do not typically undergo as rapid expansion as those using a CD28 costimulatory domain, so we postulate that the risk of fatal or very severe neurotoxicity may be reduced with a 4-1BB containing CAR. However, very recently (February 2017, personal communication-Seattle) one case of fatal neurotoxicity

(cerebral edema) using a CAR incorporating the 4-1BB costimulatory domain was reported. The CAR utilized in this study will incorporate a 4-1BB costimulatory domain.

1.2.2.2.3 Co-stimulatory Signaling

Another major insight gleaned from the substantial clinical experience with CD19-CAR T cells for B cell malignancies is the differential impact of distinct costimulatory domains on T cell function. It has become evident that CD28 costimulation facilitates more rapid and higher peak T cell expansion(18, 19), it also predisposes T cells to early exhaustion, which leads to poor long-term T cell persistence as a result of activation induced cell death(24). In contrast, 4-1BB costimulation is associated with a slower expansion rate, lower peak level, a diminished risk of T cell exhaustion and more prolonged persistence following adoptive transfer(24). Although remission induction rates do not appear to differ between CD28 and 4-1BB containing CD19-CARs, sustained remission likely requires T cell persistence and thus l 4-1BB CARs may confer improved long-term outcomes.

1.2.2.2.4 B-cell Aplasia

The cumulative CD19-CAR experience affirms that prolonged B cell aplasia is an expected consequence of effective, persistent CD19-CAR therapy. Similar to the management of patients with congenital absence of B cells, and patients treated with chronic rituximab, CAR-mediated B cell aplasia can be managed effectively with immunoglobulin replacement therapy(25, 26). Thus far, significant increase in infection susceptibility or other toxicity related to chronic B cell depletion has not been noted(18).

1.2.3 CD19 Immune Escape Following CD19 Directed Immunotherapy

CD19 is universally expressed at high levels on B-ALL at diagnosis and levels of expression do not change appreciably following cytotoxic therapy. Indeed, CD19-based flow cytometry provides powerful prognostic impact by measuring minimal residual disease (MRD) following standard cytotoxic therapy in B-ALL(27-29). However, with the introduction of CD19-based immunotherapies, relapse with diminished or absent surface CD19 has been increasingly observed. CD19 immune escape was first reported following blinatumumab therapy(30), and has been observed by several groups following CD19-CAR therapy for B-ALL.(16, 19, 31) In a recent report from Children's Hospital of Philadelphia of 50 patients rendered into remission with CD19-CAR therapy, 40% of patients had relapsed with a median follow-up of 10.5 months, and loss of the CD19 target accounted for 65% of the total relapses(32). While the true incidence of relapse due to CD19 immune escape has not yet been established, our CD19-CAR experience to date implicated CD19 escape as the most common cause of post-CAR relapse in B-ALL.

Investigation into the biology of CD19 immune escape has identified a complex biology guiding the loss or downregulation of CD19 expression. The two distinct pathways of tumor remodeling that have been recognized are categorized as the "isoform switch" and "lineage switch". The majority of cases fall into the isoform switch category, wherein the cells retain all other characteristics of B-ALL and there is no clear evidence for alteration in fitness. In the majority of "isoform switch" cases, mRNA specific for CD19 is retained, but is enriched for CD19 isoforms that preferentially remain intracellular, lack a transmembrane domain, and/or lack the epitopes targeted by all CD19-CARs currently under study as well as blinatumomab(33). Such B-ALL cells resultantly express reduced total surface levels of wild-type CD19 or express an

aberrant CD19 that lack the epitope targeted by CD19-CARs and blinatumumab. An alternative pathway for loss or diminution of CD19 expression involves tumor cells undergoing switching from the lymphoid to the myeloid lineage with a resultant population with myeloid markers and features that clonally relates to the parental B cell leukemia(31).

1.2.4 CD22-CAR Therapy for B-ALL

The emergence of CD19 immune escape has prompted interest in the development of immunotherapies targeting alternative B-ALL cell surface molecules. CD22 is a 130 kD sialic acid binding transmembrane glycoprotein, which belongs to the Siglec superfamily. Signaling via CD22 mediates inhibitory signals on B cell receptor signaling via immunoreceptor tyrosine-based inhibitory motifs, which are essential for maintenance of B cell tolerance(34-36). CD22 expression is restricted to cells of the B lineage. It is expressed on the surface of mature B cells, but is not expressed on pluripotent hematopoietic stem cells(37, 38). The vast majority of B-lineage malignancies express CD22 including B-ALL and B cell NHL(39-45).

Several therapeutics targeting CD22 have been studied in clinical trials with some success. An unconjugated CD22 mAb (Eptratuzumab), mediated modest clinical activity against B-ALL alone or when combined with chemotherapy in single arm studies(46-48). CD22 is rapidly internalized following antigen binding, leading to development of CD22 antibody drug conjugates and immunotoxins. Inotuzumab, an CD22 mAb conjugated to calicheamicin, mediated complete responses in 81% of patients with relapsed/refractory B-ALL(49). Moxetumumab, an anti-CD22 genetically linked to pseudomonas exotoxin mediated clinical activity in hairy cell leukemia(50) and B-ALL(51), but benefits were transient due to the agent's short half-life, and substantial immunogenicity, which precluded repetitive cycles. 90Y-conjugated epratuzumab mediated complete responses in 3 of 17 B-ALL patients treated, but this was associated with associated with pancytopenia due to radiotoxicity.(48)

Our group sought to develop a chimeric antigen receptor targeting CD22. A variety of binding domains, hinge regions and costimulatory domains were compared for efficacy based upon killing of cell lines in vitro and the capacity to eradicate human B-ALL xenografts in immunodeficient mice. The lead candidate incorporates the fully human scFv m971, which recognizes a membrane proximal epitope on CD22(41)·(52);. Among other therapeutics targeting CD22 currently or previously in clinical trials, none have utilized this binding domain. The lead candidate also has a very short hinge region, and incorporates the TCR zeta signaling domain and the 4-1BB endodomain, which was selected based upon superior functionality compared to the CD28 endodomain(24).

Clinical testing of this first chimeric antigen receptor targeting CD22 is under study at the Pediatric Oncology Branch (POB) of the National Cancer Institute (NCI) in children and young adults with B-ALL. Results from the first sixteen patients treated are encouraging. The first dose level tested of 3 x 105/kg was chosen to be lower than the current standard CD19-CAR dosing because this was a first in human study. Six patients received this dose with one experiencing an MRD negative CR. A second patient demonstrated signs of clinical activity as evidenced by a substantial reduction in circulating blasts, but the patient subsequently developed rapid disease progression and did not achieve a CR. At the second dose level, 1 x 106/kg, 8 of 10 patients experienced an MRD negative CR. Each of the remissions induced at the 1 x 106 dose level were associated with persistent CD22-CAR T cells in the peripheral blood. One of the patients

from this initial cohort remains in an ongoing remission at 18 months post CAR therapy with ongoing detection of CAR T cells. At dose level three, 3 x 106/kg, the first patient developed grade 4 hypoxia following CAR T cell infusion. A second patient was safely treated at this dose level without a DLT. Based on the single DLT at the third dose level and clinical activity appreciated at dose level 2, a decision was made to expand dose level 2 to further explore activity and safety. Eight additional patients have since been treated at dose level 2 (1 x 106/kg) to further explore activity and safety. Nine patients (56%) achieved a complete remission, with 8 out of 10 responders (80%) receiving dose levels ≥ 1 x 106 CD22 CAR/kg. Of the sixteen patients enrolled, nine had CD19 neg/dim B-ALL as a result of previous CD19 directed immunotherapy, and six of these patients achieved complete remission to CD22-CAR. Updated results of this CD22 CAR trial were recently published upon. 58 patients have been treated to date, with 50/58 (86.2%) of patients having had CRS. Of the 57 infused patients evaluable for response, 40/57 patients achieved a complete response, of whom 35 were MRD negative(53).

From this small series, we conclude that the CD22-CAR shows many characteristics that are similar to the CD19-CAR. Response occurs rapidly within 28 days and correlates with CD22-CAR expansion. Cytokine release syndrome did occur, but the maximal severity was Grade 2. The CD22-CAR T cells traffic to the spinal fluid at levels similar to that observed with CD19-CAR. Although a cyclophosphamide/fludarabine based preparative regimen was used, we observed minimal neurotoxicity (Grade 1) in two patients, all of which was reversible. Two patients experienced grade 5 toxicity at the expansion cohort. This include one patient who developed bacterial infection following recovery from CRS and neutrophil recovery and ultimately died from multi-organ failure due to infection. An additional patient developed grade 5 ARDS in the setting of CRS as expected, since the CD22-CAR incorporates a 4-1BB costimulatory domain, patients maintained CAR persistence and persistent B cell depletion at 90 days, with some evidence for persistent CAR cells out to 9 and 18 months seen in those patients who are in the longest remission. This CD22 CAR is the first alternative to CD19 for CAR based immunotherapy for B-ALL. Given that CD22 is expressed on numerous other B cell malignancies, the results raise the prospect that this CD22-CAR may also provide a novel therapeutic for application across the spectrum of B cell malignancies.

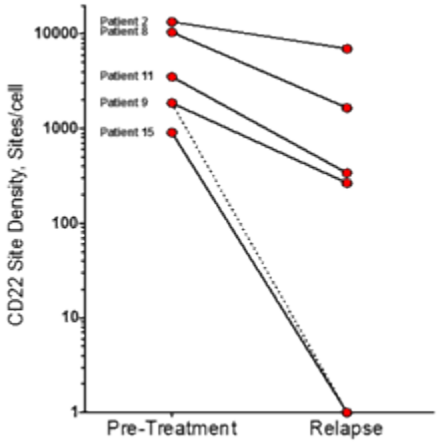
The CD22-CAR also provides the first evidence for a salvage immunotherapy, in the event of relapsed disease or resistance to front line immunotherapy.

1.2.5 CD22 Immune escape following CD22 targeted immunotherapy

In the current CD22-CAR T cell trial being conducted by POB NCI, alterations in CD22 site density have been observed in a few of the relapsed patients. One notable example is being seen, following CD22 directed antibody therapy (inotuzumab), where several patients have emerged with partial loss of CD22 on leukemia blasts after this therapy. Similarly, following anti-CD22 CAR therapy, one patient, at the 6-month restaging evaluation revealed a decrease in CD22 site density from over 10,000 sites/cell prior to CD22-CAR therapy, to approximately 1000 sites/cell at relapse in the presence of circulating anti-CD22 CAR T-cells (see **Figure 1**, Patient #8). Another patient, known to be CD19 negative at the time of CD22-CAR infusion, developed relapsed leukemia at 5 months post-CD22 CAR therapy that was partially positive for both CD19 and CD22 (see **Figure 1**, Patient #9). Another patient who was known to be CD19 negative prior to CD22 CAR therapy and had received inotuzumab prior to receiving her CD22 CAR T cells, had a bimodal CD22 population noted in her peripheral blood flow with 89% of blasts

expressing CD22; bone marrow flow cytometry showed complete CD22+ population at that time. Despite this, at 1-month post CD22 CAR therapy, she had a complete MRD negative remission, with 41% circulating CD22 CAR T cells in her bone marrow. At her 2-month restaging visit, despite a high level of circulating CD22 CAR T cells, she relapsed with CD19-/CD22- ALL (see **Figure 1**, Patient #15). Additionally, another patient who was previously refractory to blinatumomab and CD19 CAR, attained a complete remission but had a decrease in CD22 site density, from approximately 3,500 sites per cell prior to CD22 CAR therapy and post infusion, CD22 site density of less than 500 sites/cell at 1 month (see **Figure 1**, Patient #11). The mechanism for CD22 surface expression alteration is unknown at this time, however constant CAR immune pressure as seen in CD19-CAR treated patients may contribute to this phenomena.

Figure 1: CD22 Site Density with CD22-CAR T Cell Therapy



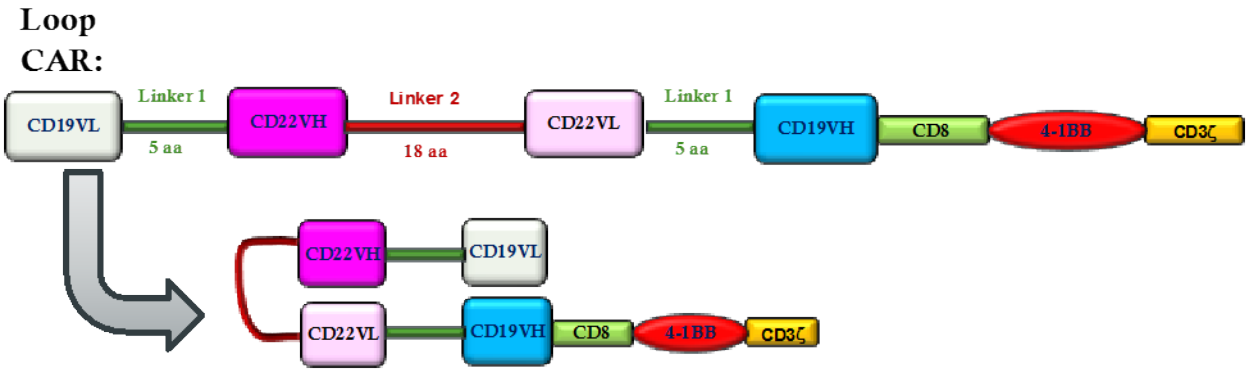
In summary, the reported clinical trial experience using CAR-T cells for management of B cell malignancies and the toxicity profile, supports use of this treatment strategy for children and young adults with no alternative curative options. We believe the lower CRS and neurologic toxicity rates observed using 4-1BB costimulation along with evidence that 4-1BB provides persistent CAR-T expression with durable responses supports the use of 4-1BB costimulation in our CAR19/CD22 construct.

1.2.6 Development of a Bi-specific CD19/CD22-CAR for B-cell Malignancies

Sequential treatment with CD19-CAR followed by CD22-CAR reserved for patients who develop CD19 neg/dim resistance provides one approach to immune targeting of B-ALL. However, there is concern that a similar loss of CD22 expression might also occur as a result of an immune response directed solely toward one target. Furthermore, we noted that one patient treated with CD22-CAR developed recurrence at 3 months with increased expression of CD19 at that timepoint. Therefore, we posit that the long-term effectiveness of CARs could be enhanced if the CAR could simultaneously target two antigens, such as CD19 and CD22.

To this end, we developed a chimeric antigen receptor with transmembrane and signaling domains that are essentially identical to the CD22-CAR, but in place of an scFv targeting singular CD-22, we incorporate scFvs identical to those incorporated into the original CD19-CAR (fmc63) and the CD22-CAR described above (m971), **Figure 2**.

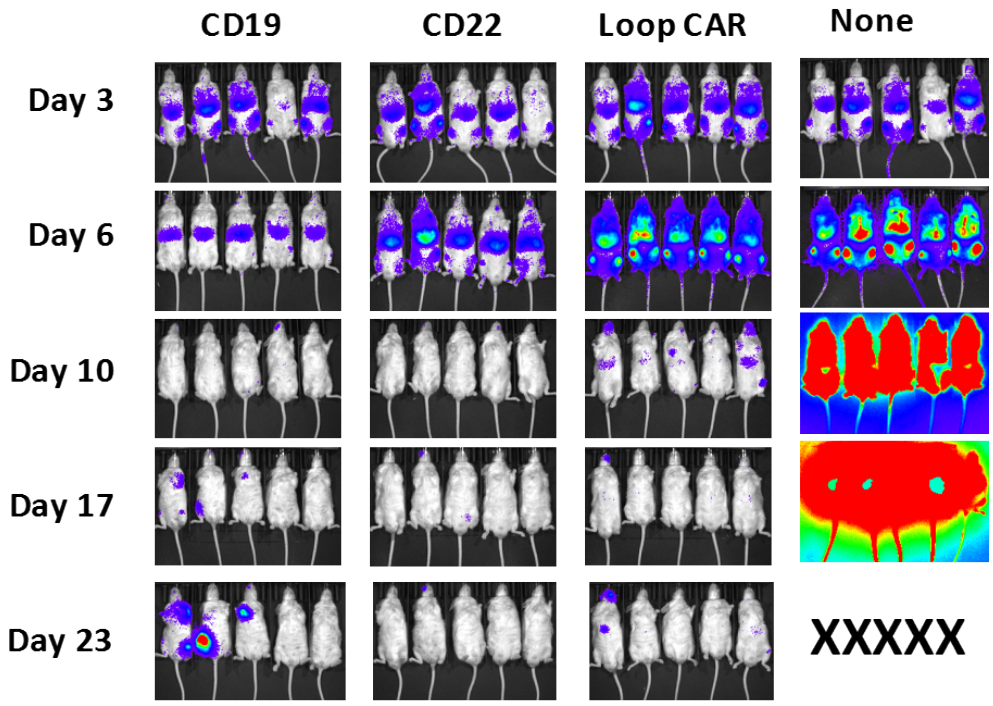
Figure 2: Loop CAR Construction Using CD19 & CD22 scFv From Single CAR Construct



The anti-leukemia activity of bispecific loop CAR T cells was established and compared to CARs targeting the CD19 and CD22 antigens. Lentiviral vectors containing supernatants were generated for the CD19, CD22, and bispecific CAR constructs and T cells were transduced to express respective CARs. Negative controls using mock (non-transduced) T cells were cultured and activated in parallel. We then tested *in vivo* CAR cytotoxicity. NSG immunodeficient mice were injected with the NALM6 leukemia cell line that has been permanently transfected to express the luciferase gene (termed NALM6-GL, gift of S. Grupp and D. Barret, Children's Hospital of Pennsylvania). NALM6-GL leukemia growth within a xenograft model can be detected by imaging the mice with a sensitive CCD camera that can measure photons produced by the luciferase enzyme following injection of the mice with the luciferin substrate. On day 3, upon establishment of detectable leukemia, mice were injected with CAR T cells transduced with either CD19, CD22, bispecific loop CAR, or control. The bispecific loop CAR had comparable activity in xenografts compared to CD19-CARs and CD22-CARs (**Figure 3**). This bivalent, bispecific CAR has the advantage of being able to recognize CD19+CD22+ cells, as well as CD19+CD22– and CD19–CD22+ cells.

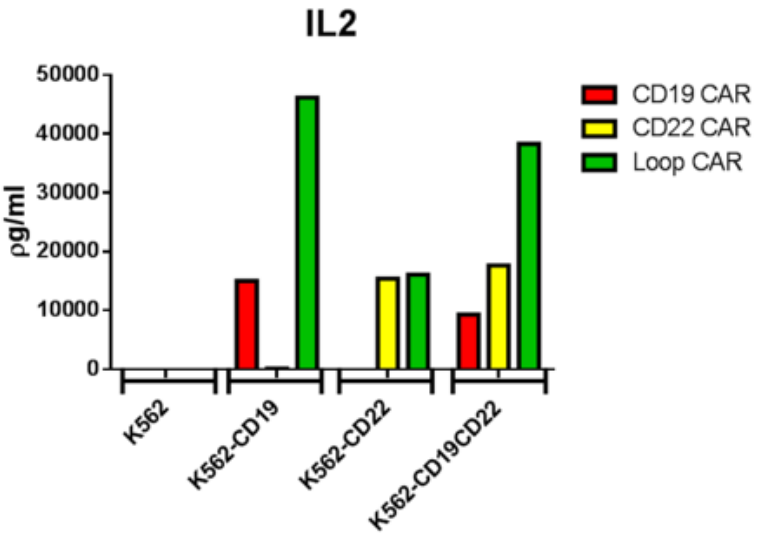
CML cell line K562 was artificially transduced with CD19 or CD22 or both to express the target antigens. K562 cells served as the negative control. 1E5 CAR T cells were washed 3 times and then co-incubated with 1E5 target cells in RPMI media at 37^0C. After 14 hours, culture supernatant was harvested and the production of the cytokines was measured with ELISA kits. IL-2 production by the Loop CAR was comparable to that seen with the CD19 CAR (**Figure 4**).

Figure 3: Potent in vivo Activity of Bispecific CAR T cells against CD19+CD22+Leukemia



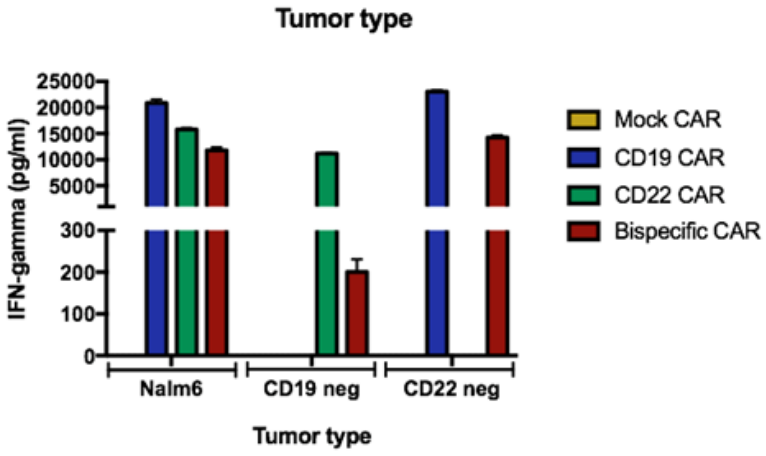
NSG (immunodeficient) mice were injected with 1 x 10^6 NALM6-GL cells on day 0 and images taken on day 3 demonstrate, with luciferin substrate, the presence of leukemia in all animals. On day 3, each mouse group was injected with 3 x 10^6 CAR T cells transduced with either CD19, CD22, Bispecific, or control. Mice were re-imaged weekly and equivalent anti-leukemic activity was seen in Bispecific Loop CAR.

Figure 4: Cytokine production by ELISA in vitro



Interferon-gamma production was also seen with use of the bispecific CAR against both CD19 negative and CD22 negative samples (**Figure 5**).

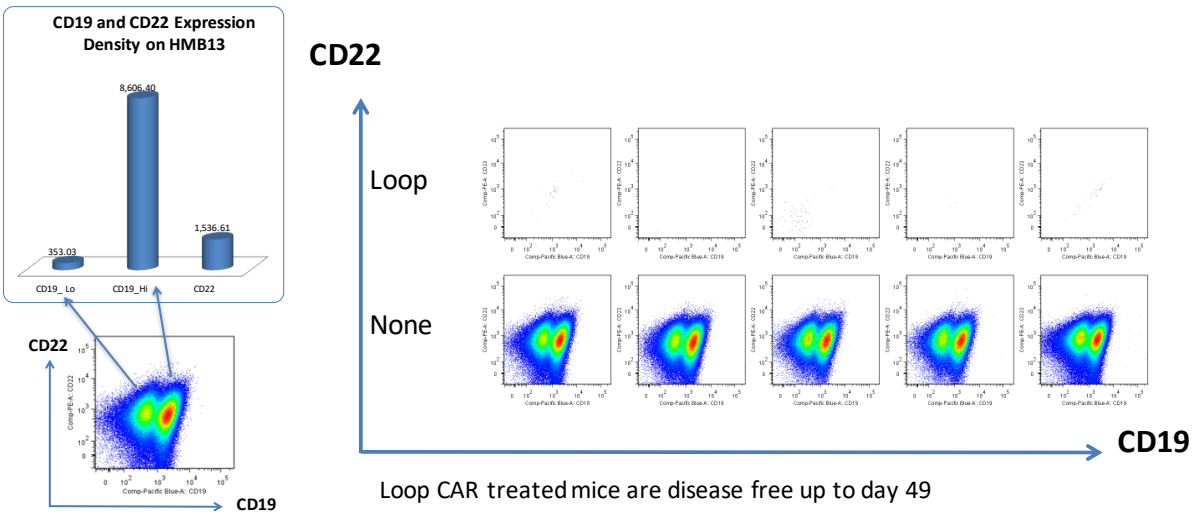
Figure 5: IFN-gamma production with CARS



Additional testing was performed pre-clinically to determine if the bispecific loop CAR had activity against CD19 dim populations. After treatment with blinatumomab, a patient relapsed with a $CD19^{low}$ and $CD19^{high}$ leukemia. A patient derived xenograft model (PDX) was created with this leukemia phenotype. At day 0, 1 x 10^6 leukemia cells where injected into two NSG mouse groups, experiment vs control. At day 9, when leukemia was detected by flow cytometry, bispecific loop CAR T cells and non-transduced mock T cells were injected into the experimental and control groups respectively. The bispecific loop CAR demonstrated excellent activity and cured this post CD19 directed immunotherapy relapse.

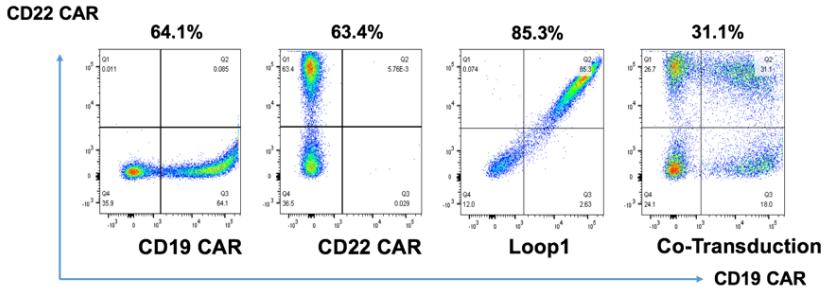
Patient samples post CD19-directed immunotherapy shows CD19^{low} and CD19^{high} population in addition to CD22 population. NSG mice were injected with 1 x 10^6 leukemia cells and treated on day 9 with 3 x 10^6 CAR T cells of bispecific loop CAR or non-transduced T cells. The bispecific loop CAR was able to eradicate PDX sample and mice remained disease free up to 49 days (**Figure 6**).

Figure 6: Relapsed Patient Sample Cured in Xenograft Model After Treatment with Bispecific Loop CAR T cells.



Other strategies that could provide a dual-targeting strategy include either 1. Co-transduction of both CARs into one T cell or 2. Co-infusion strategy by which both anti-CD19 CAR and anti-CD22 CAR T cells would be simultaneously infused. Both were considered as alternative approaches to the currently proposed bi-specific CAR strategy. In regards to the first strategy of co-transduction, although a reasonable concept, preclinical studies performed in the Fry lab (POB, NCI) demonstrate that this approach leads to a much lower transduction efficiency than desired and is not a clinically feasible approach at this time. (**Figure 7**, Personal communication-Fry lab).

Figure 7: Transduction Efficiencies of CAR Strategy Options

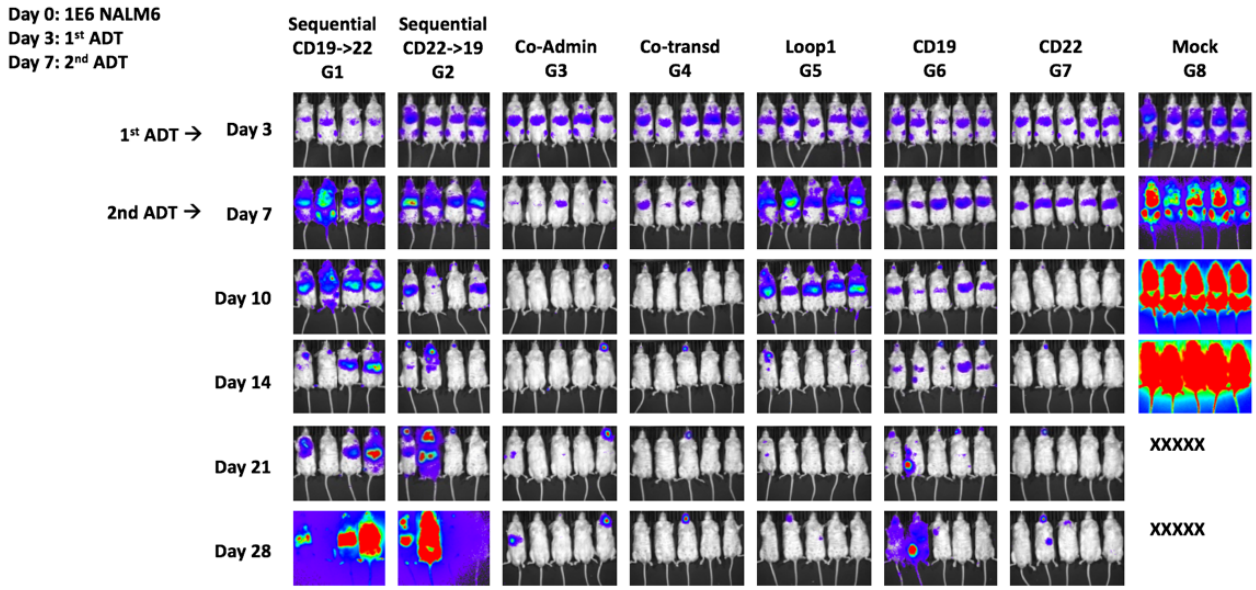


The vectors for CD19, CD22, and the bispecific Loop1 CAR were produced by transient transfection of the 293T lenti packaging cell line. Human PBMCs from a healthy donor were activated with CD3/CD28 microbeads for 24 hours. Activated T cells were then transduced with the vector individually or co-transduced with both CD19 and CD22 vectors together. Surface expression of the CD19 CAR and CD22

CAR were analyzed on day 8. Co-transduced T cells have much lower expression of both CD19 and CD22 CARs compared to the bispecific CAR. The expression of CD19 and CD22 CARs on co-transduced T cells are not at an equal molar ratio. In contrast, Loop1 CAR has an almost 1:1 ratio in expression of CD19 and CD22 CAR, which displays as a diagonal plot.

In regards to a co-infusion model (with either simultaneous or sequential infusion of anti-CD19 and anti-CD22 directed CAR cells), several concerns emerge. First, a sequential infusion approach, which may be preferred to avoid cumulative infusion related toxicity, is ineffective *in vivo*, as demonstrated below (**Figure 8**).

Figure 8: Effectiveness of CAR Therapy Administration Options



NSG mice were challenged with 1E6 of NALM6 leukemia on day 0. On 3 day, mice in group 4 to 7 received 3E6 of CAR$^+$ T cells. Mice in group 1 and group 2 received sequential treatment with 1E6 CD19 or CD22 CAR$^+$ T cells on day 3 and 3E6 CAR$^+$ T cells of the other CAR on day 7. Mice in group 3 received co-administration of a total of 6E6 CAR T cells with 3E6 of CD19 CAR and 3E6 of CD22 CARs on day 3. Mice in group 4 received almost 10E6 of total T cells due to the low expression on the co-transduced T cells. Bioluminescent intensity represents tumor burden.

Simultaneous administration, while effective, in this model, may have implications in the clinical setting with cumulative toxicity of two different CAR cell expansions—but certainly could be explored further. The co-transduction model, shown above, while effective is notably being given at much higher doses to achieve this efficacy and is not yet a clinically feasible strategy.

Thus, in proceeding forward with a dual-targeted approach, to explore the safety of a single CAR with dual targeting capabilities avoids concern for cumulative toxicity and demonstrates *in vivo* clinical activity.

1.3 PRODUCTION OF CD19/CD22 CAR EXPRESSING T CELLS

This protocol will test the safety and activity of the bi-specific CD19/CD22-CAR in patients with relapsed, refractory B cell malignancies. Major elements of the treatment regimen, including the processing of the cells, the lymphodepleting preparative regimen and dose escalation scheme will closely mimic that clinical development of CD19-CAR and CD22-CARs. Briefly, fresh or

cryopreserved peripheral blood mononuclear cells collected via apheresis will be enriched for T cells using a CD4/CD8 immunomagnetic bead enrichment. They will then be activated by co-culture with immunomagnetic particles expressing anti-CD3 and anti-CD28 mAb. One day following activation, replication incompetent lentiviral vector particles containing the CD19/CD22.BB.z construct will be added to the culture for transduction. Cells will be incubated for minimum of 9 days with IL-2 (200IU/mL) then harvested and administered fresh or frozen for subsequent infusion. PI has the option to extend the culture to day 12. Cells will be required to meet standard release criteria including transduction efficiency based off of protein-L flow ≥15%, viable T cell content ≥80%, flow identity testing using anti-CD19 idiotype and CD22-Fc/Siglec2 ≥ 10%, in process sterility, mycoplasma, endotoxin, as well as no evidence for replication competent lentivirus. CAR T cell products are manufactured on the CliniMACS Prodigy (Miltenyi Biotec), an automated cell processing device designed for closed-system, GMP-compliant manufacturing of cell therapy products. Manufacturing unit operations on the Prodigy are carried out in validated closed-system, presterilized, single-use disposable sets to prevent contamination and cross-contamination. All procedures will take place using good manufacturing process guidelines

1.4 Safety Considerations for Bispecific CD19/CD22 CAR Therapy

1.4.1 Risk of chemotherapy

Toxicities resulting from fludarabine and cyclophosphamide in the doses proposed in the current study are well known and are what have been used in the prior anti-CD19 CAR and anti-CD22 CAR protocols conducted at the Pediatric Oncology Branch (POB) of the NCI. Such a preparative regimen is designed to decrease the number of endogenous T cells, including T regulatory cells that may otherwise suppress CAR T cell cytotoxicity, and to induce increased availability of homeostatic cytokines thereby allowing for better engraftment of the transferred CAR T cells. The dose limiting toxicity (DLT) 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 but is unlikely given the relatively low dose administered in this trial and given that mesna will be used prophylactically as a uroprotective agent. Tumor lysis syndrome (TLS) following fludarabine and cyclophosphamide administration can occur in patients with advanced bulky disease. To prevent this, 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 (e.g., Bactrim for PCP prophylaxis) during treatment and for a minimum of 6 months following treatment.

1.4.2 Risk of Autoimmunity

Autoimmune toxicity is a risk of adoptive cell therapy trials for cancer and can occur if the transferred populations recognize the target antigen on normal tissues. Thus far, on-target, off-tumor toxicity of both CD19-CAR and CD22-CAR T cells has been restricted to B cell aplasia, which can be managed with immunoglobulin replacement therapy. Given that the CD19/CD22-

CAR tested in this study incorporates scFvs that have already been tested in clinical trials, it is unlikely that unexpected autoimmune toxicity will occur, but patients will be monitored closely for the occurrence of unexpected toxicity and if it is observed, appropriate supportive care and modification to the clinical trial will be instituted.

1.4.3 Risks of Gene Therapy

Risks of gene therapy include insertional mutagenesis or emergence of replication-competent lentivirus (RCL). 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 or lentiviral 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 lentivirus, 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.

Of note, 10 participants on this study had RCL testing performed in real time pre- and post-infusion and there was no RCL detection in any of the samples tested. Based on this data, a proposal was submitted by the protocol team and approved by the FDA to eliminate culture based testing on the infused product, and to eliminate real time testing of patient samples, with a plan to archive samples that will be available to be tested at a later date if there is a clinical concern. An additional 14 patient samples have been collected and will be stored and tested if clinically indicated at a future date. (Note: Testing removed with protocol revisions in September 2022.)

1.4.4 Risk of Cytokine Release Syndrome

Cytokine release syndrome occurs in the setting of a variety of immunotherapies including following antibody infusions and also following adoptive cell therapy. The clinical picture, now described by many groups, is essentially indistinguishable from infection/sepsis, with fever being the hallmark sign. The pathophysiology is related to excess cytokine levels, released either directly from the adoptively transferred cells or indirectly induced by the immune activation. Severe cytokine release syndrome can be associated with signs and symptoms of macrophage activation syndrome and hemophagocytic histiocytosis. Grading and management of CRS in this protocol will follow the guidelines set forth by consensus criteria established in 2019 by the ASTCT, formerly known as American Society for Blood and Marrow Transplantation (ASBMT) (54) which includes diligent supportive care and search for infection, with immunosuppression using anti-IL6R mAbs, IL6 mAbs, and/or corticosteroids reserved for more severe cases. Using such a risk-adapted system for management of CRS has diminished morbidity and mortality associated with this syndrome. (See **Appendix D**)

1.4.5 Risk of Neurotoxicity

As discussed above, a sizable minority of patients treated with CD19-CAR therapy develop reversible neurotoxicity that may range from mild cognitive confusion, to visual hallucinations, to lack of responsiveness to commands. Although the pathophysiology of neurotoxicity is not well understood, current concepts hold that it is a non-specific effect of immune activation rather than a direct, on-target effect of CD19-CAR T cells. While immunosuppression appears to effectively reverse cytokine release syndrome, treatment with anti-IL6R for neurotoxicity does not appear to be effective and may even lead to transient worsening due to elevations in circulating IL-6 induced by the mAb. Corticosteroids may have some beneficial effects, but controlled trials have not been undertaken and therefore the true effectiveness of corticosteroids for management of severe neurotoxicity remains unclear.

Furthermore, we postulate that the risk of fatal or very severe neurotoxicity may be reduced with a 4-1BB based on the experience thus far that suggests that 4-1BB costimulatory domain-based CARS do not typically undergo as rapid expansion as those using a CD28 costimulatory domain. Indeed, lethal neurotoxicity has rarely been reported using a CAR incorporating a 4-1BB costimulatory domain. The CAR utilized in this study will incorporate a 4-1BB costimulatory domain.

Patients will be monitored carefully for neurotoxicity throughout this study (See section **3.12** for additional details). Additionally, we will also have prospective neurologic evaluations performed on this protocol to provide additional input and insight into the treatment and management of the patients who do develop neurotoxicity. All patients will be treated prophylactically with levetiracetam (Keppra), unless contraindicated, and patients with significant neurotoxicity will be monitored closely and treated aggressively as outlined in section **4.7**. Prospective neurocognitive testing will only be performed in patients with isolated CNS disease (see section **1.5.5**; updated with protocol revisions in September 2022).

1.4.6 Risk of Disseminated Intravascular Coagulation (DIC) and Hemophagocytic lymphohistiocytosis (HLA)/ macrophage activation syndrome (MAS)

DIC and HLH/MAS are known complications of CAR therapy,(55, 56) the underlying etiology of which is unclear, but believed to be due to an inflammatory response related to CAR Therapy. DIC can present as asymptomatic lab findings but can be more severe and present with signs of clinical bleeding. Understanding the pathophysiology of DIC is being further explored as an exploratory analysis (see section **1.1.3**) and the early clinical experience with DIC on this protocol suggests that it is a self-resolving process that can be ameliorated with use of steroids. Similarly, HLH/MAS—which can manifest as a constellation of symptoms including fever, elevated ferritin, hypertriglyceridemia, pancytopenia and hepatic function abnormalities may also self-resolve or can be treated with immunosuppressive agents (steroids, chemotherapy, Anakinra are a few examples)(57). HLH/MAS may also present with features of coagulopathy, so it is currently unclear if the DIC is a manifestation of HLH/MAS, but certainly all of these appear to be linked to the CAR-T cell induced inflammatory milieu. Patients treated on this protocol will be closely monitored for these findings as they are clinically relevant and may require intervention to reduce risks.

1.4.7 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(58). Flow cytometry typically permits detection of recurrent or persistent disease with sensitivity on the order of 1 cell in 10^4, while PCR can detect disease on the order of 1 cell in 10^5. 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 10^6) that could be used predictively for relapse(59-61). Adding this testing to our current CD19/CD22 protocol can yield information on very low levels of MRD in our patients and help us prospectively and retrospectively analyze data acquired for these patients after CAR therapy.

1.5 PROTOCOL SUMMARY AND RATIONALE

Most aggressive cancers eventually relapse or become refractory to current conventional treatments, necessitating the immediate development of new treatment modalities. The most recent advances in cancer therapy involve the development of new immune stimulating agents designed to target T-cells to cancer cells and initiated cell death signaling pathways. However, limitations to this type of immunotherapy include lack of tumor specificity, insufficient T-cell recruitment/numbers, and the existence of endogenous immunosuppressive agents which significantly attenuate or altogether block T-cell activity.

Chimeric Antigen Receptor (CAR) expressing T-cells is a new therapy wherein a patient's own T-cells are harvested and subsequently genetically modified in order to target cell surface antigens on specific cancer cells. In addition to their specificity, these CAR T-cells can be modified to be highly proliferative and possess the ability to negate immunosuppressive mechanisms making them ideal agents against highly aggressive cancers.

Specific to this study, bivalent CARs expressing both CD19 and CD22 will be utilized in order to avoid the immune escape that occurs when only a single antigen is targeted.

1.5.1 Clinical Trial Update on CD19/CD22 CAR T cells

As of September 2022, 26 patients with B-cell acute lymphoblastic leukemia (B-ALL) and 2 patients with B-cell non-Hodgkin lymphoma (NHL)have been treated on this study across three different dose levels and the results of the ALL cohort are described below.

Fifteen (58%) of those with ALL developed cytokine release syndrome (CRS), with 3 (11.5%) having ≥ grade 3 CRS and all were effectively treated with supportive care, and some patients required administration of tocilizumab and/or steroids. Additionally, of the 26 patients treated, only 2 experienced neurotoxicity (grade 3) which was reversible with systemic steroids and intrathecal hydrocortisone.

Importantly, 17/26 patients with B-ALL had complete remission of their disease. Based on the results to date, dose level 3 is being utilized in the expansion pase of the study, as it has a tolerable side effect profile and is associated with anti-leukemia response(62).

1.5.2 Dose Escalation

The experience to date with CD19 CAR and the POB NCI experience thus far with CD22-CAR trials is that dose level 1 x 10^6 transduced CAR T cells/kg is an effective dose. Given that this is a new CAR construct, our proposal is to start as a lower dose level at 3 x 10^5 transduced CAR T cells/kg.

1.5.3 Rationale for Amendment, version date 01/11/2020

Based on the experience to date (n=11), this CAR T-cell construct is well-tolerated but at the current dose levels being utilized, we are seeing limited expansion, persistence and efficacy. Although initial dose escalation was planned using our prior CAR T-cell experiences, to further explore the functionality of this construct, we would like to add an additional dose level on this trial. For several CD19 CAR-T cell constructs, including the FDA approved construct, early CAR-T cell expansion and prolonged B-cell aplasia > 6 months was associated with complete responses and long-term CAR persistence and response. Thus, we propose to add an additional dose level, DL 4: 1 x 10^7 transduced T cells/kg.

Thus far, no subject has experienced DLT at any dose level and cytokine release syndrome (CRS) has been limited [DL1 (3 x 10^5 transduced T cells/kg (n=4); DL2: 1 x 10^6 transduced T cells/kg (n=4); DL3: 3 x 10^6 transduced T cells/kg (n=3)] and only 2 of 11 subjects has had $\geq$ grade 2 CRS, both of whom had full recovery.

We hypothesize that a higher dose level could safely be administered with a potential to optimize CAR T-cell persistence and efficacy and that this dose level can be adequately manufactured. Additional dose-escalations are not planned due to concerns for dosing feasibility.

In CD19 CAR treated patients, disease burden correlated with toxicity profile and severity of CRS; patients with higher disease burden had higher grade CRS(18, 19, 63). Thus, we have had minimal clinically significant cytokine release syndrome (CRS) at DL1-DL3, with only 2/11 patients treated having > grade 2 CRS.

1.5.4 Rationale for Amendment, version date 09/08/2020

Protocol update: (as of 9/15/20) A total of 17 patients have been enrolled on study, and 15 patients have been treated to date (one patient had intercurrent illness that precluded treatment, and one additional patient has yet to be infused). Amongst the 17 patients, 1 patient has B-cell lymphoma, and the others have B-cell ALL.

Table 1: Enrollment by Dose Level, Response, and Toxicity Summary

Dose level (Dose transduced CAR T cells/kg)	Enrolled (n)	Previous CAR therapy n, (%)	DLT (n)	CR (n, % CR rate)	Persistence of Circulating CAR-T cells at 2 months (n, %)
1 (3 x 10e5)	4	4, (100%)	None	0	None

Dose level (Dose transduced CAR T cells/kg)	Enrolled (n)	Previous CAR therapy n, (%)	DLT (n)	CR (n, % CR rate)	Persistence of Circulating CAR-T cells at 2 months (n, %)
2 (1 x 10e6)	4	1, (25%)	None	2 (50%)	1 (25%)
3 (3 x 10e6)	9*	1, (14%)#	1, (14%)#	5 (71.4%)#	None
*2 patients have not been treated. # denominator is 7 patients treated at dose level 3. Dose level 3 was expanded to treat at least 6 patients due to DLT as described below. No additional DLTs were seen. DL3 will now be used for expansion phase of trial..					

One subject has experienced DLT at dose level 3 ($3x10^6$ transduced T cells/kg) in the form of grade 3 neurotoxicity which manifested as delirium/confusion and altered mental status. This subject was treated with steroids and had rapid reversal of neurotoxicity. Dose level 3 has now been expanded to treat at least 6 patients. Cytokine release syndrome (CRS) ≥grade 3 remains limited [DL1 (3×10^5 transduced T cells/kg (n=4); DL2: 1×10^6 transduced T cells/kg (n=4); DL3: 3×10^6 transduced T cells/kg (n=7)] with only 2 of 15 subjects having had ≥ grade 2 CRS, both of whom had full recovery.

Thus, based on the response rate at dose level 3, and limited toxicity profile, we will use dose level 3 in the expansion phase of the trial.

Additional changes in this amendment include:

- Increasing the age limit to 35: The current adolescent and young adult (AYA) definition used by the NCI (and widely accepted in the oncology community) includes patients up to 39 years of age. We will target an age ≤35 years old as many young adults are relapsing with B cell malignancies, that are biologically/ genetically similar to pediatric B cell malignancies, and may be amenable to treatment with CAR T-cell therapy, however are not eligible to receive the FDA approved product which is currently limited to those < 25 years of age. All prior POB HSCT protocols have treated patients up to 35. The pediatric oncology branch has expertise in treating AYA patients with refractory malignancies and has gained significant experience and comfort in treating this young adult population with CAR T-cell therapy.
- Addition of an option to administer oral mesna and oral hydration for patients receiving outpatient lymphodepleting chemotherapy. The option to give oral supportive care (hydration and mesna) is considered standard of care and a well-established practice in the Children's Oncology Group Consortium Supportive Care Guidelines.
- Increasing the time from donor lymphocyte infusion (DLI) to enrollment/treatment on study with CAR T cells due to a recent incident in a patient where the patient received DLI 4 weeks prior to enrollment and developed graft-versus-host-disease (GVHD). The GVHD is primarily attributable to DLI, however, it is possible that CAR T-cells could have contributed to triggering the GVHD event.

1.5.5 Rationale for Amendment, version date 05/17/2021

1.5.5.1 Modify Dosing Calculation Practices in Obese Patients

Based on our ongoing efforts in CAR T-cell therapy in children and young adults, and both the 1) recent increase in the upper age limit and 2) consideration of an non-responding obese patient recently treated on a parallel CAR T-cell trial (and review of our own data), we have made the decision to revise our current protocol guidance for dosing in obesity.

To date, there are no well-established guidelines for CAR T-cell dosing in obesity, and more importantly, there has been no clear evidence for increased toxicity with CAR T-cells based on obesity, however, dose-dependent responses and anti-leukemia efficacy has been well-established by our team and others. Our current practice (established in 2012) incorporates dosing on a per kg/basis with dosing/weight adjustments for obesity for a BMI $\geq$ 30. However, in review of our practices and national obesity metrics, we believe that this guideline warrants revision.

Based on both institutional and national practices, consideration of CAR T-cell dosing limitations, and to more appropriately account for pediatric and adult obesity, we will be making the following changes:

- Maximum cell dosing will be capped at 100 kg weight (currently there is no cap on weight)
- Cell dose adjustments for obesity will be made for BMI $\geq$ 35
  - Practical weight (as per our current protocol) will be utilized for dose-adjustments in obesity
  - IBW for pediatric patients will be used for pediatric based dosing and obesity considerations (this was previously missing in our protocol)

By setting a dose cap, based on weight, incorporating pediatric IBW considerations and setting dose adjustment parameters for a BMI > 35, we believe these changes will be safe, account for CAR T-cell dosing feasibility and also optimize dosing in obese patients.

1.5.5.2 Utilization of ASTST consensus criteria for CRS grading

Protocol will be clarified to only use ASTCT consensus criteria (54) for CRS for grading. Prior Lee will not be used and any CRS grading per Lee (22) in the initial cohort of patients will be reconciled and re-graded per ASTCT. This will allow for harmonization of CRS grading across constructs.

1.5.5.3 Inclusion of patients with isolated CNS disease and/or CNS3 disease

With experience in 18 subjects on this protocol, only one subject developed reversible, grade 3 immune-effector cell associated neurotoxicty syndrome (ICANS) and no subjects experienced irreversible ICANS (i.e. cerebral edema). In comparison to both institutional(20) and extramural(63-65) experience with the anti-CD19(19, 66) and anti-CD22 CAR(53, 67), the neurotoxicity profile of this particular CAR appears to be well tolerated. To date, 3 patients on this protocol have enrolled with active CNS disease (2 patients with flow cytometric CNS disease and 1 patient with CNS2 disease), and all subjects tolerated the infusion without any severe ICANS. Additionally, anti-leukemia activity in the CNS was seen and CAR T cell trafficking to the CSF space has been demonstrated, with a median %CAR T cells at day 28 of

1.1% (range:0-49.7%) Based on the experience to date on this current protocol, the POB's prior experience with treatment and management of patients with active CNS involvement by disease (CD19 CAR, 12-C-0112), (CD22 CAR, 15-C-0029), incorporation of prospective neuro-cognitive testing and knowledge of trafficking of CAR cells to the cerebrospinal fluid, we are proposing to allow for the inclusion of patients with isolated CNS disease and/or CNS3 disease at dose level 3 (3 x 10^6 CAR T cells/kg) to explore toxicity and response.

1.5.5.4 Allow for reinfusion of CAR T cells in patients with loss of B-cell aplasia and/or loss of CAR T cell persistence

Although CD19 and CD22 CAR T cells have a high response rate in children and young adults with R/R B-ALL, 40-50% of patients relapse post CAR infusion(18, 53, 63, 68, 69). B-cell aplasia is a surrogate marker of ongoing CAR T cell activity and persistence, and some studies have demonstrated that early loss of B-cell aplasia, generally defined < 6 months post infusion, can be useful in predicting a patients' risk of CD19+ relapse(63, 68)(Pulsipher, data presented at TCT virtual meeting 2021). Indeed, one study demonstrated that loss of B-cell aplasia adversely affected the risk of CD19+ relapse, with a hazard ratio 34 (95% CI, 2.1-552; p=0.01). Loss of CAR persistence has also been correlated with relapse(63). Reinfusion of CAR T cells has been a strategy to try and maintain more durable remissions, with some demonstrated success(68, 70). Indeed, there is an ongoing phase 2 clinical trial to assess efficacy and safety of administration of tisagenlecleucel reinfusion in patients who experience loss of B-cell aplasia (NCT04225676)(71). Additionally, in our current and previous POB CAR T cell trials (CD19 and CD22), we have demonstrated safety in reinfusing patients who relapse post-initial infusion. Thus, based on the experience to date, we are proposing to allow for reinfusion of CD19/CD22 CAR T cells in patients with loss of B-cell aplasia, and/or loss of CAR T cells as measured by flow cytometry (defined in section **3.11**). Patients will receive the same dose level of CAR T cells as their initial infusion, and will receive increased lymphodepleting chemotherapy as outlined in section **3.9.2.2**. In cases where a subject is reinfused for loss of CAR T cells or loss of B-cell aplasia (e.g without evidence of relapsed, active disease), we will monitor duration of engraftment of CAR T cells and/or recurrence of B-cell aplasia as a separate event as time from the reinfusion. These subjects will continue to have duration of leukemia/lymphoma response captured from the time of initial infusion.

1.5.5.5 Increase the age limit to be consistent with other POB CAR trials and with current adolescent and young adult (AYA) definition

The age limited has been increased to 39 to keep consistent with other POB CAR trials and be consistent with current AYA definition which includes patients up to 39 years of age.

1.5.6 Rationale for Amendment, version date (09/16/2022)

1.5.6.1 Addition of exploratory objectives

Additional exploratory objectives evaluating the microbiome and metabolism have been added. Please see section **1.7.9** and **1.7.10** for further information.

1.5.6.2 Discontinuation of prospective neurocognitive testing in all patients, with the exception of patients with isolated CNS disease.

The rationale for removal of neurocognitive testing is that this exploratory endpoint has been completed and recently published (62). In summary, in the 20 patients treated and included in the

neurocognitive endpoint, 17 of 20 patients completed pre- and post-infusion assessments and a majority of patients had stable or improved neurocognitive scores in the 5 domains tested. Of the 146 paired test scored from patients, 132 (90.4%) paired test scores were stable or improved over time. Additionally, comparison of mean scores on neurocognitive measures pre- and post-infusion demonstrated stable or improved mean scores in all patients.

As of September 2022, we have seen minimal neurotoxicity, and will continue to closely monitor all subjects for neurotoxicity with appropriate evaluations and interventions in place. See **Appendix B 2**: Neurotoxicity Assessments Calendar for revised neurologic evaluations.

1.5.7 Rationale for Amendment (05/08/2023)

1.) Addition of exploratory objective evaluating the role for siltuximab in management of CRS.

Please see section **1.7.11** for further details.

2.) Addition of alternate lymphodepletion regimen due to a national shortage of fludarabine.

Fludarabine and cyclophosphamide are the most commonly used lymphodepletive preparative regimens used prior to CAR T-cell infusion. As of May 2022, fludarabine has been reported to be in short supply across the United States. In the event that fludarabine is not available or there is limited supply, we propose to use pentostatin and cyclophosphamide as lymphodepletion prior to CAR T-cell infusion.

Pentostatin is a purine analog that is noted to have lymphocyte-depleting properties(72-76). Pentostatin has previously been shown to deplete lymphocytes including multiple T-cell subsets within a week of administration(77). The combination of pentostatin and cyclophosphamide has previously been shown to be safe when used to treat chronic lymphocytic leukemia (CLL)(72, 76). One patient was treated with pentostatin and cyclophosphamide prior to administration of autologous anti-CD19 CAR T cells(78), The transferred T cells proliferated vigorously and persisted at high levels for several months(78), The patient obtained a prolonged complete remission(78). This group also utilized pentostatin/cyclophosphamide as one of 3 different lymphodepletion regimens prior to CD19 CAR T-cell infusion in 14 patients with CLL and reported that 8/14 patients responded, although there is no detailed information regarding those who received pentostatin(79). Pentostatin has been safely used after allogeneic stem cell transplantation to treat graft-versus-host disease(75). Pentostatin is generally thought to cause less myelosuppression relative to lymphocyte depletion when compared to other agents such as fludarabine(72, 74, 76). Pentostatin synergizes with the alkylating agent cyclophosphamide to cause lymphocyte depletion in mice, and pentostatin has also been shown to suppress T cell function in T cells that are not depleted(74, 80). Recently published work out of NIH by Dimitrova et al demonstrated that pentostatin/cyclophosphamide was a well tolerated reduced intensity conditioning regimen for pediatric transplant patients with primary immunodeficiencies, and that patients did not exhibit increase in infections and had low incidence of organ toxicity(81).

We propose to utilize a lower dose of pentostatin of 2mg/m^2/dose as this dose was used in patients with hairy cell leukemia whom were deemed high risk patients (those who had infections or had myelosuppression at presentation)(82) and most patients who will enroll on this trial have myelosuppression due to prior therapies. This modest dose to the current

cyclophosphamide backbone offers synergy without increased risk of toxicity. See section **3.9.2** for regimen details. If and when pentostatin is utilized, we will specifically evaluate outcomes in relationship to CAR T-cell toxicity, efficacy and CAR T-cell expansion.

1.6 PEDIATRIC ENROLLMENT

The first patient treated in the first cohort will be greater than 18 years of age to provide an initial safety evaluation prior to administration in pediatric patients. Safety data from adult patients treated on the Stanford study "Phase 1 Dose Escalation Study of CD19/CD22 Chimeric Antigen Receptor (CAR) T Cells in Adults with Recurrent or Refractory CD19/CD22-expressing B Cell Malignancies" (PI: Crystal Mackall, MD), information regarding any toxicity will be shared and may similarly inform dose escalation safety data on this study for the initial treatment of pediatric patients on this trial. Pediatric patients will only be treated after at least 1 patient ≥ 18 years of age or older will undergo investigational cellular therapy without DLT (observed for 28 days after infusion) prior to treating a pediatric patient (< 18 years of age).

1.7 CORRELATIVE STUDIES BACKGROUND

CAR T cell therapy targeting a single tumor associated antigen has mediated striking remissions in B cell leukemia and lymphoma. The clinical experience to date has demonstrated that anti-leukemic effects are associated with CAR T cell expansion(19)·(83) and that sustained remission is associated with CAR T cell persistence(84). In patients who have relapsed in the presence of persistent singular CD19 specific or CD22 specific CAR T cells, relapse has often been characterized by CD19 dim/negative or CD22 dim/negative phenotypes respectively. The complex interplay of tumor, T cell and intrinsic CAR properties that influence these outcomes are not well understood. We aim to utilize this safety study as an opportunity to collect correlative data that will permit extensive study of both the B cell and T cell compartment prior to and following CAR T cell therapy. We aim to integrate multi-dimensional technologies to permit complex analyses of the apheresis product, the CAR T cell product pre-infusion and in vivo expanded CAR T cells following antigen encounter. We additionally aim to investigate properties of B cell tumors that render them susceptible to CAR T cell cytotoxicity and study physiologic and malignant B cell remodeling under the pressure of multi-targeted CAR therapy.

1.7.1 Factors Impacting Loss of CAR T cells

Persistence of CAR T cells is associated with superior anti-leukemia effects in the setting of CD19-CAR T cell therapy for leukemia. We hypothesize that there are three distinct causes of T cell loss following CD19/22-CAR T cell therapy. First, in a subset of patients, there is poor CAR T cell expansion following infusion of a product that appears to meet appropriate release criteria. We hypothesize that such products can be identified by the presence of exhausted or terminally differentiated cells that lack progenitor capacity. To explore the potential to identify such products *a priori*, we will analyze apheresis samples and manufactured products to enumerate the frequency of naïve, T stem cell memory, T central memory and T effective memory subsets. We will also analyze apheresis and manufactured products for the presence of markers of T cell exhaustion including PD-1, Tim3 and LAG-3. Phenotypes will be correlated with the degree of expansion following CAR T cell infusion.

Second, we hypothesize that a subset of patients will undergo efficient early T cell expansion but will demonstrate loss of CAR-T cell persistence associated with the acquisition of a terminally differentiated phenotype and/or characteristics of T cell exhaustion. To test this, we will

evaluate CAR expressing T cells *in vivo* following adoptive transfer to identify characteristics associated with poor persistence. These phenotypic analyses will include flow cytometry and mass cytometry with a goal of identifying T cell phenotypes associated with poor persistence *in vivo*. We will also undertake epigenetic analyses to analyze correlations between T cell phenotype, persistence and enhancer profiles. Where possible, we will also compare the phenotype of cells harvested from blood, bone marrow, CSF and lymphoma to analyze whether the tissue microenvironment has substantial impact on T cell exhaustion, phenotype or function.

Finally, we hypothesize that a subset of patients lose their CAR T cell populations as a result of immune rejection. To test this, we will analyze for the presence or development of anti-CAR mediated T cell responses using cell based assays, as well as overlapping peptide libraries to localize the targets of anti-CAR reactivity.

1.7.2 Impact of Persistent CD19/22 Immune Pressure on Malignant and Normal B Cell Differentiation

CD19 and CD22 are acquired during the process of normal and malignant B cell differentiation. Persistent immune based pressure targeting these two antigens has the potential to induce profound changes on both normal and malignant B cell differentiation. In this study, we will use mass cytometry, flow cytometry, exome sequencing, RNAsequencing and ATAC-Seq to monitor changes in the normal and malignant B cell differentiation in the presence of persistent CAR T cells targeting these antigens. We will explore whether this dual targeted therapeutic is capable of inducing loss of either or both antigens in malignant cells and whether such antigen loss B-ALL cells is associated with a particular differentiation state, a particular genotype and/or are associated with increased or diminished fitness as evidenced by clinical behavior, or where possible growth patterns *ex vivo*. Similar analyses will focus on patients with remission marrows, analyzing whether normal B cell development can proceed in the absence of CD19 and/or CD22 subpopulations. This will provide a novel opportunity to characterize the impact of CD19 and/or CD22 loss on normal lymphoid and/or myeloid cells development.

1.7.3 Utility of minimal residual disease assessment

A reliable biomarker that detects disease before it is evident clinically or on imaging offers potential to improve long-term disease control and survival. Necrosis and apoptosis of lymphoma cells lead to release of tumor DNA in blood circulation(85), DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells(85). Next-generation sequencing and high-throughput technology can non-invasively detect and quantify the circulating free DNA (cfDNA)(86). The VDJ immunoglobulin genes contain unique sequences that are markers of clonality(87). These clonal markers can be used to assess MRD after the treatment and have been shown to prognostic value in DLBCL post-treatment surveillance(86) and other lymphoid malignancies(88),(89).

The role of MRD testing and cfDNA assessment in patients treated with CAR-T cells has not been well studied. In this study, we will correlate cfDNA assessment with marrow flow cytometry to identify the timing of relapse identification using these two approaches.

1.7.4 Explore the level CD19/CD22 surface expression and CD19/CD22 site density on leukemic blasts and correlate with clinical response to CAR-T cells

Based on preliminary experience in anti-CD22 and anti-CD19 CAR therapy, there does appear to be a threshold for CD19/CD22 antigen expression that is needed in order to effectively target leukemic blasts. In this study, CD19/CD22 expression and site density at baseline and at the first restaging will be performed as an optional evaluation, when samples are available, and evaluated at the National Cancer Institute (Flow Cytometry Lab: Dr. Maryalice Stetler-Stevenson) where there is the expertise and ability to characterize CD19, and particularly CD22 expression. In parallel, exome sequencing and RNA sequencing (Shern lab POB) will be performed to analyze the correlation in observed changes at the protein level with genetic and transcriptional changes at the antigenic locus.

1.7.5 Use of endothelial cell and coagulation markers to evaluate for thrombotic events

The endothelium has a crucial role in maintaining hemostatic balance by synthesizing, storing, and releasing many proteins that have opposing roles. For instance, it is responsible for vasoconstrictor, vasodilator, anticoagulant, and procoagulant proteins and when damage occurs to the endothelial cell, the delicate balance can be disrupted(90). Few complex cases of hematologic diathesis have been seen in patients treated with CAR T cell therapy. By adding additional research tests to evaluate endothelial cell markers, platelet activation, and fibrinolysis inhibitors, we will be able to study if certain biomarkers can play a role in predicting which patients treated with CAR T cells will have thrombotic or micro-thrombotic disease. Some of these tests have been shown to be predictive of thrombotic events in other patient populations. For instance, in cases of veno-occlusive disease, hepatic venous endothelial dysfunction is thought to be an inciting event that causes thrombosis, with low levels of activated protein C, and increased secretion of PAI-1 contributing to thrombosis(91). Additionally, prothrombin fragment 1+2 along with D-dimer was shown to be increased in patients with venous thromboembolisms versus those without ($P<0.001$)(92). Furthermore, there is recent evidence that endothelial activation may be involved in the toxicity profile(93), particularly neurotoxicity and that this may bear resemblance to HUS/TTP pathophysiology. These research studies can potentially yield important information that can have therapeutic implications for our patients and enhance our understanding of the mechanism related to CAR or cytokine mediated hematologic toxicities.

1.7.6 Retrospective Grading of CRS as per the newly established ASTCT CRS Consensus Grading **COMPLETED**

In an effort to standardize comparability in toxicity profiles across various CAR constructs and targets, there has been a recently established working group which has published consensus guidelines on the grading of CRS(54). Prior to this effort, there were multiple CRS grading criteria that were used, that make it difficult to determine comparability. At the NCI, we have always used the initial criteria that were developed and published by our NCI colleagues, in collaboration, with other institutes. However, to more uniformly inform the field, we will now grade CRS by the newly established guidelines, which will be put in our follow-up manuscript. These new guidelines will be used in conjunction with our current treatment algorithm which have been well established based on our experience to date. All patients on this trial to date have had both Lee and ASTCT CRS grading performed.

1.7.7 To explore apheresis and CAR- T cell product characteristics that may be associated with clinical outcomes (e.g., CAR T-cell persistence, toxicity and/or efficacy)

With significant interpatient heterogeneity and realization of the impact of manufacturing changes on CAR T-cell related outcomes—including toxicity, efficacy and CAR T-cell persistence, this aim is to retrospectively evaluate apheresis and CAR T-cell product characteristics. Remaining products that are not used for clinical purposes will be retrospectively and prospectively analyzed utilizing multifactorial analysis to evaluate characteristic of the product that may correlate with outcomes. Analysis will be primarily performed in the Taylor Lab (POB), and in the CCE. Data will be reviewed in the context of clinical outcomes.

1.7.8 To explore CAR T-cell expansion by T-cell subsets and immunophenotypic evaluation of markers of T-cell activation and/or exhaustion

Characterization of the immunophenotype of CAR T-cell expansion will be informative to correlate with CAR T-cell persistence and/or toxicity and has not been well explored. We will incorporate systematic analysis of CAR T-cell profiling to look at characteristics of the CAR T-cell, including T-cell subsets and immunophenotypic profiling to correlate with clinical outcomes.

1.7.9 Gut Microbiome Assessment of Patients Undergoing CAR-T Cell Therapies

The human gut microbiome, or community of microorganisms that are found in the intestines, is implicated in the etiology, progression and response to treatment in several different disease processes including obesity, inflammatory diseases and cancer(94). Specifically in cancer, the gut microbiome has a role in: 1) tumor initiation and progression, both through a direct effect on tumor cells and indirectly through manipulation of the immune system; 2) determining a response to cancer therapies and to predict progression and survival; and, 3) therapy, with modulation of the microbiome being able to potentiate the effect of certain immunotherapies and decrease toxicity(95, 96). There is currently minimal human data regarding the role of the gut microbiome in CAR T cell therapy. Murine models show microbial short chain fatty acids improve anti-tumor activity of specific CAR T cells and that modulation of the microbiome may produce an increased anti-tumor effect(97, 98). One adult study (unpublished) showed an enrichment in certain gut bacterial taxa correlating with clinical response and toxicity, and there have been no pediatric studies looking at the role of the microbiome.

Within this current CAR T cell treatment protocol, we propose a pilot observational microbiome assessment of patients undergoing CAR T cell therapy at the NIH Clinical Center through stool collection to assess how the gut microbiome may be associated with a) cytokine release syndrome, b) neurotoxicity, c) clinical response, and d) infection. Results of this study may help give a more complete understanding of response and complications of CAR T cell therapy and possibly identify microbiome therapeutic targets to improve response rates and decrease complications. We plan on collecting stool samples pre lymphodepleting (LD) chemo, post LD and pre-CAR-T cell infusion (optional) as well as post- timing differing with and without an occurrence of CRS as well as with any relevant clinical event. The goal of this exploratory objective is to evaluate the gut microbiome in patients receiving CAR-T therapy and correlate with cytokine release, neurotoxicity, infection, antibiotic use, and clinical response.

1.7.10 Metabolomic Analysis in Patients Undergoing CAR-T Cell Therapies

Over the last decade, growing evidence has accumulated to show the importance of metabolism on immune responses(99). Metabolomic analysis also emerged as a major tool in the field of immunotherapy and immunopathology. Metabolomic profiles integrate host and microbiota-derived metabolites as well as xenobiotics or nutrients factors and have been associated with high impacts on checkpoint inhibitor therapy, graft versus host disease, and auto-immune diseases(100-102). Preclinical studies on CAR-T cells highlighted the important relationship between T-cell metabolic fitness, microenvironment metabolites levels and anti-tumor function(103, 104). Importantly, the question of how metabolite levels influence efficacy and toxicity in pediatric patients treated with CAR-T cell therapy remains unanswered.

This study will measure metabolite levels in patient plasma/urine metabolites levels as a function of CAR-T cell therapy in a targeted and untargeted manner, with the goal of defining profiles associated with efficacy (complete response, duration of response, overall survival), toxicity (cytokine release syndrome, immune effector cell-associated neurotoxicity syndrome, hemophagocytic lymphohistiocytosis-like syndrome), CAR expansion and persistence. Targeted analysis will focus on redox balance (ascorbate, NAD(H), FAD(H), glutathione), amino-acids (arginine, tryptophane, glutamine, methionine), nucleoacids (ATP, adenosine), TCA cycle metabolites, hepcidin, Acteyl-CoA and lipids. To this end, peripheral blood and urine samples will be obtained from patients at the indicated time points pre- and post-CAR-T cell therapy. All samples will be prepared under the direction of Dr. Naomi Taylor and will then be evaluated for the metabolites indicated above. The goal of this analysis is to evaluate how metabolite levels influence efficacy of CAR-T cells, and toxicity in pediatric patients treated with CAR-T cell therapy.

1.7.11 To evaluate the efficacy of siltuximab in treatment of cytokine release syndrome

CRS is the most commonly seen toxicity in CAR T-cell therapy, and it occurs as a result of non-specific immune activation, which is closely related to pro-inflammatory cytokines, in specific IL-6. In the global registration trial using tisagenlecleucel, tocilizumab (an IL-6 receptor antagonist) was administered in 39% of patients, and it was noted to reverse CRS, and did not affect clinical efficacy, namely CAR T-cell expansion, complete remission rates, or persistence of CAR T-cells(63). Tocilizumab received FDA approval in 2017 for treatment of CAR T-cell related CRS(105). However, anecdotal evidence has suggested that neurotoxicity or ICANS may worsen or can develop after toclilizumab administration due to transient rise in serum IL6 that results in decreased clearance via endocytosis by the antagonized IL6R(22, 106-109). Indeed patients treated on our CD19-28z CAR trial who received tocilizumab had significantly elevated levels of CSF IL-6 on D28 as compared to those that did not receive tocilizumab and a pre-clinical model in a rhesus macque demonstrated that IV tocilizumab had poor CNS penetration (107, 108).

During the COVID-19 pandemic, an international shortage of tocilizumab prompted medical providers to use alternative agents such as siltuximab, to treat COVID-19 and demonstrated that siltuximab was well tolerated and an effective alternate that reduced CRS associated inflammation and COVID-19 mortality(110-112). In an effort to find additional therapies to optimize CAR T-cell toxicity profiles, several ongoing studies are now evaluating the use of siltuximab, an IL-6 antagonist that directly binds free IL-6, for prevention or treatment of CRS or

ICANS (NCT04975555, NCT05665725). Recently presented data demonstrated that 5/6 patients who received siltuximab for CRS had resolution of CRS of 1 hour (range 1-13 hrs) from first siltuximab infusion, and only 1 patient required rescue tocilizumab. Additionally, 3/4 patients with ICANS had resolution of ICANS with siltuximab and corticosteroid treatment(113).

Siltuximab will be used as first line CRS management in both pediatric and adult participants treated with CD19/CD22 CAR T cells. Dosing is based on the FDA approved dose for adults which is 11mg/kg.

Based on the limited experience with use of siltuximab in pediatric patients receiving CAR T-cells, this dose appears to be well-tolerated with potential for benefit in the management of CRS, particularly in the setting of neurotoxicity. Specifically, on the multisite CAR T cell trial targeting GD2 for patients with relapsed/refractory osteosarcoma or neuroblastoma (NCT04539366), 3 pediatric participants have received siltuximab at 11mg/kg (2 of whom received siltuximab at the NIH CC) for CRS without notable side effects (PI: Kaplan, personal communication). On several phase I/II CAR T cell studies treating pediatric and young adult patients with relapsed/refractory hematologic malignancies, physicians at the Children's Hospital of Philadelphia and St. Jude Children's Research Hospital are also giving siltuximab at 11 mg/kg without notable complications (PIs: Grupp, Talleur, personal communication).

On this current trial, 19/23 patients treated at the current dose level (3x10^6 transduced CAR T-cells/kg) have achieved complete remissions. Six patients (26%) treated at this dose level experienced ≥ grade 2 CRS and received tocilizumab as first line therapy for CRS. Additionally, 3 patients experienced grade 3 ICANS, 2 of whom received tocilizumab prior to the development of ICANS. All patients recovered from acute CRS and ICANS toxicities.

Given the established safety profile of this CD19/CD22 CAR construct, the opportunity to prospectively study additional IL6 directed agents, including those which may have the potential to decrease rates of neurotoxicity (as siltuximab directly binds IL6, theoretically decreasing the ability of high levels of IL6 to cross the blood brain barrier) serves to expand the arsenal of medications which can be used to treat CRS. A treatment algorithm has been developed whereby standard agents such as tocilizumab and/or steroids will be employed if participants continue to have worsening or refractory CRS/ICANS after siltuximab administration (see **Figure 9** in protocol), to prevent more severe complications. Treatment will be as per **Appendix D**: Guidelines for Grading and Management of Suspected Cytokine Release Syndrome, **Figure 9**.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

Study subjects with CD19+CD22+expressing B cell malignancies who have relapsed or are treatment refractory may enroll as defined by the following inclusion and exclusion criteria.

2.1.1 Inclusion Criteria

2.1.1.1 Diagnosis

Participant must have a B cell ALL (inclusive of CML with ALL transformation) or lymphoma and must have relapsed or refractory disease after at least one standard chemotherapy regimen 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, recurred after SCT, or have disease activity that prohibits SCT at the time of enrollment. Participants who have undergone autologous SCT will be eligible, and participants that have undergone allogeneic SCT will be eligible if, in addition to meeting other eligibility criteria, they have no evidence of GVHD and have been without immunosuppressive agents for at least 30 days. Participants with Philadelphia chromosome + ALL must have failed prior tyrosine kinase inhibitor.

Participants 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. For those being considered for reinfusions, measurable or evaluable disease is not required at the time of reinfusion.

2.1.1.2 CD22/CD19 expression

CD19 expression must be detected on greater than 15% of the malignant cells by immunohistochemistry or greater than 90% by flow cytometry. The choice of whether to use flow cytometry or immunohistochemistry will be determined by what is the most easily available tissue sample in each participant. In general, immunohistochemistry will be used for lymph node biopsies, flow cytometry will be used for peripheral blood and bone marrow samples. CD22+ B cell malignancy is required and CD22 expression levels will be documented when available, but a specific level of expression is not an eligibility requirement; it may be documented as positive or negative.

2.1.1.3 Age

Greater than or equal to 3 years of age (and at least 15 kg) and less than or equal to 39 years of age at time of enrollment (≥ 3 years to ≤ 39 years). NOTE: The first participant in each dose cohort must be ≥ 18 years of age.

2.1.1.4 Clinical Performance

Clinical performance status: Participants ≥ 16 years of age: Karnofsky ≥ 50%; Participants < 16 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.

2.1.1.5 Participants must have adequate organ and marrow function as defined below:

– leukocytes	≥750/mcL*
– platelets	≥50,000/mcL*
– total bilirubin	≤2 X ULN (except in the case of subjects with documented Gilbert's disease > 3x ULN)
– AST(SGOT)/ALT(SGPT)	≤10 X institutional upper limit of normal
– creatinine	≤ the maximum for age listed in the table below
	OR
– creatinine clearance	≥60 mL/min/1.73 m^2 for participants with creatinine levels above institutional normal.

Age	Maximum Serum

(Years)	Creatinine (mg/dL)
≤ 5	≤ 0.8
6 to ≤ 10	≤ 1.0
>10	≤ 1.2

* 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.

2.1.1.6 Subjects with CNS disease are eligible, with exceptions as noted in the exclusion criteria **2.1.2.2**

2.1.1.7 Contraception

2.1.1.8 Individuals of child-bearing or child-fathering potential (IOCBP or IOCFP) must be willing to practice birth control from the time of enrollment on this study and for 12 months after receiving the preparative regimen for IOCBP and for 4 months after receiving the preparative regimen for IOCFP.

2.1.1.9 Cardiac function: Left ventricular ejection fraction ≥ 45% or fractional shortening ≥28%, and no clinically significant ECG findings

2.1.1.10 Pulmonary Function

2.1.1.10.1 Baseline oxygen saturation >92% on room air at rest

2.1.1.10.2 Participants with respiratory symptoms must have a DLCO/adjusted > 45%. For children who are unable to cooperate for PFTs they must not have dyspnea at rest or known requirement for supplemental oxygen.

2.1.1.11 Ability of subject, parent(s)/guardian(s), Legally Authorized Representative (LAR), or Durable Power of Attorney (DPA) to understand and the willingness to sign a written informed consent document.

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.

2.1.2.2 Subjects with radiologically detected active CNS lymphoma or isolated CNS disease which are eligible for definitive CNS directed radiation therapy will be excluded.;

2.1.2.3 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;

2.1.2.4 .Pregnant or nursing individuals.

2.1.2.5 Subjects will be excluded related to the following prior therapy criteria:

Systemic chemotherapy, anti-neoplastic investigational agents, or antibody based therapies ≤ 2 weeks (6 weeks for clofarabine or nitrosoureas) prior to apheresis with the following exception: • No time restriction with prior intrathecal chemotherapy, steroid therapy, hydroxyurea or ALL maintenance type chemotherapy (vincristine, 6-mercaptopurine, oral methotrexate, or a tyrosine kinase inhibitor for participants with Ph+ ALL) provided there is recovery from any acute toxic effects.
Radiation therapy ≤ 3 weeks prior to apheresis with the following exception: • No time restriction with radiation therapy if the volume of bone marrow treated is less than 10% and the subject has measurable/evaluable disease outside the radiation window.
History of allogeneic stem cell transplantation prior to apheresis that meet the following criteria: • Less than 100 days post-transplant • Evidence of active graft-versus-host disease (GVHD) • Taking immunosuppressive agents within 30 days prior to apheresis. • Less than 6 weeks post donor lymphocyte infusion (DLI)
History of prior CAR therapy or other adoptive cell therapies prior to apheresis that meet the following criteria: • Less than 30 days post-infusion • Circulating CAR T cells (or genetically modified cells) ≥5% by flow cytometry in peripheral blood

2.1.2.6 HIV/HBV/HCV Infection:

- Seropositive for HIV antibody. (Participants with HIV are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy in the future should study results indicate effectiveness.)
- Positive for Hepatitis B surface antigen (HbsAG).
- Evidence of active Hepatitis C (evidenced by detectable HCV RNA)

2.1.2.7 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;

2.1.2.8 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;

2.1.2.9 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.

2.2 RECRUITMENT STRATEGIES

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

- This study will be posted on NIH websites and on NIH social media forums.
- Listed on clinical trials.gov;
- Listed in PDQ;
- In addition, participants treated on other institutional trials 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 IRB for review.

2.3 SCREENING EVALUATION

2.3.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

1. Email, written, in person or telephone communications with prospective subjects
2. Review of existing medical records to include H&P, laboratory studies, etc.
3. Review of existing MRI, x-ray, or CT images
4. Review of existing photographs or videos
5. Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

A waiver of consent for these activities has been requested in section **11.6.4**.

2.3.2 Screening activities performed after consent has been obtained

The following activities will be performed only after the subject has signed the consent for this study. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility.

The following screening tests must be performed within 4 weeks of signing the consent unless otherwise noted.

2.3.2.1 B-cell malignancy confirmation and CD19-CD22 expression

Expression will be evaluated by immunohistochemistry or by flow cytometry in a CLIA approved laboratory and documentation at screening will be required (testing is permitted to be conducted at any time since most recent disease presentation). Confirmation of disease will be based on review of pathology reviewed and confirmed at NIH as feasible.

2.3.2.2 Participant Medical History

The participant's complete history and a thorough review of medical records and by interview will be collected and recorded. Concurrent medical signs and symptoms must be documented to

establish baseline severities. A disease history, including the month of initial diagnosis and list of all prior treatment, responses, and duration of response to the prior treatment also will be recorded.

2.3.2.3 Clinical Evaluation

A complete physical examination will be performed. The exam will include general appearance of the participant, height and weight, vital signs, examination of the skin, eyes and ears, nose, throat, lungs, heart, abdomen, extremities, musculoskeletal system, and nervous system. Performance status will be scored on all subjects. Subjects under the age of 16 years will be scored according to the Lansky Scale. Subjects who are ≥16 years of age will be scored according to the Karnofsky Performance Status (See **Appendix A**). Body surface area (BSA) should be calculated at baseline.

2.3.2.4 Laboratory studies

- Evaluation for HIV seropositivity to consist of ELISA and, if positive, confirmation by Western blot. The investigator, in the event of a positive finding, will make appropriate counseling available.
- Evaluation for Hepatitis B surface antigen (HbsAG) and anti-HCV antibodies.
- DTM Viral Markers (Transplant donor) and Rapid Plasma Reagin (RPR) will be drawn **within 4 weeks** of apheresis per DTM requirements. If apheresis has already been performed on another protocol, this testing will not be required.
- Additional viral titers and PCR (e.g., CMV/EBV IgG/IgM) testing may also be done per PI discretion.
- 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.
- β-HCG serum or urine pregnancy test on all IOCBP.
- Peripheral blood CD3 count obtained by TBNK or PID panel
- PT/PTT

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

- Chemistries: 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, CRP, Total Protein, Uric Acid, ferritin, triglyceride level
- Immunoglobulins
- CBC with differential and platelet count
- Urinalysis

2.3.2.5 Disease Evaluation

- Based on participant's primary disease, not all listed evaluations need to be completed, per PI discretion. Appropriate imaging methods of any sites relevant to the subject's disease (e.g., CT Scan, PET/CT, or MRI).
- Bone marrow aspirate including flow cytometry (bone marrow biopsy as feasible)
- Spinal fluid for cell count, cytospin. Only in participants with a history of CNS disease at PI discretion. CSF for flow cytometry may be done per PI discretion, as feasible.
- Other tissue biopsy may be performed as clinically indicated for immunohistochemistry and/or flow cytometry of a fine needle aspirate
- Peripheral blood for flow cytometry
- Pulmonary Function Evaluation
- Pulse oximetry
- Pulmonary function testing (spirometry and DLCO) will only be required in participants with respiratory symptoms (e.g. dyspnea at rest or known requirement for supplemental oxygen).
- For children who meet the above criteria but are unable to cooperate for PFTs, the criterion is: No evidence of dyspnea at rest, no exercise intolerance and no requirement for supplemental oxygen therapy.

2.3.2.6 Cardiac Function

- ECG
- Assessment of cardiac function (ejection fraction/shortening fraction) echocardiogram or cardiac MRI.

2.4 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

2.4.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of an eligibility criterion may be rescreened.

2.4.2 Treatment Assignment Procedures

2.4.2.1 Cohorts

Number	Name	Description
1	Dose Escalation	Up to 17 subjects previous to amendment, version date 01/11/2020 **CLOSED**
1a	Dose Escalation: CAR naïve or previously CAR treated with interim transplant	Up to 13 CAR naïve or previously CAR treated with interim transplant or highest dose administered is determined (as of amendment, version date 01/11/2020) **CLOSED**
1b	Dose Escalation: previously CAR treated but not transplanted	Up to 13 previously CAR treated but not transplanted or highest dose administered is determined (as of amendment, version date 01/11/2020) **CLOSED**
2a	Dose Expansion: CAR-T naïve or previously CAR treated with an interim transplant	Up to 30 CAR naïve previously CAR treated with an interim transplant subjects treated at MTD. 15 enrolled initially (counting those treated at MTD in cohort 1) then expanded to 30 if sufficient response
2b	Dose Expansion: prior CAR-T therapy without an interval transplant	Up to 30 subjects with prior CAR-T therapy without an interval transplant treated at MTD. 15 enrolled initially (counting those treated at MTD in cohort 1) then expanded to 30 if sufficient response
2c	Dose Expansion: Isolated CNS disease	Up to 12 subjects with isolated CNS disease

2.4.2.2 Arms

Number	Name	Description
1	Dose Escalation	CD19/CD22-CAR-transduced T cells at escalating doses
2	Dose Expansion	CD19/CD22-CAR-transduced T cells at MTD or highest dose administered

2.4.2.3 Arm Assignment

Subjects in Cohort 1, 1a and 1b will be directly assigned to Arm 1.

Subjects in Cohorts 2a, 2b, 2c will be directly assigned to Arm 2.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a single-site phase I study in pediatric and young adult participants with CD19+CD22+ expressing B cell hematologic malignancies who have relapsed or refractory disease after at least one standard chemotherapy and one salvage regimen. All participants enrolled on this trial have been deemed incurable by standard therapies. Autologous PBMC will be obtained by leukapheresis and transduced with CD19/CD22-CAR lentiviral vector. Cryopreserved PBMC stored from participation in other institutional cell therapy or cell collection studies may be used to generate the cellular product on this study as long as they meet the criteria described in the IND.

The first participant treated in each cohort will be evaluated for 21 days prior to the treatment of the second participant in a cohort to provide an initial safety evaluation prior to administration in subsequent participants at that dose level. We believe this staggering regimen is adequate to demonstrate safety in children particularly given the similarity of this CAR construct to those that have been used safely in pediatric participants receiving the CD22 CAR T cells and CD19 CAR T cells. The preparative regimen cyclophosphamide and fludarabine has been used successfully in many CAR clinical trials with acceptable toxicity profile.

Participants will receive a lymphodepleting preparative regimen of fludarabine and cyclophosphamide followed by infusion of CD19/CD22-CAR T cells. Participants will be evaluated sequentially after treatment for toxicity, antitumor effects and for persistence of CAR in blood samples and functionality of transduced T cells. Additional blood and bone marrow samples will be collected to complete correlative study analysis.

Initially participants will receive 3 x 10^5 transduced T cells/kg (± 20%) as outlined in section **3.7**. If no adults have received this dose prior to initiating enrollment on this study, at least 1 participant ≥ 18 years of age or older will undergo investigational cellular therapy without DLT (observed for 28 days after infusion) prior to treating pediatric participant, as per section **3.7**. If 2/6 participants experience DLT at dose level 1, dose -1 will be explored (1 x 10^5 transduced T cells/kg (± 20%)) as described in section **3.7**. The MTD, or highest cell dose studied if MTD is not reached, will be expanded in two groups (children and young adults who have previously received CAR T cells versus those that are CAR T cell-naïve) for a total of 12 evaluable participants in each group to further explore the safety, feasibility and clinical response activity.

With a limited dose escalation and/or de-escalation if needed, utilizing a 3+3 Phase 1 design, utilizing a biologically active and well tolerated dose for both CD19 and CD22-CAR trials. If tolerated and safety has been assessed, we will have a very limited dose escalation to 3 x 10^6/kg.

Based on the data from the first 11 participants treated on study, at the three current dose levels, we propose adding an additional dose level, DL 4: 1 x 10^7 transduced T cells/kg, to explore due to limited expansion and limited persistence of CAR T cells with favorable toxicity profile.

Additionally, participants that have received previous CAR therapy are eligible to enroll on trial, however, based on our experience to date on this trial and others, participants may have limited responses to subsequent infusions with CAR-T therapy, especially when the same antigen is targeted.

As this is a dual, bispecific construct targeting CD19/CD22, our plan is to enroll participants in two groups: CAR naïve and CAR pretreated as toxicity profile and response rates may be different between these two groups. Additionally, based on our experiences to date, we have utilized intensified lymphodepletion as a strategy to reduce the likelihood of immune mediated rejection, and potentially improve response rate in participants who have had prior CAR T-cell exposure, and this strategy will be utilized for the CAR pretreated cohort. This intensified lymphodepletion has been well tolerated and represent a moderate increase above our standard dosing. Starting at dose level 4, participants will be enrolled in separate cohorts based on prior treatment.

3.2 APHERESIS FOR CELL ACQUISITION

Participants will undergo apheresis according to institutional standards, as estimated by recipient weight and target cell harvest dose in the institution apheresis facility. If PBMCs have been cryopreserved in DTM-CC for a different cell therapy or cell collection study and meet the requirements for this study, they may be used to generate CD19/CD22-CAR T cells on this study.

A target cell number extrapolated based upon the expected expansion and planned cell dose of transduced T cells/kg will be collected. Citrate anticoagulant may be used in subjects according to institutional SOPs. For subjects, less than 18 kg, low-dose heparin may be added. Prophylactic intravenous $CaCl_2$ and $MgSO_4$ infusions may be administered by the apheresis clinical team per institutional standard operating procedures. Institutional guidelines will be followed for venous access and apheresis procedures.

3.3 CELL PROCESSING

Cellular Product: Autologous T cells cultured with interleukin-2; transduced with lentiviral vector (CD19/CD22.BB.z) expressing CD19/22 Chimeric Antigen Receptor (Anti-CD19/CD22 CAR); following Fludarabine and Cyclophosphamide

Cell therapy production will be conducted according to the DTM-CCE SOPs and must meet the requirements for a GMP facility. Fresh or cryopreserved peripheral blood mononuclear cells (PBMC) (depending on the timing of apheresis relative to cell culture, participant condition and scheduling availability) will be enriched for T cells using a CD4/CD8 immunomagnetic bead enrichment. They will then be activated by co-culture with immunomagnetic particles expressing anti-CD3 and anti-CD28 mAb. One day following activation, replication incompetent lentiviral vector particles containing the CD19/CD22.BB.z construct will be added to the culture for transduction. Cells will be incubated for approximately 9 days with IL-2 then harvested and administered fresh or frozen for subsequent infusion. Cells will be required to meet standard release criteria including transduction efficiency ≥10%, T cell content ≥70%, sterility and minimum levels of LPS as well as no evidence for replication competent lentivirus. All procedures will take place using good manufacturing process guidelines.

The release criteria will be based upon analyses for each dose of CD19/CD22-CAR T cells and will include:

Test	Method	Criteria
Cell viability[1]	Trypan blue exclusion	≥70%

Test	Method	Criteria
Cell number[1]	cell counter	within 20% of planned dose level
% CAR+ cells[2]	Anti-CD19 CAR idiotype, CD22 Fc	≥10%
Endotoxin[1]	Gel Clot	≤ 5 EU/mL
Mycoplasma[2]	Mycoplasma test	Negative
VSV-G[2]	q-PCR	Negative
Sterility testing[3]	gram stain, culture	Negative

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

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

[3]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 and results will be in process at the time of infusion, therefore they may not be definitive.

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

3.4 Impact of Disease Progression or Delay in Initiation of Protocol Specified Therapy after Enrollment

Participants must have evaluable disease at the time of protocol enrollment. PBMC will be collected and every effort will be made to initiate cell culture within 60 days so minimal time transpires for a participant's disease to progress significantly. However, for most participants with active leukemia, 60 days is still an unacceptable period to wait without any additional therapy. Furthermore, there may be delays in cell manufacturing that are beyond our control that need to be accounted for. With the goal of optimizing a subject's clinical condition, should a subject develop evidence of disease progression after enrollment, or require intermittent doses of low-dose chemotherapy to maintain disease stabilization or prevent disease progression, but before initiation of protocol specified lymphodepleting chemotherapy, the subject may be treated with disease-directed standard of care (SOC) (i.e., non-investigational) chemotherapy as clinically indicated and initiation of protocol-specified lymphodepleting chemotherapy will be delayed. Participants may receive interim SOC therapy either with their local physician or at NIH. If the participant receives therapy with his/her local physician, neither the therapy nor the AEs from this therapy will be recorded or reported on this protocol (except as part of prior history at baseline) as this information is external to the study. If the participant chooses to receive interim SOC therapy at NIH, the participant will be enrolled to protocol 04-C-0165 Standard of Care and Interventions protocol and the therapy and associated AEs will be reported on protocol 04-C-0165.

After completion of interim SOC therapy and in order to initiate the protocol-specified lymphodepleting therapy, there must be 7 days from the last dose of standard chemotherapy (with the exclusion of steroids and hydroxyurea where there is no washout.)

For all participants that have received any form of SOC interim therapy, the following labs will be repeated prior to starting lymphodepleting chemo: CBC, chemistries including sodium, potassium, chloride, total CO2, creatinine, glucose, urea nitrogen, albumin, alkaline phosphatase, ALT/SGPT, AST/SGOT, total bilirubin, Lactate Dehydrogenase (LDH),uric acid, ferritin, and triglycerides. These labs can be collected as baseline labs prior to starting lymphodepleting chemotherapy.

Also, per PI discretion, the participant may have repeat disease evaluations performed (method determined by participants' type and location of disease) in order to proceed with lymphodepleting therapy and planned CAR infusion. For participants who only receive maintenance like-ALL therapy, as defined in protocol section **2.1.2.5**, or steroids for the purpose of disease stabilization or to prevent disease progression, restaging evaluation will only be performed if clinically indicated per PI discretion. Baseline data will be collected. Data regarding the interim SOC therapy will be captured in prior therapy CRFs, but no AEs will be collected or reported for this protocol until the participant initiates study therapy.

3.5 Impact of Intercurrent Illness After Enrollment

Should a subject develop a serious or life-threatening condition or infection (not including progressive disease) after enrollment and either before or after lymphodepleting therapy, the subject will be treated for their condition as clinically indicated and administration of anti-CD19/22-CAR T cells will be delayed. Cells will be maintained in culture for up to an additional 72 hours 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. If the participant is able to receive fresh, non-cryopreserved cells within 72 hours of the planned original dose on Day 0, administration should follow the procedure as outlined in section **3.10** and the participant will remain evaluable for toxicity and efficacy. If more than 72 hours from planned cell harvest elapses, the cells will be cryopreserved and, if clinically indicated, the subject may receive them up to 7 days from the planned infusion date and be considered evaluable. Alternatively, the subject may have the option of receiving the cells at a later point once the condition has resolved, but these participants will not be evaluable for toxicity and efficacy and will be replaced in the accrual numbers.

3.6 Dose Limiting Toxicity (DLT) / Treatment Limiting Toxicty (TLT)

Adverse events that are considered disease-related (not suspected of relationship to CD19/CD22-CAR T cells) will not be considered dose-limiting toxicities. Only those AEs suspected to be related to pre-infusion chemotherapy and/or CD19/CD22-CAR T cells (any component of the treatment regimen) will be used in the definition of DLT/TLT. As the dose escalation portion of the study has completed, Treatment-limiting toxicities will now be captured during the dose expansion phase. Toxicities occurring after initiation of the chemotherapy preparative regimen but prior to CD19/CD22-CAR T 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 preparative regimen 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. Participants who are fully assessable are those that have completed the non-myeloablative preparative regimen and received the CD19/CD22-CAR T cell infusion. DLT/TLT will only be attributed to the preparative regimen if it prevents infusion of CD19/CD22-CAR T cells.

The definition of DLT/TLT in these studies uses NCI's Common Terminology Criteria for Adverse Events (CTCAE v5).

3.6.1 Definition of DLT/TLT

Adverse events that are at least possibly related to the CD19/CD22-CAR T cells with onset within the first 28 days following cell infusion will be considered DLT/TLTs according to the following 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 (i.e., neutropenia or thrombocytopenia with the exception of lymphopenia) lasting greater than 30 days will be considered a DLT/TLT. Lymphocyte count and subsets will not be considered in the definition of DLT/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 DLT but evaluable for all other aspects of the study.

3.6.1.2 Non-Hematological Toxicity:

- Any grade 4 adverse event at least possibly related to research or IND, with exclusion of transient, laboratory abnormalities without clinical consequence, will be considered DLT/TLT.
- CRS Grade 4 in severity, or Grade 3 in severity for more than 7 days
- Infusions reactions ≥ Grade 2 lasting more than 24 hours
- Grade 4 Cerebral Edema
- ≥ Grade 3 Neurotoxicity
- ≥ Grade 3 Disseminated Intravascular Coagulation

The MTD is a dose level immediately below the level at which the enrollment is stopped due to DLTs, 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 participants develop DLT, the MTD will have been exceeded.

If no additional subject develops a DLT, the MTD will not have been exceeded and the cohort will be evaluated for response. If < 3 responses are present in this dose cohort the next dose level can be administered after the four-week safety assessment period of the last participant 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

Cell dose will be body weight-based up to 100kg. All participants > 100kg, with a BMI <35, will be cell dosed at 100kg. All participants with BMI greater than 35 will have doses calculated using practical body weight (PBW) (see **Appendix E** for calculation), with dosing also capped at 100kg.

Initially 3 to 6 participants will receive 3 x 10^5 transduced T cells/kg (± 20%). If this dose has been deemed to be safe in adults (1 adult participant has received this dose without DLT) pediatric participants may be enrolled and treated. If no adults have received this dose (or subsequent dose levels) prior to initiating enrollment at the specified dose level on this study, at least 1 participant ≥ 18 years of age or older will undergo investigational cellular therapy without DLT (observed for 28 days after infusion) prior to treating a pediatric participant. A four (4) week (28 days) safety assessment period will follow regimen completion (defined as infusion of CD19/CD22-CAR T cells) of the first participant in the first cohort (and subsequent cohorts if needed). Subsequent participants in that dose cohort and subsequent cohorts may be treated after a one week (7 day) safety assessment period. Four weeks (28 days) must elapse after completion of cell infusion in the final participant in each cohort to allow for safety assessment of the dose cohort and response determination before treating participants on the next dose cohort level, if escalation is needed.

In addition, at the discretion of the principal investigator, an additional 1-2 participants may be enrolled at a single dose level to obtain information on efficacy and safety provided that no more than 1 participant at that dose level has experienced a DLT.

If < 3 out of 6 participants demonstrate an objective response (CR or PR) at Dose Level 1, AND < 2 out of 6 participants experiencing DLT, the dose will be escalated to Dose Level 2: 1 x 10^6 transduced T cells/kg (± 20%) in subsequent participants. Safety, including enrollment staggering and efficacy will again be assessed as described above for Dose Level 1. If needed, the dose may be escalated to Dose Level 3, then 4 should previous dose levels prove to be safe but not efficacious.

Dose Escalation Schedule	
Dose Level	**Dose of CD19/anti-CD22-CAR T cells**
Level -1	1 x 10^5 transduced T cells/kg (± 20%)
Level 1	3 x 10^5 transduced T cells/kg (± 20%)

Dose Escalation Schedule	
Dose Level	**Dose of CD19/anti-CD22-CAR T cells**
Level 2	1 x 10^6 transduced T cells/kg (± 20%)
Level 3	3 x 10^6 transduced T cells/kg (± 20%)
Level 4	1 x 10^7 transduced T cells/kg (± 20%)

If cell growth limitations preclude administration of the number of cells targeted for the assigned cohort level, a second or subsequent culture may be initiated and the participant can receive interim standard therapy as per protocol section **3.4** while awaiting additional cells to see if they can reach the specified dose level. Ultimately, the participant will receive as many cells as possible and the participant will be enrolled in the appropriate cohort for the number of cells infused, allowing for an additional two participants to be enrolled per cohort due to cell growth limitations. Based on prior experience we do not anticipate many product failures. If a DLT occurs in an additional participant (beyond the first 3 enrolled) entered at a lower dose due to cell growth limitations, accrual will continue at the previously planned dose level for subsequent participants. If a minimum of 3x10^5/kg CD19/CD22-CAR-transduced T cells cannot be obtained for infusion (or 1 x 10^5/kg if dose level -1 is enrolling), the participant will still receive the cell infusion, as the effective dose of cells is not well defined, and the participant will be evaluable for feasibility but not analyzed for MTD, although toxicities will be assessed and reported separately. If the third dose level is completed without DLT and without clinical response, consideration may be given to adding additional dose cohorts in a protocol amendment. After treatment of the first 11 participants, in the first 3 dose levels, without any DLT, and limited clinical response, we propose adding an additional dose level, (DL 4: 1 x 10^7 transduced T cells/kg).

If cell growth limitations preclude administration of the number of cells targeted for the assigned dose level in 3 participants (out of 6) in a dose level, that dose will be considered not feasible as there will be inadequate number of participants in that dose level to evaluate safety. Enrollment to that dose, and any higher doses will cease. This will be considered the 'highest cell dose' studied and will be the dose level that will be studied further in the expansion cohort.

3.8 DOSE EXPANSION COHORT

To gain further experience with the safety, feasibility and clinical activity of the CD19/CD22 CAR T cells in this participant population and to determine if there are differences in the feasibility related to manufacturing or administering CD19/CD22-CAR T cells in children and young adults who have previously received CAR T cells versus those that are CAR T cell-naive, the MTD (or highest cell dose studied) will be expanded in two groups of subjects to a total of 15-30 participants in each expansion cohort (i.e., will include those subjects treated at MTD during the dose escalation phase). If 50% or more of the participants experience a clinical response (best overall response), this therapeutic regimen will be of sufficient efficacy to warrant additional study.

3.9 LYMPHODEPLETING CHEMOTHERAPY REGIMEN

3.9.1 Criteria for Initiating Lymphodepleting Regimen

The criteria for initiating the conditioning regimen is as follows:

Participants must have

- no evidence of clinically significant infection,
- no clinically significant cardiac dysfunction,
- serum creatinine must be <2 x ULN,
- no acute neurological toxicity >grade 1 (with the exception of peripheral sensory neuropathy).

Should an event exceed these criteria immediately prior to conditioning chemotherapy, conditioning chemotherapy must be delayed until the event resolves to ≤ grade 1 or baseline.

3.9.2 Drug Administration

3.9.2.1 CAR naïve or CAR pre-treated with interval transplant participants

Participants will receive the lymphodepleting regimen as follows: (This may be given as an inpatient or outpatient as necessary).

Drug	**Dose**	**Supportive Care**	**Days**
Fludarabine	25 mg/m^2 per day$^{\%}$ IV infusion in 50-100 mL of 0.9% sodium chloride over a minimum of 30 minutes, daily for 3 days.		-4, -3, -2
Cyclophosphamide	900 mg/m^2 per day IV infusion over a minimum of 60 minutes, once on Day -2 after fludarabine	**Pre-cyclophosphamide hydration:** IV pre-hydration at a rate of at least 100 ml/m2/hr of Normal Saline (NS) for a minimum of 2 hours prior to cyclophosphamide or alternatively, rapid pre-hydration can be given at 750 mL/m2 of NS over 1 hour. **Post-cyclophosphamide hydration:** IV or oral hydration until the completion of mesna at a rate of at least 100 mL/m^2/hour.	-2

Drug	Dose	Supportive Care	Days
		Mesna used as described below	
% To conserve vials in times of drug shortages, individual (i.e., daily) fludarabine doses may be rounded up or down by ≤15% with approval of the PI to reach the targeted cumulative dose of 75 mg/m^2 (+/- 10%) over 3 days.			

3.9.2.2 CAR pre-treated without an interval transplant or participants receiving a reinfusion:

Participants will receive intensified lymphodepletion as follows:

(This may be given as an inpatient or outpatient as necessary):

Drug	Dose	Supportive Care	Days
Fludarabine	30 mg/m2 per day$ IV infusion in at least 50mL of 0.9% sodium chloride over a minimum of 30 minutes, daily for 4 days.		-5, -4, -3, -2
Cyclophosphamide	600 mg/m2 per day IV infusion over a minimum 60 minutes, daily for 2 days, dosed after fludarabine.	**Pre-cyclophosphamide hydration:** IV pre-hydration at a rate of at least 100 ml/m2/hr of NS for a minimum of 2 hours prior to cyclophosphamide or alternatively, rapid pre-hydration can be given at 750 mL/m2 of NS over 1 hour. **Post-cyclophosphamide hydration:** IV or oral hydration until the completion of mesna at a rate of at least 100 mL/m^2/hour.Mesna as described below.	-3 and -2
$ To conserve vials in times of drug shortages, individual (i.e., daily) fludarabine doses may be rounded up or down by ≤15% with approval of the PI to reach the targeted cumulative dose of 120 mg/m^2 (+/- 10%) over 4 days.			

3.9.2.3 Alternative Lymphodepletion Regimen (when Fludarabine is unavailable)

Alternative Lymphodepletion Strategy
Standard LD Pentostatin 2mg/m^2 Day -4 [%] Cyclophosphamide 900 mg/m2 Day -2
Increased LD Pentostatin 1.5mg/m^2 Day -5, -4[%] *(cumulative 3mg/m^2)* Cyclophosphamide 600 mg/m2 Day -3, -2 *(cumulative 1200mg/m^2)*
[%] Administer IV over 20 to 30 minutes or as a bolus infusion. Hydrate with 500 to 1,000 mL of 5% Dextrose in 0.45% NS or equivalent before infusion, and 500 mL of 5% Dextrose or equivalent after infusion.

3.9.3 Supportive Care Measures

3.9.3.1 Mesna

Mesna will be administered per institutional/national standards on days when cyclophosphamide is administered.

3.9.3.1.1 Inpatient Mesna

Mesna will be administered at a dose that equals 60% of the daily cyclophosphamide dose by continuous IV infusion on days when cyclophosphamide is given. Mesna will be prepared in 5% dextrose (or other composition appropriate for the clinical situation) and will be infused over 9 hours starting concurrently with the cyclophosphamide.

3.9.3.1.2 Outpatient Mesna

First dose: All participants will receive the first dose of mesna IV given concurrently with the cyclophosphamide, at a dose that is 20% of the cyclophosphamide dose. IV Mesna will be prepared in 5% dextrose (or other composition appropriate for the clinical situation).

Subsequent doses: The second and third doses of mesna can be given either IV or orally (note: the oral mesna dose is **twice** the IV mesna dose).

Subsequent IV mesna : Each dose will be 20% of the cyclophosphamide dose, given at 4 and 8 hours after the start of cyclophosphamide (for a total daily mesna dose equal to 60% of the daily cyclophosphamide dose).

Subsequent oral mesna: Participants able to tolerate oral mesna may receive the last two bolus doses orally. Each oral mesna dose will be 40% of the cyclophosphamide dose, given at hours 2

and 6 after the start of cyclophosphamide (for a total daily mesna dose equal to 100% of the daily cyclophosphamide dose.) Oral mesna solution is 100 mg/mL and should be diluted prior to administration to improve palatability. Participants will receive instructions from pharmacy describing how to dilute mesna for consumption in the outpatient setting (e.g. dilute each dose in 1 ounce of water, carbonated beverages, fruit juice, milk, etc.). Participants who vomit within 2 hours after taking oral mesna should repeat the dose or receive IV mesna instead.

3.9.3.2 Anti-emetics

Routine anti-emetic prophylaxis and treatment should be employed. Corticosteroids may not be used (except for physiologic replacement as outlined in section **2.1.2.5**, or in IT chemotherapy as outlined in section **4.1**).

3.10 CD19/CD22-CAR T CELL INFUSION – DAY 0

3.10.1 Cell Infusion Criteria

Subjects must meet the following criteria in order for cells to be infused (based on labs obtained within 1 day of cell infusion):

- CD19/CD22-CAR T cells must have met release criteria (section **3.3**)
- Subject has no evidence of hemodynamic instability
- Subject has not developed a new requirement for supplemental oxygen therapy
- 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
- Liver transaminase (ALT and AST) $\leq$10 x ULN based on age- and laboratory specific normal ranges
- There is no evidence of clinically significant cardiac dysfunction, uncontrolled, significant tumor lysis syndrome, serum creatinine >2 x ULN, and no acute neurological toxicity > grade 1 (with the exception of peripheral sensory neuropathy).

Should an event not meet these criteria immediately prior to receiving CD19/CD22-CAR T cell, the cell infusion must be delayed until the event resolves. If the CD19/CD22-CAR T cell infusion is delayed >2 weeks, conditioning chemotherapy MAY be repeated**, unless investigator deems this unnecessary**. In addition to the above criteria, special considerations should be made prior to cell infusion if the subjects' temperature is $\geq$ 38.0 C within 48 hours prior to cell infusion.

3.10.2 Inpatient and Outpatient Care Post-Cell Infusion:

Participants may be admitted for cell infusion to NIH in accordance with CC and nursing policies. Participants will be monitored a minimum of twice weekly until Day 14 as inpatients or outpatients but must be admitted with the first fever or with clinical manifestations of CRS. Participants should remain outpatient in close proximity to the NIH for at least twice weekly evaluations until completion of Day 28 (+/- 4 days) restaging evaluations unless participant does not show signs of cytokine release syndrome or CAR expansion.

3.10.3 Pre-medications

Participants will receive the following medications prior to cell infusion (it is recommended that these pre-medications be given 15-90 minutes prior to the cell infusion, but they may be administered up to 4 hours prior to the 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. Premedications may be omitted based on clinical conditions at the discretion of the PI or AI.

3.10.4 Cell Administration

Cells are delivered to the participant care unit by a staff member from the institutions' Cell Processing Service. 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 participant's chart, as is done for blood banking protocols.

The cells are to be infused intravenously (IV) over 5-20 minutes via syringe(s).

For cryopreserved cells, 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.

3.10.5 Required Monitoring During Cell Infusion

Monitoring will include vital signs (temperature, blood pressure, heart rate, respiratory rate) prior to infusion, at the end of the infusion, every 15 minutes (± 5 min) after the end of the infusion X 2, every 30 minutes (±10 min) after the end of the infusion X 2. Vital signs may be evaluated more frequently if clinically indicated.

3.10.5.1 Supplemental oxygen will be available at the bedside

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

3.11 Option for Additional Dose(s) of CD19/CD22-CAR T Cells

On the day cells are infused, 90% of any remaining cells that have been produced above and beyond the number of cells needed for a subject's dose level will be cryopreserved using standard techniques. 10% of the remaining cells will be used for research. Subjects will have the option for additional infusions of CD19/CD22-CAR T cells (including preparative chemotherapy regimen, toxicity assessment and research blood sampling) if the following criteria are met:

3.11.1 Eligibility Criteria for Subsequent Cell Infusions

3.11.1.1 Inclusion Criteria

3.11.1.1.1 Response to previous infusion. Subjects who had a PR, or SD with clinical benefit may elect to receive another infusion of cells. 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.) Subjects that initially had a CR may receive a reinfusion if any of the following criteria is met:

- Evaluable disease recurs;
- If participant has loss of B cell aplasia defined by an absolute CD19+ > 50/mcL and rising in the peripheral blood (2 timepoints needed), and/or 1% CD19+ hematogones in the bone marrow;
- If a participant has loss of CAR T cell persistence (<5% circulating CAR T cell in peripheral blood by flow cytometry) < 6 months post-initial infusion.

3.11.1.1.2 At least 30 days have passed since the previous cell infusion.

3.11.1.1.3 At least six weeks from most recent DLI infusion.

3.11.1.1.4 Circulating levels of CD19/CD22-CAR T cells must be <5% in peripheral blood by flow cytometry.

3.11.1.1.5 Participants must have been previously infused with CD19/CD22 CAR T cells on study and have at least 1 additional dose cryopreserved. (The cell dose based on CAR transduced cells) for the reinfusion will be the same dose as the initial infusion dose)

3.11.1.1.6 Any toxicity (regardless of causality) after the previous CD19/CD22-CAR T cell infusion must resolve such that subjects meet the following eligibility criterian (based on labs obtained within 1 day of cell infusion):

3.11.1.1.7 CD19/CD22-CAR T cells must have met release criteria (section **3.3**)

3.11.1.1.8 Subject has no evidence of hemodynamic instability

3.11.1.1.9 Subject has not developed a new requirement for supplemental oxygen therapy

3.11.1.1.10 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

3.11.1.1.11 Liver transaminase (ALT and AST) $\leq$ 10 x ULN based on age- and laboratory specific normal ranges

3.11.1.1.12 There is no evidence of clinically significant cardiac dysfunction, uncontrolled, significant tumor lysis syndrome, serum creatinine >2 x ULN, and no acute neurological toxicity > grade 1 (with the exception of peripheral sensory neuropathy).

3.11.2 Exclusion Criterion for Subsequent Cell Infusions

Participants who incurred TLT after receiving the first cell infusion will not be eligible to receive additional cell infusions unless IRB and FDA approval is granted on a case by case basis.

3.11.3 Additional Antineoplastic and Lymphodepleting Chemotherapy

Participants may receive additional antineoplastic and lymphodepleting chemotherapy prior to the reinfusion of CD19/CD22-CAR T cells. See section **3.9.2** for lymphodepletion dosing.

3.11.3.1 Rationale for Intensified Lymphodepletion

Given the historically limited response with second or subsequent infusions, which is believed to be due to an anti-CAR immune mediated rejection of the infused CAR-T cells that develops after the first infusion, in subjects who meet the above eligibility criteria for subsequent infusion and who have evidence for either a) limited CAR-T cell expansion with the first infusion; b) rapid loss of CAR-T cells suggesting limited persistence; c) loss of B cell aplasia as defined above; d) incomplete response where a reinfusion to improve upon the initial response could be considered; incorporation of an intensified lymphodepleting regimen consisting of 4 days of fludarabine 30 mg/m2/dose (days -5, -4, -3, -2) and 2 days of cyclophosphamide 600 mg/m2/dose (days -3, -2) may be utilized along with the same initial dose of CAR-T cells infused. All subjects who receive subsequent infusion must meet LD initiation criteria as per section **3.11.1**.

Based on institutional experience with a similar regimen on the POB Anti-CD22CAR protocol 15-C-0029, this regimen is well tolerated in the setting of CAR-T cell therapy. The goal of the intensified regimen would be used to reduce the likelihood of immune rejection of the infused CAR-T cells and improve the response. As per section **3.11.1.1.5,** all toxicities will be monitored and if two or more participants develop unexpected grade 4 toxicity from the chemotherapy regimen, then utilization of this regimen will be paused pending further discussion and review.

On the day of infusion, the cellular product will be thawed and administered IV immediately (as per institutional guidelines) 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.

3.11.3.2 Post Infusion Monitoring

Any subject who receives subsequent infusions of CD19/CD22-CAR T cells will NOT be evaluable for TLT purposes of this study as they will be beyond the 28-day observation period for TLTs after their first cell infusion. However post-infusion monitoring will be the same as for the 1st infusion and all toxicities, including secondary reactions, will be recorded and reported. If two or more participants develop Grade 4 toxicity at any time following the second CD19/CD22-CAR T cell infusion that is felt to be possibly, probably or likely related to the CD19/CD22-CAR T cells, then reinfusions will be paused pending discussion with the FDA and IRB regarding continuing reinfusions as part of the experimental regimen.

3.12 Option for Remission Maintenance

For those participants who achieve a complete remission post CAR T-cells and do not proceed immediately to a consolidative transplant post CAR T-cell therapy and are being monitored for long-term response and CAR persistence, there are well-established parameters suggestive of impending relapse. These metrics include a) resurgence of normal precursor B-cells (hematogones); b) resolution of B-cell aplasia; c) loss of CAR T-cell persistence and/or d) detection of minimal residual disease.

In these circumstances, participants may have the option to receive a reinfusion or standard chemotherapy to maintain remission and prevent relapse, while remaining on study. This “maintenance” approach to remission induction is well-established in the treatment of acute

lymphoblastic leukemia and may include standard low-dose ALL directed maintenance-type chemotherapy regimens (e.g., 6-mercaptopurine, oral methotrexate, steroids and vincristine, or for instance utilization of a tyrosine kinase inhibitor in Ph+ ALL, amongst other standard low-dose options). All regimens will be captured and recorded. Decisions regarding utilization of specific standard chemotherapeutic agents will be based on prior exposure and toxicity profile.

If the participant chooses to receive relapse prevention therapy, the participant will be enrolled to protocol 04-C-0165 Oncology Care & Interventions.

Participants will be taken off-protocol as per section **3.16** if there is clear progressive disease or initiation of alternative therapy for treatment of disease progression, with exception for those participants who will be moving towards a subsequent infusion.

3.13 Neurologic and Neurocognitive Evaluation

A neurologic assessment (**Appendix H**) will be performed as feasible, at least twice on study, before preparative regimen and after cell infusion (Day 28 ± 4 days) to monitor prospectively for signs of neurotoxicity; additional timepoints may be added per PI discretion (e.g. for those participants who experience cytokine release syndrome, a timepoint at the onset and resolution of CRS may be indicated).

Only for those with isolated CNS disease: Additionally, 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/CD22-CAR T cell infusion and at timepoints given in section **15.2.2**. These tests will only be administered to subjects who speak English or Spanish. A parent/adult observer (or adult participant if the parent/adult observer is not available) also will be asked to complete a Background Information Form (about 5 minutes to complete) and a brief neuro-symptom checklist assessing the severity and duration of any neurologic symptoms over the past week (about 2 minutes to complete) (**Appendix H**). The neuro-symptom checklist can be administered to parent/adult observers (or adult participants) who do not speak English using an interpreter. Administration of the test and neuro-symptom checklist will be optional during reinfusion. Neuro cognitive evaluations should ideally be performed prior to initiation of seizure prophylaxis with levetiracetam (Keppra). See **Appendix H** for more details.

A repeat cognitive evaluation (**Appendix H**) will be administered at approximately 3 months post cell infusion (if participant returns for follow up visit) (only in participants with isolated CNS disease).

3.14 Study Calendar

See **Appendix B**: Study Calendar

3.15 Protocol Accrual Suspension

The study will be halted, and the FDA and IRB will be notified if any of the following conditions are met:

- DLT occurs in dose level -1 cohort.
- Development of EBV lymphoma or polyclonal lymphoproliferative disease (PLPD).
- Any Grade 5 event at least possibly related to the research product.

3.16 TELEMEDICINE

Telemedicine is the use of interactive audio, video, audio-visual, or other telecommunications or electronic technology by a licensed health care practitioner to deliver clinical services. This protocol will allow the team to practice telemedicine to communicate with participants in real time, to be able to monitor and collect data, as well as the ability to share the participants' health information with other health professionals. Providers may include primary providers, specialists/consultants and nurses. Other members of the healthcare team may also be present to aid with the communication devices, scheduling or records management.

The participant or participant's legal representative will be informed prior to the use of a telemedicine encounter and consent will be obtained as outlined in section **11.6**.

Telemedicine visits will be arranged through our NIH Clinical Center Health Information Management Department and will be scheduled using NIH-approved remote platforms. Telemedicine visits may be used for follow-up visits if deemed appropriate by the PI. All telemedicine visits must be documented in CRIS like a normal onsite visit and the note should indicate that this visit was performed virtually.

History and Physical exams as well as vital signs, weight, performance status, adverse event capture as directed in in **Appendix B** may be completed by remote visit with a member of the study team (e.g., if the participant is not able to return to the NIH CC). Remote visits will be conducted in compliance with NIH guidelines and FDA regulations.

3.17 LOCAL EVALUATIONS

For protocol-specified timepoints post Day 28 as directed in **Appendix B** a participant may be asked to come to the NIH CC for an in-person assessment or be referred to their local provider, if clinically indicated, and/or at the discretion of the investigator. All physical exams, assessments, labs, and imaging used for follow-up or restaging visits may also be performed with the participant's local physician as determined by the PI. For in-person assessments, physical examinations may be omitted at the discretion of the investigator. For laboratory evaluations conducted with local providers, interlaboratory variability is not a concern as the lab tests are all routine. In the case of any visits with participants' local providers, records will be obtained for the research records.

3.18 COST AND COMPENSATION

3.18.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.18.2 Compensation

Participants will receive a payment of $20 for each stool sample collected (following collection), up to a total of $100. Participants will only be paid for each completed stool collection. Compensation will be processed per NIH guidelines through Research Volunteer System. Please see guidance at:

https://clinicalcenter.nih.gov/recruit/crvp/payment.html

For pediatric participants, check will be given to a parent/guardian after each collection.

If a participant withdraws from study, no future compensation will be made, but prior compensation will not be revoked.

If a sample is collected for clinical/non-research reasons the participant will not be compensated.

3.18.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.19 Criteria for Removal from Protocol Therapy and Off Study Criteria

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

3.19.1 Criteria for removal from protocol therapy

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

- Treatment limiting toxicity (TLT) due to preparative regimen toxicity that precludes cell infusion.
- Treatment limiting toxicity (TLT) due to cell infusion (unless criteria are met for a reinfusion per section **3.11**). 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 **3.6.1**, the participant will not generally have the option of receiving a reinfusion of cells (unless exception is granted by the IRB and FDA).
- General or specific changes in the participant's condition render the participant unacceptable for further treatment on this study in the judgment of the investigator.
- Progressive disease (unless criteria are met for a reinfusion per section **3.11**).
- Pregnancy
- Initiation of alternative therapy (unless criteria are met for a reinfusion per section **3.11** or if alternative therapy is being used to maintain remission as per section **3.12**).

3.19.2 Off-Study Criteria

- Screen Failure

- Participant withdrawal from protocol (in which case the reason will be documented, if possible)
- Lost to follow-up
- Death
- Conclusion of the 15 years of long term follow up and/or participant is off therapy and enrolled in long-term follow up protocol for participants receiving gene therapy
- Participant does not receive cell infusion

3.19.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 4 visits scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 12 weeks and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 CONCURRENT THERAPY FOR EXTRAMEDULLARY LEUKEMIA OR CNS LYMPHOMA:

Participants must be in CNS remission or have CNS-2 disease without clinical evidence of cranial nerve involvement at the time of protocol enrollment. 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 participants with ALL at times of restaging spinal taps. Further, intrathecal chemotherapy will NOT be administered in any participant who develops CNS toxicity 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

Intrathecal chemotherapy will be dosed by age according to institutional guidelines, (for example, the following):

Age (years)	MTX (mg)	HDC (mg)	ARA-C (mg)	Volume (ml)
3-8	12	12	24	8
≥9	15	15	30	10

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

Any combination of therapy as clinically indicated may be administered at the PI or AI's discretion.

4.1.3 Administration

Administration should be performed according to institutional guidelines/SOP: Delivery should be isovolumetric (ml CSF out = ml drug in) via lumbar puncture (LP) in the lateral decubitus position. Participants should remain in prone or Trendelburg position for 30 minutes post LP to facilitate drug circulation throughout the CNS.

4.1.3.1 History of CNS involvement post cell infusion guidelines

Intrathecal chemotherapy will NOT be administered in any participant who develops neurologic toxicity until at least 2 weeks after complete resolution. (exception: intrathecal hydrocortisone may be administered as indicated for treatment of severe neurotoxicity or intrathecal therapy may be administer for progressive CNS leukemia)

4.1.4 Leucovorin

Leucovorin may be given as 10 mg/m^2/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 participants 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). Additional suggested guidelines are provided in **Appendix J**.

Special Statement about IVIG: Since B cell antigen-targeted immunotherapy has been demonstrated in other studies to also eliminate normal B cells. For this reason, it is recommended that serum IgG levels be monitored before cell infusion and every 3-6 weeks after infusion (not required). If IgG < 500, IVIG will be administered at a dose of 500 mg/kg with the appropriate pre-medications and per institutional guidelines as clinically indicated.

4.3 Prophylaxis and Treatment of Tumor Lysis Syndrome in Children

Pediatric participants 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 according to institutional standards SOP (for example: at a dose of approximately 100 mg/m^2/dose p.o. 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/m^2/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/m^2/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.

4.4 Blood Product Support

Using daily CBC's as a guide, the participant 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.

In participants with symptomatic coagulopathy, attempts will be made to keep platelets >50,000/mm3 and fibrinogen ≥ the lower limit of normal.

4.5 Hydroxyurea

Participants 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 1 day prior to CAR T-cell infusion.

4.6 Cytokine Release Syndrome

Grading and management of CRS in this protocol will follow the guidelines in **Appendix D**, **Table 3**, which includes diligent supportive care and search for infection, with immunosuppression using anti-IL6R mAbs and/or corticosteroids reserved for more severe cases.

4.7 Neurologic Toxicity

Participants will be monitored closely with neurologic checks with vital sign according to institutional standards during hospitalization. Levetiracetam (Keppra) or alternative seizure prophylaxis will be started anytime prior to CAR cell infusion in participants with CNS disease, for those at high-risk for neurotoxicity, or in those who have had a prior history of seizures, as per PI discretion. Adults will receive 500 mg BID, which may be escalated per standard guidelines to a maximum of 2 gm BID; Children will initiate levetiracetam at 20 mg/kg in 2 divided doses (10 mg/kg BID). If levetiracetam is contraindicated, an alternative anti-seizure regimen may be used after discussion with the protocol team. The daily dose may be increased in consultation with neurology in the setting of neurotoxicity. Participants with significant

neurotoxicity may be treated with corticosteroids. Subjects will start levetiracetam at any time prior to the cell infusion and will be maintained on levetiracetam through the period of cytokine release syndrome. Levetiracetam may be discontinued by as early as day 21 (or after the resolution of CRS, whichever is later), or sooner if clinically indicated.

5 BIOSPECIMEN COLLECTION

Blood, bone marrow and CSF samples will be collected for correlative studies as outlined below and in **Appendix I**. A single-pass apheresis may be performed if an apheresis catheter is in place at Day 28 ± 7 days.

5.1 CORRELATIVE STUDIES FOR RESEARCH

Note: Platforms and procedures may be adjusted based upon current technology and/or collaborations in place at the time of actual analyses

5.1.1 Overall goal of study correlatives

To explore secondary and exploratory objectives on this protocol and better understand the T cell and tumor properties that influence CAR T cell efficacy and persistence and permit disease relapse and also explore the depth of treatment response and explore multiple aspects of the toxicity profile.

5.1.2 Specific Aims

- Assess the impact of T cell subset composition as delineated using flow cytometry, mass cytometry and ATAC-Seq on CAR T cell expansion and persistence
- Use exome sequencing and RNA sequencing (Shern lab POB) to analyze the correlation in observed changes in antigen expression at the protein level with genetic and transcriptional changes at the antigenic locus. Sample collection, storage and testing will be performed on subjects who have consented to protocol 10-C-0086 "*Comprehensive Omics Analysis of Pediatric Solid Tumors and Establishment of a Repository for Related Biological Studies*".
- Where possible, use TCR sequencing to fate map cells contained in the apheresis of manufactured product to persistent CD19/22-CAR T cells as an exploratory aim to identify subsets with a greater likelihood of T cell persistence in the setting of adoptive cell therapy. Sample collection, storage and testing will be performed on subjects who have consented to protocol 10-C-0086 "*Comprehensive Omics Analysis of Pediatric Solid Tumors and Establishment of a Repository for Related Biological Studies*".
- Characterize antigen expression on relapsed B-ALL and B cell lymphoma following multi-specific CAR-mediated targeting
- Utilize the CAR-mediated arrest of B cell maturation to gain insight on physiologic and pathophysiologic B cell development, maturation arrest and alternative survival pathways in normal and malignant B cells.
- Explore the level CD19/CD22 surface expression and CD19/CD22 site density on leukemic blasts, when feasible and correlate with clinical response to CAR-T cells

- To explore CAR T-cell product characteristics that may be associated with clinical outcomes (e.g., CAR T-cell persistence, toxicity and/or efficacy)
- To explore CAR T-cell expansion by T-cell subsets and immunophenotypic evaluation of markers of T-cell activation and/or exhaustion

5.1.3 Sequence-based detection of MRD

- Selected participant's samples (e.g. participants who have persistent CAR cells in the blood, and who appear MRD negative by flow cytometry) will have bone marrow samples sent to Adaptive Biotechnologies Corporation to undergo analysis by sequence-based detection of MRD. This testing will generally be performed for research purposes and results will not be available in real-time. In certain circumstances, these samples may be sent for clinical monitoring also.

5.1.4 Hematology studies

- Hematology studies will include standard clinical laboratory studies, including PT/PTT, fibrinogen, D-Dimer, peripheral blood smear, and complement studies.

5.1.5 Optional hematology studies to be performed only as feasible in those with evidence of clinically significant DIC include:

- Additional studies specifically for the evaluation of hemolytic uremic syndrome and/or thrombotic thrombocytopenic purpura and or MAS/HLH, including sMAC, and other complement studies, as well as ferritin and soluble IL2R.

5.1.6 Gut Microbiome (optional)

Stool samples may be obtained as outlined in **Appendix I:** Biospecimen Collection Schedule and Forms and used for gut microbiome evaluation. Analyses may include the following:

- Microbiome sequencing (including 16S rRNA gene sequencing, shotgun metagenomic sequencing, and potential evaluation of the resistome, virome, and parasitome).
- Fecal flow (sorting and 16S and/or shotgun sequencing of IgA bound organisms, which is an indication of pathogenicity).
- Lipocalin, fecal caprotectin and α-1-anti-trypsin levels (to evaluate mucosal inflammation and malabsorption).
- Metabolomics (to evaluate intestinal metabolomic profiles, and when combined with microbiome/metagenomic studies, may suggest some functional role for candidate microbes of interest).
- Measurement of secreted IgA and antimicrobial peptides.
- Culturing/PCR for specific organisms.
- Stool proteomics.
- Use of stool samples in mouse models.
- Storage for future research.
- Blood and urine for host responses in microbiome assessment.

5.1.7 Metabolomic Analysis (optional)

Peripheral blood and urine samples may be collected as outlined in **Appendix I** to analyze NAD(H), FAD(H), glutathione), amino-acids (arginine, tryptophane, glutamine, methionine), nucleoacids (ATP, adenosine), TCA cycle metabolites, hepcidin, Acteyl-CoA and lipids.

5.1.8 Sample Collection Schedule

The samples to be collected and schedule for sample collection is detailed in **Appendix I**. In addition, **Appendix I** contains the sample information for submitting CD22 surface expression to the NCI for FACS analysis.

5.2 MONITORING GENE THERAPY TRIALS: PERSISTENCE, AND RCL, AND TOXICITY

The Federal Drug Administration requires monitoring of all participants who have received CAR therapy products. Participant's peripheral blood samples need to be obtained and undergo analysis for persistence and RCL PCR including detection of p24 antigen and reverse transcriptase activity Product Enhanced Reverse Transcriptase [PERT]). Persistence will be performed by peripheral blood flow cytometry at the National Institutes of Health. RCL PCR testing will be performed at the Indiana University Vector Production Facility (IU VPF). For the purposes of this protocol, this will be considered gene-therapy follow-up.

Participants will be co-enrolled on NIH protocol 15-C-0028: Follow-up Evaluation for Gene-Therapy Related Delayed Adverse Events after Participation in Pediatric Oncology Branch Clinical Trials. Participants will have blood samples collected prior to cell infusion (at baseline) for RCL PCR. After infusion of CAR cells, participants will have all subsequent gene therapy monitoring (persistence, RCL and toxicity) completed as directed by protocol 15-C-0028.

Note: All previously collected samples collected for RCL testing of participants on this protocol with this amendment (09/16/2022) will be stored at NCI-Frederick and only tested as clinically indicated (e.g., development of a new cancer thought to be related to therapy with CD19/22 CAR Tcells). If testing is required, samples will be shipped to Indiana University Vector Production Facility for testing.

5.3 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The study will remain open so long as sample or data analysis continues. Samples will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

If the participant withdraws consent the participants data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

5.3.1 Sample Data Collection

All samples sent to the Biospecimen Processing Core (BPC) will be barcoded, with data entered and stored in the Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined BPC personnel, who are issued individual user accounts. Installation of Labmatrix is limited to specified computers. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to participants without Labmatrix access. Data will be recorded for each sample, as appropriate (e.g., participant ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location). Participant demographics associated with the Clinical Center participant number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.3.2 Sample storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the CPP and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the CPP. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a participant withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed or returned to the participant, if so requested. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

Sample barcodes are linked to participant demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to participant information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

The study will remain open and status reported to the NIH Intramural IRB until all samples have been analyzed, reported or destroyed. Any use of these samples for purposes not described in this protocol will require prospective NIH Intramural IRB review and approval.

5.3.3 Hourigan Laboratory (NIAID)

Participant biospecimens, collected for the purpose of research under this protocol will be analyzed as per **Appendix I** . Samples will be tracked in the laboratory database with access limited to qualified study members approved by the Principal Investigator. Each team member accesses the database with their own individual login credentials and an audit trail tracks data entry and any alterations to the database.

Sample inventory and chain of custody is tracked for all biospecimens. Samples are linked to the subject by the Subject ID, which cannot be traced back to the participant. Also recorded, are the trial name/protocol number, date obtained, current location, any derivative products, and whether the sample has been exhausted. All received samples will receive a unique bar code number, generated by the database that cannot be traced back to the participant. Vial labels do not have personally identifiable information for the participant. Vials will not be traceable back to participants without authorized access.

Samples are stored in freezers at -80°C. These freezers are located onsite at NIAID and location is recorded in database. Access to samples from a protocol for research purposes will be by permission of the Principal Investigator.

5.3.4 Taylor Laboratory (POB)

Participant biospecimens, collected for the purpose of research under this protocol will be analyzed as per **Appendix I**. Samples will be tracked in the laboratory database with access limited to qualified study members approved by the Principal Investigator. Each team member accesses the database with their own individual login credentials and an audit trail tracks data entry and any alterations to the database.

Sample inventory and chain of custody is tracked for all biospecimens. Samples are linked to the subject by the Subject ID, which cannot be traced back to the participant. Also recorded, are the trial name/protocol number, date obtained, current location, any derivative products, and whether the sample has been exhausted. All received samples will receive a unique bar code number, generated by the NCI Labmatrix database that cannot be traced back to the participant. Vial labels do not have personally identifiable information for the participant. Vials will not be traceable back to participants without authorized access.

Samples are stored in freezers at -20°C, -80°C, or -140°C/liquid nitrogen vapor phase according to stability requirements. These freezers are located onsite at POB and location is recorded in Labmatrix. Access to samples from a protocol for research purposes will be by permission of the Principal Investigator.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR Part-11 compliant data capture system provided by the NCI CCR 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. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day -4, through day 30. After 30 days, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the participant's outcome.

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, this will be reported expeditiously per requirements in section **7.2.1**.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

- Coded, linked data in an NIH-funded or approved public repository (clinicaltrials.gov).
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
- Identified or coded, linked data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

- An NIH-funded or approved public repository (clinicaltrails.gov).
- BTRIS (automatic for activities in the Clinical Center)
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

When will the data be shared?

- Before publication.
- At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Policy

The genomic data sharing policy does not apply to this protocol as participants are co-enrolled on 10-C-0086 "Comprehensive Omics Analysis of Pediatric Solid Tumors and Establishment of a Repository for Related Biological Studies".

6.3 RESPONSE CRITERIA

For the purposes of this study, participants should be re-evaluated for response as outlined in section **15.2**. In addition to a baseline scan, confirmatory scans should also be obtained 4 weeks following initial documentation of objective response.

Response criteria are outlined in **Appendix F**, for participants with lymphoma and participants with leukemia.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each participant while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc).

Cytokine release syndrome will be graded as per the grading system in **Appendix D** of this protocol.

CAR-T related neurotoxicity will be graded using CTCAE and the grading system in **Appendix G**.

7 NIH REPORTING REQUIREMENTS / DATA SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements. Note: Only IND Safety Reports that meet the definition of an unanticipated problem or present new information that might affect the willingness of participants to enroll or remain on the study will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention **s**hould be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to NCICCRQA@mail.nih.gov within one business day of learning of the death.

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 CD19/C22 T-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 CD19/C22 T-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 NIH Required Data and Safety Monitoring Plan

7.5.1 Principal Investigator/Research Team

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

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each participant 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 Safety Monitoring Committee (SMC)

This protocol will be periodically reviewed by an intramural Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NIH Intramural 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 based on the risks presented in the study. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period.

The SMC review will focus on unexpected protocol-specific safety issues that are identified during the conduct of the clinical trial.

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 SPONSOR SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a participant or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2)).

8.1.2 Serious Adverse Event (SAE)

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 event (see section **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient or subject convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- 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.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32).

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor with the exception of any listed in section **8.4**.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in section **8.4**.

All SAE reporting must include the elements described in section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form

and instructions can be found https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions.

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 WAIVER OF EXPEDITED REPORTING TO CCR

Expected SAEs, occurring between Day 0 and Day 28, that will not require expedited reporting include:

- Grade 1 and 2 CRS events
- Grade 3 CRS events that do not resolve to Grade 2 in 72 hours (about 3 days)
- Inpatient admission/hospitalization for evaluation and treatment of CRS.
- ICU transfer for evaluation and treatment of CRS

CRS will be graded per 2019 ASTCT, see section **15.4.**

As participants are increasingly and more routinely treated as outpatients for CAR T-cell therapy, inpatient admissions with fever are expected and anticipated events. Similarly, the event of a simple transfer to the ICU to optimize management and provide more careful monitoring, in and of itself, is also not unexpected.

The PI will submit a safety report of all grade 3-5 events, regardless to causality to the study intervention, every 6 months. The report will include a line listing of all events including the PID, date of onset of the event, date of resolution of the event, date of dosing for each study intervention in relationship to the event, attribution of the event, grade of the event, CTCAE term of the event and the System Organ Class of the event. In addition, a listing of all adverse events including the number of participants experiencing each adverse event, cumulative and per grade, and per causality assessment will be included. The report will indicate the date of the data cut off, total number of participants treated to date, and dictionary for any coded field. The report is cumulative and new information for the last 6 months period should be highlighted. The report should be sent to OSROSafety@mail.nih.gov.

The Sponsor might request case summaries for those events if, upon review, the Sponsor determines that an aggregate safety report is required (21CFR312.32(c)(1)(iv)).

8.5 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATORS

Reporting will be per the collaborative agreement.

8.6 REPORTING PREGNANCY

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions.

8.6.1 Maternal exposure

If a participant becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of

when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy become known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies should be followed up and documented.

8.6.2 Paternal exposure

Male participants should refrain from fathering a child or donating sperm during the study and for 4 months after the study therapy.

Pregnancy of the participant's partner is not considered to be an AE. The outcome of all pregnancies occurring from the date of the first dose until 4 months after the last dose should, if possible, be followed up and documented. Pregnant partners may be offered the opportunity to participate in an institutional pregnancy registry protocol (e.g., the NIH IRP pregnancy registry study) to provide data about the outcome of the pregnancy for safety reporting purposes.

8.7 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.8 SPONSOR PROTOCOL DEVIATION REPORTING

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements.

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL 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 and secondary study endpoints; adherence to the protocol, regulations, ICH E6, 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 a 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.

Clinical site monitoring is conducted to ensure:

- that the rights of the participants are protected;
- that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures; and,
- the quality and integrity of study data and data collection methods are maintained.

Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Sponsor and Regulatory Oversight Support (SROS) Services contractor. Clinical site monitoring activities will be based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. OSRO will determine the intensity and frequency of monitoring based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. The Sponsor will conduct a periodic review of the CMP to confirm the plan's continued appropriateness. A change to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

OSRO SROS Monitoring visits and related activities will be conducted throughout the life cycle of each protocol. The first activity is before the study starts to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will occur at the study site(s). Monitoring visit reports will describe visit activities, observations, and associated action items or follow-up required for resolution of any issues, discrepancies, or deviations. Monitoring reports

will be distributed to the study PI, NCI CCR QA, CCR Protocol Support Office, coordinating center (if applicable), and the Sponsor regulatory file.

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESIS

10.1.1 Primary Objective

Assess the safety of administering escalating doses of autologous CD19/CD22-CAR engineered T cells that can be feasibly produced to meet established release specifications in children and young adults with B cell ALL or lymphoma following a cyclophosphamide/fludarabine conditioning regimen.

In addition to evaluating up to 6 participants at a given dose level with respect to toxicity, the number of participants which can successfully manufacture the targeted dose number will be determined. Participants will be enrolled on a given dose level until adequate participants are enrolled to produce the correct number of cells for safety evaluation at that dose level.

10.1.2 Secondary Objective

Evaluate whether CD19/CD22-CAR T cells can mediate clinical activity in children and young adults with B-ALL, isolated CNS ALL, or lymphoma.

10.2 SAMPLE SIZE DETERMINATION

10.2.1 Sample size related to safety endpoint

The primary objectives of this study are safety and feasibility. Three to 6 participants will be enrolled in three dose cohorts to determine the maximum tolerated dose. The study will allow for up to 3 participants to be replaced in each of the dose cohorts 1 through 3 (9 additional participants) due to inability to achieve target doses, for a maximum of 27 participants. Up to 2 extra participants may be enrolled on a single dose level, per amendment, version date 06/10/2019. Prior to amendment, version date 01/11/2020, 4 participants had been enrolled on dose level 1 and 4 had been enrolled on dose level 2; participants are currently enrolling onto dose level 3, and with allowance for replacement of participants, up to 9 participants may be enrolled on dose level 3. Beginning with amendment, version date 01/11/2020, participants will enroll in two cohorts based on their prior CAR-T and/or transplant experience (CAR naïve or CAR pre-treated with an interim transplant; previous CAR-T treatment with no interim transplant). As a result, dose level 4 will contain up to 18 total participants (2 cohorts with up to 9 participants per cohort). Thus, up to 4+4+9+18=35 participants may enroll in the 4 dose levels during dose escalation. In addition, the study will allow for 6 total inevaluable participants (participants enrolled but who cannot receive cells, either due to physical deterioration or withdrawn consent during cell growth). Thus, 35 + 6 + 2 (2 extra participants by PI discretion, per amendment, version date 06/10/2019) yields a maximum of 43 participants who will be enrolled to determine MTD and feasible dose. If dose level 4 exceeds DLT, future participants will be enrolled into lower dose levels but into the two separate strata.

The endpoint for Safety of CD19/CD22-CAR T cells is evidenced by the incidence and severity of dose limiting toxicities (DLTs) (i.e. laboratory abnormalities, changes in vital signs, and changes in physical examination) following chemotherapy preparative regimen and infusion of CD19/CD22-CAR T cells, recorded and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 5 at three dose levels until the maximum tolerated dose (MTD) is determined. If Dose level 3 can be feasibly manufactured and is administered in up to 6 participants without evidence of DLT or efficacy, consideration will be given to amending the trial to examine higher dose levels.

The dose escalation procedure follows a 3+3 design as described in section **3.7**, beginning with dose level 1, based on the DLT count and response rate in each cohort, with escalation also constrained by the feasibility of producing the doses called for. The purpose of the design is not to invert the dose-toxicity curve at a target DLT rate, but rather to proceed with appropriate caution to dose level 3, which we expect will be reached without observing any DLT. Safety monitoring will continue throughout the study in the expanded cohort of up to 30 participants in each expansion group at the final MTD (or highest dose tested).

After determining the MTD, an expansion cohort will enroll initially 15 participants at the MTD (or maximal dose studied) in two cohorts (one cohort will be CAR naïve or CAR pre-treated with an interim transplant ,and the other cohort will be those who have received prior CAR T-cells without an interval transplant), including up to 6 from each of the two the dose escalation cohorts treated at DL4, if applicable, to further define safety, persistence, efficacy and duration of response. If sufficient responses occur, the two cohorts may be expanded to 30 participants per cohort.

Up to 12 participants will be enrolled at the MTD for cohort 2c isolated CNS disease. For participants with isolated CNS disease, they will be evaluated at the current dose, dose level 3 ($3x10^6$ cells/kg) in order to explore toxicity and response. Initially 6 eligible participants will be enrolled and if 0/6 have a neurologic TLT and 0-1 of 6 have any other type of TLT, then this cohort will be expanded to 12 total participants, provided that no more than 4 participants cumulatively experience any TLT, in order to further describe the toxicity of the participants.

10.2.2 Sample Size Related to Feasibility Endpoint

Feasibility will be defined as the successful manufacturing of CD19/CD22-CAR T cells that meet established release criteria to satisfy the targeted dose level. Although we anticipate reaching the targeted cell dose during manufacture, feasibility of manufacturing cells remains a primary objective in this participant population. Participants will be enrolled on a given dose level until adequate participants are enrolled to produce the correct number of cells for safety evaluation at that dose level. Specifically, dose escalation will proceed as long as 3 or more of the first 3 to 6 participants in a dose level are able to produce adequate cells for evaluation. For example, this might mean that 6 to 9 participants will need to be enrolled at a dose level to result in 6 for the safety evaluation. However, if less than 3 of 6 participants at a given dose level are able to have adequate cells produced, evaluation of that level and beyond for safety and feasibility will not take place. If cell growth limitations preclude administration of the targeted cohort cell dose, the participant will receive as many cells as possible, and be considered part of the lower dose cohort. If a minimum of 1.0×10^5 CD19/CD22-CAR-transduced T cells per kg cannot be obtained for infusion, the participant may be treated but will not be evaluable for

toxicity or response but will be considered a feasibility failure. Specifically, if after the first 6 participants have been enrolled at a given dose level, more than 3 are unable to have adequate CD19/CD22-CAR T cells produced (that meet COA for infusion), accrual to that dose level will stop and the dose escalation phase of the study will also end, 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 for a given, which would be desirable. The evaluation of participants in the expansion cohort will take place using the highest dose level at which feasibility, as well as safety, was identified. In the expansion cohorts, the fraction which are able to manufacture the targeted dose level will also be monitored and beginning with the 6th participant in an expansion cohort, if at any point fewer than half of the enrolled participants are able to manufacture an acceptable level of cells, the accrual to the expansion cohort will end.

Thus, 4 + 4 + 9 + 18 + 6 + 2 + 30 + 30 +12 =115 + 25 (screen failures) + (10 inevaluable) = 150 is the accrual ceiling. The actual maximum enrolled may be reduced by up to 6 + 6 if both cohorts at DL4 enroll 6 evaluable participants apiece and can be included in the expansion cohort evaluations, which instead yields a maximum of 128 participants who may be enrolled under those circumstances.

Given the number of participants seeking out adoptive cell therapy trials the rate limiting factor at this time is the time and labor-intensive nature of manufacturing the CD19/CD22-CAR T cells. We anticipate enrollment of 2-4 participants per month given the number of participants with B-ALL treated at the NCI.

10.3 Populations for Analyses

Modified intention to treat. All participants who receive at least a single dose of the treatment will be included in the analyses.

10.4 Statistical Analyses

10.4.1 General Approach

Safety analyses will consist of reporting AEs by type of toxicity and grade for all participants enrolled on the study. Feasibility will be determined by identifying if adequate CD19/CD22-CAR T cells have been produced.

10.4.2 Analysis of the Primary Endpoints

The primary endpoints are safety. Safety analyses will consist of tabulations of grades of toxicity by type of toxicity.

10.4.3 Analysis of the Secondary Endpoints

The secondary endpoints include feasibility and to evaluate whether CD19/CD22-CAR T cells can mediate clinical activity in children and young adults with B-ALL, isolated CNS ALL, or lymphoma. For evaluation of feasibility, the number of participants which can successfully manufacture the targeted dose number will be determined. For efficacy in ALL and lymphoma participants, this will be evaluated in two groups of participants, those who have previously received CAR therapy and those that are CAR naïve. Efficacy will be important in determining if a phase 2 study is warranted.

The toxicity data for isolated CNS cohort will be summarized in a descriptive manner, and the response rate will be reported along with a 95% confidence interval.

A futility analysis will be conducted when 15 participants at the MTD in each group (2a and 2b) have reached the 28 day bone marrow assessment (for lymphoma the efficacy will be determined at 3 months post-CAR infusion due to the delayed treatment effect in lymphomatous disease), and if 5 or fewer participants have a CR, further enrollment to that group in the study will stop, and the 80% upper confidence limit will be formed. This fraction will have an upper one-sided 80% confidence limit of 0.48, which is below the desired 50% and thus would justify stopping accrual at this point. Assuming no futility stopping, no failure of manufacturing the required doses, and no DLT observed, the study will reach a maximum size of 30 participants treated at dose 3 in each group, and confidence intervals will be formed. With 0 DLT out of 30 participants, based on the one-sided upper Confidence Limits, we can rule out true DLT rates as high as 6%, 8% and 10% with 80%, 90% and 95% confidence (respectively).

The precision of estimate of the probability of dichotomous outcomes (as measured by 90% half-widths of two-sided 90% confidence intervals) relating to evidence for efficacy is no worse than 0.17, under the above conditions.

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.

10.4.4 Safety Analyses

All participants who receive experimental treatment (non-myeloablative chemotherapy and CD19/CD22-CAR T cell infusion) will be analyzed for safety and efficacy.

Subjects not treated for any reason will be included in the disposition tabulation but will be excluded from the safety and efficacy analysis.

The safety and tolerability of CD19/CD22-CAR T cell regimen will be assessed by:

- Suspected adverse events, and
- Suspected serious adverse events

As evidenced by:

- Changes in clinical laboratory tests (clinical chemistry, hematology, etc.).
- Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature).
- Changes in physical exams. Signs and symptoms assessed may require additional testing as clinically indicated such as ECG, PFT, radiographic studies, etc.
- Participant reported signs and symptoms

Safety data will be analyzed per standard methods and interpreted descriptively for each dose cohort. Safety data will be summarized for each dose cohort separately and for all dose cohorts combined. Adverse events will be assessed using the CTCAE version 5 for type and severity of

event. Serious Adverse Events will be summarized for each dose cohort and for all dose cohorts combined. Reasons for discontinuation of study therapy will be tabulated.

Laboratory testing includes hematology, serology, serum chemistry, and urinalysis. Baseline laboratory testing will be those results obtained prior to initiating the nonmyeloablative chemotherapy regimen. The study will utilize local lab for all clinical laboratory testing. Laboratory data will be tabulated based on the following result class.

- Normal: result is within the local lab normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form.

Vital signs collected immediately prior to receiving study drug will be the baseline vital signs. Observed vital sign values and change from baseline in vital signs at each visit will be summarized without formal statistical testing.

Vital sign result may also be tabulated based on the following result class.

- Normal: result is within the normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form. Number and percent of subjects within each result class will be tabulated by time point for each vital sign.

Findings of physical examinations will be tabulated by dose cohorts without formal statistical analysis.

10.4.5 Baseline Descriptive Statistics

General demographic and clinical characteristics will be reported for the participants evaluated in the study.

10.4.6 Planned Interim Analyses

A futility analysis will be conducted when 15 participants at the MTD in each group (or highest dose studied) have reached the 28 day PET imaging and/or bone marrow assessment, and if 5 or fewer participants have a CR, further enrollment to that group in the study will stop, with the upper 80% Confidence Limit of 0.476 not reaching the 0.48 minimum overall response rate expected for the "competing" treatment with active therapy such as autologous HCT for chemotherapy responsive participants of 40%.

10.4.7 Sub-Group Analyses

None planned.

10.4.8 Tabulation of individual Participant Data

None planned.

10.4.9 Exploratory Analyses

a) Evaluate whether participants receiving CD19/CD22-CAR T cells relapse with loss or diminished expression of CD19 and/or CD22.
b) Measure persistence of CD19/CD22-CAR T cells in the blood, bone marrow and CSF, and explore correlations between CD19/CD22-CAR T cell properties and CAR T cell efficacy and persistence.
c) Analyze alterations in early B cell development induced by immune pressure exerted via CD19/CD22-CAR T cells.
d) Assess neurologic and cognitive effects of the anti-CD19/CD22-CAR engineered T cell infusion pre- and post-infusion.
e) Explore the level CD19/CD22 surface expression and CD19/CD22 site density on leukemic blasts and correlate with clinical response to CAR-T cells
f) Explore the role of additional hematologic evaluation to enhance our understanding of the hematologic diathesis that can be seen in association with CAR or cytokine mediated toxicity**.**
g) Explore whether sequence-based genomic analysis can detect minimal residual disease (MRD) in participants who have persistence of CD19/CAR22+ T cells, and who are MRD negative by flow cytometry.
h) To retrospectively grade CRS as per the newly established ASTCT Consensus Guidelines that were published in 2019 **COMPLETED**
i) To identify immunophenotypic changes which may predispose to non-response or relapse using CyTOF in paired specimens from participants enrolled on this study.
j) To explore CAR T-cell product characteristics that may be associated with clinical outcomes (e.g., CAR T-cell persistence, toxicity and/or efficacy)
k) To explore CAR T-cell expansion by T-cell subsets and immunophenotypic evaluation of markers of T-cell activation and/or exhaustion
l) To describe the toxicity profile, CAR T cell expansion and persistence, overall response rate, and overall survival after reinfusion of anti CD19/CD22 CAR T cells in participants who do not have active disease, but who have early loss of CAR T cells, or loss of B cell aplasia < 6 months post initial infusion.
m) Evaluate the efficacy of siltuximab as a treatment for cytokine release syndrome and/or immune effector cell associated neurotoxicity syndrome.
n) Evalaute the impact of non-fludarabine based lymphodepletion on CAR T-cell efficacy, toxicity and CAR T-cell expansion
o) Evaluate the gut microbiome in participants receiving CAR-T therapy and correlate with cytokine release, neurotoxicity, infection, antibiotic use and clinical response.
p) Conduct metabolomic analysis to evaluate how metabolite levels influence efficacy of CAR-T cells, and toxicity in pediatric participants treated with CAR-T cell therapy.

10.4.9.1 Statistical Analysis of Cognitive Testing Data

Brief cognitive evaluations will be conducted pre- and post-infusion to assess any changes that might be related to the CD19/CD22 CAR treatment. These cognitive evaluations will consist of brief tests of verbal and visual memory, attention, processing speed, and executive functions. In addition, we will ask one parent or adult observer (e.g., adult sibling, spouse, other close relative with the participant) to complete a questionnaire evaluating the participant's daily executive skills

and a brief symptom checklist to assess the severity and duration of any neurologic symptoms that occur post-infusion (see **Appendix G**: Guidelines for Evaluation, Grading and Treatment of CAR-T Related Neurotoxicity).

To analyze the data for the secondary cognitive objective, paired t-tests will be used 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, analysis of variance with repeated measures will be used 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.

Note: As of this amendment 09/16/2022, neurocognitive testing will be limited to participants with isolated CNS disease.

10.4.9.2 Persistence of CD19/CD22-CAR T Cell Analyses

- Measure persistence of adoptively-transferred CD19/CD22-CAR-transduced T cells in the blood and, where possible, the bone marrow and CSF of participants.

Peripheral blood, bone marrow aspirate, and CSF will be collected when available and separately analyzed for the presence of CD19/CD22-CAR T cells. The percentage of all CD3+ cells in a sample that are positive by flow cytometry for CD19/CD22-CAR containing T cells will be analyzed and reported as time from T cell infusion.

11 HUMAN SUBJECTS PROTECTIONS

11.1 RATIONALE FOR SUBJECT SELECTION

The participants to be entered in this protocol have B cell malignancies that 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 participant 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.

Pregnant individuals are excluded from this study because the study agents have the potential for teratogenic or abortifacient effects. In addition, because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with the study agents, nursing should be discontinued.

11.2 PARTICIPATION OF CHILDREN

The age range of participants eligible for this trial is greater than or equal to 3 years of age but less than or equal to 39 years of age. Physicians, nurses, and multidisciplinary support teams of the POB, NCI and Clinical Center will provide participant 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.

11.3 Participation of Subjects Unable to Give Consent

Adults who are unable to consent may enroll in or be retained on this protocol because some participants may have disease that affects the central nervous system or cognitive ability and enrollment might be compromised without their involvement. In addition, the protocol offers a prospect of direct benefit (section **11.4**) and should therefore excluding these participants would compromise their ability to share in the benefits of the research. All subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study.

Capacity to consent will be evaluated by the Principal or Associate Investigator(s). For adults, whose ability to consent is uncertain, The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) to assess ongoing capacity of the subjects and to identify an LAR.

Please see section **11.6.1** for consent procedure.

11.4 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 **1.4**. The goal of this study is to improve upon the number of participants who may benefit from adoptive cell therapy by using participants' own transduced T-cells without the need to identify anti-tumor T-cells uniquely from each participant as was required in many cancer therapy protocols. The success of this effort cannot be predicted at this time. Because all participants 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.

11.4.1 Risks of CD19/CD22-CAR T Cells

Although the risk profile of CD19/CD22-CAR T Cells cannot be completely known at this time, the risks can be anticipated based on extensive experience with CD19-CAR T cell infusions and the POB NCI experience with CD22-CAR T cell infusions. The potential significant risks are described in section **1.4**. The risk profile of CD19 CAR cells is well established as multiple groups have tested CD19 CAR T cells in leukemia and lymphoma. Results from a number of the trials have been published with response rates of 70-90% for acute lymphoblastic leukemia being reported in the majority of studies. The majority of AEs reported as treatment-related have been reversible and grade 1 or 2 in severity. Significant problems that have been considered treatment related, include Cytokine Release Syndrome (CRS), visual hallucinations, neurological toxicity including confusion, dysphasia, hallucinations and death. One episode of cardiac arrest has occurred, and grade 4 neutropenia has been observed outside the expected window.

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

1. 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.
2. 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 $CaCl_2$ and $MgSO_4$ 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.
3. Transient mild thrombocytopenia is common after apheresis, but bleeding is unlikely.
4. Dilutional anemia occurs during apheresis, but this is unlikely to be clinically significant.
5. Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

11.4.3 Risks of the preparative regimen 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 **12** for summary of toxicities.

11.4.4 Risk of Blood Sampling

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting. Up to 94 mLs of blood may be collected at any given timepoint, and up to 448 mLs of blood may be collected in any given 8-week period as this is necessary for participant's safety. For pediatric participants, the amount of blood drawn for research purposes from pediatric participants (those under 18 years of age) will not exceed these limits nor exceed 5 mL/kg in a single day, and 9.5 mL/kg over an 8 week period.

11.4.5 Risk of Urine and Stool Collection

There is no physical risk associated with urine or stool collection.

11.4.6 Electrocardiogram (EKG) and Echocardiogram

Side effects of EKG are skin irritation where EKG electrodes are placed. Side effects of an echocardiogram are discomfort from the transducer being firmly placed against the chest.

11.4.7 Pulmonary Function Tests (PFTs)

These tests are safe and side effects are unlikely, but may include brief light headedness or slight soreness of the chest.

11.4.8 Bone Marrow Biopsy

Bone marrow biopsy is minimally invasive and is typically a very safe procedure. Usually the hipbone is numbed with anesthesia. Using a needle, the solid and liquid portion of bone marrow

is taken out. This procedure causes some pain. Very rarely, infection or bleeding may occur at the needle site.

11.4.9 Lumbar Puncture

Risks of lumbar puncture include pain at the site where the needle goes, in and the spinal fluid is taken, and headache. There is a small risk of infection or bleeding.

11.4.10 Risk of Central Venous Catheter (CVC)

Risks include bleeding, bruising, blood clot or infection at the site where the catheter is put in. In rare cases, placing a CVC has resulted in collapse of a lung. If this happens, the lung would be quickly re-inflated using a tube put into your chest. Sometimes catheters may become infected or clogged. If this happens the catheter may need to be replaced. The CVC will be flushed once a day to prevent it from becoming clogged. The nursing staff will show you how to do this yourself when you return home.

11.4.11 Risk of Cognitive Testing

Participants may experience feelings of frustration while taking the tests. The tests are meant to be challenging. Participants will be able to take breaks as necessary.

11.4.12 Risk of MRI

People are at risk for injury from the MRI magnet if they have some kinds of metal in their body. It may be unsafe to have an MRI scan if a participant has pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips (metal clips on the wall of a large artery), metal prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, tattoos, an implanted delivery pump, or shrapnel fragments. Welders and metal workers may have small metal fragments in the eye. Participants will be screened for these conditions before having any MRI scan. Participants who have a question about metal in their body, should inform the staff. Participants will be asked to complete an MRI screening form before each MRI scan.

In addition, all magnetic objects (like watches, coins, jewelry, and credit cards) must be removed before entering the MRI scan room.

People with fear of confined spaces may become anxious during an MRI. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss. Everyone having a research MRI scan will be fitted with hearing protection. If the hearing protection comes loose during the scan, participants should let staff know right away.

There are no known long-term risks of MRI scans.

11.4.13 Risks of gadolinium enhanced MRI

Mild symptoms from gadolinium infusion occur in fewer than 1% of those who receive it and usually go away quickly. Mild symptoms may include coldness in the arm during the injection, a metallic taste, headache, and nausea. In an extremely small number, fewer than one in 300,000 people, more severe symptoms have been reported including shortness of breath, wheezing, hives, and lowering of blood pressure. A participant should not receive gadolinium if they

previously had an allergic reaction to it. Participants will be asked about such allergic reactions before gadolinium is given.

People with kidney disease are at risk for a serious reaction to gadolinium contrast called "nephrogenic systemic fibrosis (NSF)". This condition always involves the skin and can also involve the muscles, joints and internal organs. NSF has resulted in a very small number of deaths. A blood test of the kidney function may be done within the month before an MRI scan with gadolinium contrast. Participants will not receive gadolinium for a research MRI scan if their kidney function is below the safe level.

Most of the gadolinium contrast leaves the body in the urine. However, the FDA has issued a safety alert that indicates small amounts of gadolinium may remain in the body for months to years. The long-term effects of the retained gadolinium are not unknown. Some types of gadolinium contrast drugs are less likely to remain in the body than others. In this study, we will use the gadolinium contrast drugs that are less likely to remain in the body. In this study, we will use the gadolinium contrast drugs that are less likely to remain in the body, whenever possible. Additional information called a "Medication Guide" will be given to all participants. Upon request, staff will give participants individual information about retained gadolinium that is seen on their studies.

11.4.14 Scans and Contrast

The radiation risks of the PET/CT, and CT scans are discussed below. In addition to radiation risks, these scans that employ contrast may cause allergic reactions, injection site reactions abdominal discomfort and fainting. MRIs carry no radiation risks, but are contraindicated in subjects with metal in their bodies. In subjects that receive gadolinium contrast with MRIs, allergic reactions, injection site reactions and kidney damage may occur.

An IV line may need to be inserted for administration of the contrast agent or anesthetic, which may cause pain at the site where the IV is placed and there is a small risk of bruising or infection.

11.4.15 Risks of exposure to ionizing radiation

In this research study subjects with lymphoma or extramedullary ALL may have up to five CT scans and five PET/CT scans performed (inclusive of screening and treatment) a year. Subjects undergoing CT scans and PET/CT scans will be exposed up to 11.5 rem annually. This amount of radiation is greater than the NIH Radiation Guidelines of 0.5 rem per year for participants less than 18 years old. This level of exposure is associated with an increased risk of cancer.

11.5 RISKS/BENEFITS ANALYSIS

Participants 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. Prophylactic levetiracetam will be administered to all participants with close monitoring in the event neurologic toxicity occurs. The enrollment profile and dose escalation rules will help to ensure that participants are not exposed to unacceptable risk as a safe dose level is determined.

Participants on this study may directly benefit from participation. It is anticipated that this study will provide scientific information relevant to tumor immunotherapy. Participation of adult

subjects, including those who may be become unable to consent also involves greater than minimal risk but presents the prospect for direct benefit.

11.6 Consent and Assent Process and Documentation

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent and consent for telehealth visits, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policies, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant as described below.

Manual (non-electronic) signature on electronic document

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the subject will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at:

https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

11.6.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in section **11.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in section **11.6**.

11.6.2 Consent Process for Minors

Consent will be obtained from parent(s)/guardians of minor children as described in section **11.6**.

Where deemed appropriate by the clinician and the child's parent(s) or guardian, the child will be included in all discussions about the trial and age-appropriate language will be used to describe the procedures and tests involved in this study, along with the risks, discomforts and benefits of participation. The assent process will take place in conjunction with consent; therefore, in person and remote assent are permitted under the same circumstances as in person and remote consent. Verbal assent will be obtained as appropriate for children ages > 7. Children under the age of 18, but who are age 12 or older will be asked to sign an age appropriate assent form if available in the participant's language; otherwise, the appropriate line on the adult consent document (in the minor participant's language) may be used. Children under the age of 7 years will not be required to provide assent as they typically do not have the cognitive ability to fully understand the nature of research. The consent/assent process will be documented in the child's medical record, including the assessment of the child's ability to provide assent (verbal) as applicable.

11.6.3 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) will require that consent be obtained from the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained. We request waiver of informed consent for those individuals who become lost to follow up or who have been taken off study prior to reaching the age of majority.

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

- The research involves no more than minimal risk to the subjects.
    - Analysis of samples and data from this study involves no additional risks to subjects.
- The waiver or alteration will not adversely affect the rights and welfare of the subjects.
    - Retention of these samples or data does not affect the welfare of subjects.
- The research could not practicably be carried out without the waiver or alteration.
    - 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.
- Whenever appropriate, the subjects will be provided with additional pertinent information after participation.
    - We only request a waiver of consent for those subjects who have been lost to follow-up or who have been taken off study prior to reaching the age of majority.

11.6.4 Request for Waiver of Consent for Screening Activities

Prior to the subject signing the consent for this study pre-screening activities listed in section **2.3.1** may be performed.

We request a waiver of consent for these activities as they involve only minimal risk to the subjects. A waiver will not adversely affect the rights and welfare of the subjects given that the activities are only intended to determine suitability for screening for participation in research protocols. These activities could not practicably be carried out without the wavier as central recruiting services, utilized in the NIH Clinical Center, perform pre-screening activities for multiple studies and obtaining consent for each one is beyond their resources. The subjects will be provided with additional pertinent information after participation as they will be informed whether or not they are eligible to sign a consent for additional screening.

12 REGULATORY AND OPERATIONAL CONSIDERATIONS

12.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) or Investigational Device Exemption (IDE) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

12.2 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe the site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Council for Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

12.3 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

12.4 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at the CCR NCI. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the CCR NCI will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the NIH Clinical Center.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose

information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

13 PHARMACEUTICAL INFORMATION

13.1 CD19/CD22-CAR T CELLS

The cells will be manufactured in the NIH DTM CCE according to cGMP practices.

Fresh or cryopreserved peripheral blood mononuclear cells (PBMC) (depending on the timing of apheresis relative to cell culture, patient condition and scheduling availability) will be enriched for T cells using a CD4/CD8 immunomagnetic bead enrichment in DTM CCE. They will then be activated by co-culture with immunomagnetic particles expressing anti-CD3 and anti-CD28 mAb. One day following activation, replication incompetent lentiviral vector particles containing the CD19/CD22.BB.z construct will be added to the culture for transduction. Cells will be incubated for approximately 9 days with IL-2 then harvested and administered fresh or frozen for subsequent infusion. Cells will be required to meet standard release criteria including transduction efficiency ≥10%, T cell content ≥70%, sterility and minimum levels of LPS as well as no evidence for replication competent lentivirus. All procedures will take place using good manufacturing process guidelines.

13.1.1 How Supplied

CD19/CD22-CAR T cells will be generated from autologous PBMC. The replication incompetent, bi-specific CD19/CD22-CAR lentiviral vector is manufactured by Lentigen Technology Incorporated.

Cells meeting the release criteria will be made available fresh or frozen on the day of cell infusion, along with a complete COA.

13.1.2 Stability

This cell product expires 4 hours after completion of the cell harvest.

See section **3.3** for additional information.

13.1.3 Toxicities

See section **1.2.2.2**

The following events are considered expected SAEs:

- Grade 1 and 2 CRS events
- Grade 3 CRS events that do not resolve to Grade 2 in 72 hours (about 3 days)
- Inpatient admission/hospitalization for evaluation and treatment of CRS.
- ICU transfer for evaluation and treatment of CRS

13.2 FLUDARABINE

13.2.1 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.

13.2.2 How Supplied

Fludarabine will be purchased by the NIH Clinical Center Pharmacy from commercial sources. Fludarabine Phosphate is supplied in a 25 mg/I mL vial as an intravenous solution in a 50 mg vial or as a 50 mg vial as a fludarabine phosphate powder in the form of a white, lyophilized solid cake.

13.2.3 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 2-8^{0}C; 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, ribonucleotide reductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.

13.2.4 Storage

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

13.2.5 Administration

Fludarabine concentrate is further diluted to a concentration between 0.04 and 1 mg/ml and 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 or 30 mg/m^2/dose). Please see section **3.9.2** for daily dose modifications in light if fludarabine national drug shortages. Cumulative dosing will not change thus toxicity profile should not change.

13.2.6 Toxicities

At doses of 25 mg/m^2/day or 30 mg/m^2/dose for 3 days (Please see section **3.9.2** for daily dose modifications in light if fludarabine national drug shortages. Cumulative dosing will not change thus toxicity profile should not change), 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.

13.3 CYCLOPHOSPHAMIDE

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

13.3.1 Description

Cyclophosphamide is a nitrogen mustard-derivative alkylating agent. Following conversion to active metabolites in the liver, cyclophosphamide functions as an alkylating 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.

13.3.2 How Supplied

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

13.3.3 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 2-8^{0}C.

13.3.4 Administration

It will be diluted in an appropriate solution and infused over one hour. The dose will be based on the participant's body weight, as specified in section **3.9.2.2**.

13.3.5 Toxicities

Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea and vomiting, rash and alopecia occur, especially after high-dose cyclophosphamide; fever, bone marrow failure, 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, cyclophosphamide 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.

Also, the alcohol content of cyclophosphamide injection should be taken into account when given to pediatric participants.

13.4 PENTOSTATIN

Pentostatin is an inhibitor of the enzyme adenosine deaminase, leading to cytotoxicity through disruption of DNA synthesis. Refer to FDA-approved package insert for complete product information. Please refer to package insert for more details.

13.4.1 Source

Commercially available and will be purchased by the NIH CC Pharmacy Department.

13.4.2 Administration

Recipients will receive hydration with pentostatin as specified in section **3.9.2**.

13.4.3 Toxicities

Because pentostatin is rarely associated with neurotoxicity (seizures, ataxia, encephalitis), special attention should be paid towards evaluating central nervous system toxicity.

13.5 SUPPORTIVE THERAPIES

13.5.1 Mesna

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

13.5.1.1 Description

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

13.5.1.2 Storage

Intact ampoules are stored at room temperature.

13.5.1.3 Stability

Diluted solutions for IV administration (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.

Mesna IV solution may also be administered orally. To decrease sulfur odor, parenteral solution should be diluted 1:1 to 1:10 in water, carbonated cola drinks, fruit juices (grape, apple, tomato,

orange), or plain or chocolate milk. Mesna IV solution is stable for at least 9 days undiluted in polypropylene syringes and stored at room temperature. Once diluted for oral administration, mesna injection is stable for 24 hours at 5 degrees Celsius.

13.5.1.4 Administration

For IV administration, mesna IV solution should bediluted to concentrations less than or equal to 20 mg mesna/ml fluid in D5W or normal saline and to be administered intravenously either as a continuous infusion or as an IV bolus.

For oral administration, mesna IV solution should be diluted in water, carbonated cola drinks, fruit juice, or plain or chocolate milk prior to ingestion to improve palatability. If a patient vomits within 2 hours of an oral mesna dose, the dose should be repeated, or the patient should receive IV mesna instead.

13.5.1.5 Toxicities

Toxicities include nausea, vomiting and diarrhea.

13.5.2 levetiracetam (Keppra)

(Please refer to the FDA-approved package insert for complete product information)

13.5.2.1 Description

Levetiracetam will be obtained commercially by the Pharmacy Department. Levetiracetam is an antiepileptic drug available as 250 mg (blue), 500 mg (yellow), 750 mg (orange), and 1000 mg (white) tablets and as a clear, colorless, grape-flavored liquid (100 mg/mL) for oral administration.

The chemical name of levetiracetam, a single enantiomer, is (S)-□□2-oxopyrrolidin-1-yl)butanamide, its molecular formula is $C_8H_{14}N_2O_2$ and its molecular weight is 170.21.

The precise mechanism(s) by which levetiracetam exerts its antiepileptic effect is unknown.

13.5.2.2 Storage

Store at 25°C (77°F); excursions permitted to 15-30°C (59-86°F).

13.5.2.3 Administration

See package insert for administration precautions

13.5.2.4 Toxicities

The most common toxicities include somnolence, accidental injury, hostility, nervousness and asthenia. Vomiting and anorexia have also been reported.

13.5.3 Acetaminophen (Tylenol)

Will be given as a pre-medication. This agent will be provided by the Pharmacy Department of the participating site. Please refer to the package insert for complete pharmaceutical information on this product.

13.5.4 Diphenhydramine (Benadryl)

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

13.5.5 Intrathecal Chemotherapy

The following agents will be provided by the Pharmacy Department of the participating site. Please refer to the package inserts for complete pharmaceutical information on these products.

13.5.5.1 Hydrocortisone (Cortef, Solu-cortef) NCS# 010483

13.5.5.1.1 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.

13.5.5.1.2 Toxicity

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

13.5.5.1.3 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.

13.5.5.1.4 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**)

13.5.5.1.5 Supplier

Commercially Available.

13.5.5.2 Cytarabine (cytosine arabinoside, AraC, Cytosar) NSC# 063878

13.5.5.2.1 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.

13.5.5.2.2 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.

13.5.5.2.3 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. 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.

13.5.5.2.4 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.

13.5.5.2.5 Supplier

Commercially available. See package insert for further information.

13.5.5.3 Methotrexate

13.5.5.3.1 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.

13.5.5.3.2 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 leukoencephalopathy.

13.5.5.3.3 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.

13.5.5.3.4 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.

13.5.5.3.5 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.

13.5.5.3.6 Supplier

Commercially available. See package insert for further information.

13.5.6 Allopurinol

Allopurinol will be obtained by the Pharmacy Department of the participating site. 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 p.o. 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.

13.5.7 Tocilizumab (ACTEMRA)

Will be given per **Appendix D**. This agent will be provided by the Pharmacy Department. Please refer to the package insert for complete pharmaceutical information on this product.

13.5.8 Siltuximab (Sylvant)

Will be given per **Appendix D**. this agent will be provided by the Pharmacy Department. Please refer to the package insert for complete pharmaceutical information on this product.

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15 APPENDICES

15.1 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

15.2 APPENDIX B: STUDY CALENDARS

Subjects will have clinical evaluations completed per the following guidelines with the exception noted below:

Exception #1

- If during the first restaging evaluation, the subject experiences no response (i.e., progressive disease) and no ongoing toxicity then no further clinical evaluations or follow-up is required. Participants will be followed for gene-therapy follow-up only (see section **5.2**).

Note:

- Once a participant develops PD or goes on to other therapies (including, but not limited to hematopoietic stem cell transplant), participants will be followed for Gene therapy follow-up **ONLY** (see section **5.2**). It will include evaluations for persistence of Anti-CD19/22 CAR T-cells in blood samples, detection of RCL and long-term follow-up for toxicity assessment. This follow-up will be performed as dictated in NIH protocol 15-C-0028.

15.2.1 Appendix B 1: General Clinical Calendar

PROCEDURE	Pre-Cell Infusion		Lymphodepleting Chemotherapy				CAR Infusion	Post CAR (Days 1-28)					Post CAR Follow-up (Month 2 and beyond)					
	Screening[A]	Baseline[B]	Day -5	Day -4	Day -3	Day -2	Day 0	Day 1 – Day 7	Day 8 – Day 14	Day 15 – Day 21	Day 22- Day 28	Day 28 (± 4 days)[BB]	Month 2 (± 2 weeks)[S]	Months 3 (± 2 weeks)	Month 6 (± 2 months)	Month 12 (± 3 months)	Month 18 and 24 (± 6 months)	Yearly (± 6 months)
History and PE	X	X					X	X[G]	X[G]	X[G]	X[G]	X	X	X	X	X	X	X
Vital Signs	X	X					X[C]	X[G]	X[G]	X[G]	X[G]	X	X	X	X	X	X	X
Performance Status	X	X					X					X		X				
Height	X	X											X					
Weight	X	X					X					X						
ECG	X																	
ECHO or cardiac MRI	X																	
Pulse oximetry and/or Pulmonary Function Testing	X[D]																	
CD19-CD22 Expression	X																	
HIV, HbsAg, Anti-HCV antibodies	X																	
DTM Viral Markers (Transplant donor) and Rapid Plasma Reagin (RPR) required by DTM	X[V]																	

PROCEDURE	Pre-Cell Infusion		Lymphodepleting Chemotherapy				CAR Infusion	Post CAR (Days 1-28)					Post CAR Follow-up (Month 2 and beyond)					
	Screening[A]	Baseline[B]	Day -5	Day -4	Day -3	Day -2	Day 0	Day 1 – Day 7	Day 8 – Day 14	Day 15 – Day 21	Day 22- Day 28	Day 28 (± 4 days)[BB]	Month 2 (± 2 weeks)[S]	Months 3 (± 2 weeks)	Month 6 (± 2 months)	Month 12 (± 3 months)	Month 18 and 24 (± 6 months)	Yearly (± 6 months)
β-HCG or urine pregnancy test[E]	X	X[F]																
PT/PTT	X	X[H]						X[O]	X[O]	X[O]		X						
D-dimer, Fibrinogen		X[H]						X[O]	X[O]	X[O]		X						
Complement studies (C3/C4 and Total complement or CH50)		X[H]							X[P]	X[P]		X						
Soluble IL2R		X[H]							X[P]	X[P]		X						
Chemistries[I]	X	X					X[X]	X[G]	X[G]	X[G]	X[G]	X	X	X	X	X	X	X
Immunoglobulins	X																	
CBC w/diff & platelets	X	X					X[X]	X[G]	X[G]	X[G]	X[G]	X	X	X	X	X	X	X
TBNK or PID panel[AA]	X	X[H]							X[Y]	X[Y]		X[Y]	X[Z]	X[Z]	X[Z]	X[Z]		
Urinalysis	X	X				X	X					X						
Optional hematology studies								X[Q]	X[Q]	X[Q]								
Imaging Studies	X[J]											X[J]		X[J]	X[J]	X[J]	X[J]	X[J]
Bone marrow to include flow cytometry (bone	X											X[K]		X	X	X	X	X

PROCEDURE	Pre-Cell Infusion		Lymphodepleting Chemotherapy				CAR Infusion	Post CAR (Days 1-28)					Post CAR Follow-up (Month 2 and beyond)					
	Screening[A]	Baseline[B]	Day -5	Day -4	Day -3	Day -2	Day 0	Day 1 – Day 7	Day 8 – Day 14	Day 15 – Day 21	Day 22- Day 28	Day 28 (± 4 days)[BB]	Month 2 (± 2 weeks)[S]	Months 3 (± 2 weeks)	Month 6 (± 2 months)	Month 12 (± 3 months)	Month 18 and 24 (± 6 months)	Yearly (± 6 months)
marrow biopsy as feasible)																		
Lumbar puncture for cell count, cytospin and/or flow cytometry (as feasible)	X[T]											X[L]		X[L]	X[L]	X[L]	X[L]	X[L]
Peripheral blood flow cytometry	X	X[H]						X[O]	X[O]	X[O]		X		X	X	X	X	X
Research sampling[M]		X					X	X	X	X	X	X		X	X	X	X	X
Apheresis		X[U]																
Fludarabine			X[W]	X	X	X												
Cyclophosphamide					X[W]	X												
Pentostatin			X[W, CC]	X[CC]														
Mesna					X[W]	X												
Anti-CD19/anti-CD22 CAR transduced cells							X											
Levetiracetam (Keppra)							X[N]											

PROCEDURE	Pre-Cell Infusion		Lymphodepleting Chemotherapy				CAR Infusion	Post CAR (Days 1-28)					Post CAR Follow-up (Month 2 and beyond)					
	Screening[A]	Baseline[B]	Day -5	Day -4	Day -3	Day -2	Day 0	Day 1 – Day 7	Day 8 – Day 14	Day 15 – Day 21	Day 22- Day 28	Day 28 (± 4 days)[BB]	Month 2 (± 2 weeks)[S]	Months 3 (± 2 weeks)	Month 6 (± 2 months)	Month 12 (± 3 months)	Month 18 and 24 (± 6 months)	Yearly (± 6 months)
Adverse Events (Toxicity assessments)							X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications							X	X	X	X	X	X						

A. Within 4 weeks of treatment consent unless otherwise specified
B. Within 3 days of beginning preparative regimen unless otherwise specified
C. As per section **3.10.5**, and then routinely (every 4 hours) unless otherwise clinically indicated
D. If clinically indicated
E. All IOCBP
F. All IOCBP, within 3 days of beginning lymphodepleting chemotherapy
G. At least twice weekly through Day 28
H. Within 14 days of treatment
I. Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total (Ca), Magnesium total (Mg), Inorganic phosphorus, Alkaline phosphatase, ALT/GPT, AST/GOT, Bilirubin total and direct, LDH, CRP, uric acid, ferritin, triglycerides, total protein.
J. Scans: Appropriate imaging methods of any sites relevant to the subject's disease (e.g. CT Scan, PET/CT, MRI)
K. In participants where there is no evidence for cytokine release syndrome or CAR-T cell expansion and early confirmation of disease progression would be clinically relevant, an early bone marrow evaluation may be performed Day +21 (± 4 days).
L. In participants with a history of CNS involvement, an LP for cell count, cytology, ± flow cytometry will be done. See section **4.1.3.1**. Further, intrathecal therapy will NOT be administered in any participant who develops neurologic toxicity until at least 2 weeks after complete resolution (exception: intrathecal hydrocortisone may be administered as indicated for treatment of severe neurotoxicity or intrathecal chemotherapy may be administered for progressive leukemia.
M. See **Appendix I**

N. Participants will start prior to CAR T-cell infusion, per PI discretion. Alternative seizure prophylaxis may be used as clinically indicated. This may be stopped as clinically indicated post CAR T-cell therapy
O. At least once a week through Day 28
P. On Day 10 and Day 21 (both ±4 days)
Q. Optional hematology studies to be performed weekly on Days 7-21 (± 3 days) only as feasible in those with evidence of clinically significant DIC. See section **5.1**.
R. Participants will be followed clinically until toxicity resolves to ≤ grade 1 or baseline regardless of disease status.
S. The 2-month follow-up will be done as feasible.
T. Within 30 days prior to enrollment onto treatment protocol
U. To be performed any time prior to day -7
V. Within 4 weeks of apheresis If apheresis has already been performed on another protocol, this testing will not be required.
W. Intensified Lymphodepletion only
X. Within 1 day of cell infusion
Y. Day 14, 21, 28 (± 4 days)
Z. If feasible: Monthly (± 2 weeks) for 2 months, then q3-6 months (± 1 month) until 1yr or CAR loss.
AA. Peripheral CD3 count may be calculated using flow cytometry results
BB. Studies performed within the last 4 days do not need to be repeated
CC. Pentostatin will be used if fludarabine is not available due to a national fludarabine drug shortage.

15.2.2 Appendix B 2: Neurotoxicity Assessments Calendar

Assessment	Baseline[A]	Start of CRS (±2 days)	Peak of CRS[F]	Day +10 (± 4 days)	End of CRS (±1 week)	Day +28 (± 7 days)	3 months[B] (± 1 month)
MRI/CT Brain[C]	X						
NeuroSymptom Checklist[D,G]	X			X		X	X
Neurocognitive Exam Battery[D, E, G]	X					X	X
Neurology Exam[D,]	X					X	
Handwriting Sample[C]	X	X	X		X		
ICE score (≥ 12 years old)	X			X		X	
CAPD score (< 12 years old)	X			X		X	
ICANS grade	X			X		X	

A. Within 30 days prior to starting preparative regimen
B. Only applicable in participants who remain on treatment and return for a 3-month post-infusion follow-up evaluation.
C. As clinically indicated.
D. See **Appendix G**
E. Brief battery of cognitive test evaluating memory, attention, processing speed, and executive functions. This battery of tests will only be applicable to those participants/parents who speak English or Spanish.
F. As feasible.
G. Neurocognitive assessments and neurosymptom checklists will only be performed in participants with isolated CNS disease.

15.3 APPENDIX C: GRADING OF 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
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.

15.4 APPENDIX D: GUIDELINES FOR GRADING AND MANAGEMENT OF SUSPECTED CYTOKINE RELEASE SYNDROME

Cytokine Release Syndrome (CRS): Administration of CAR T cell immunotherapy is often complicated by significant acute toxicities in the first 1-3 weeks after T cell infusion. In many cases, clinical toxicities correlate with elevated inflammatory serum cytokine levels. The signs and symptoms most often experienced by participants receiving infusions of CAR T cells include, but are not limited to, tumor lysis syndrome, fever, fatigue, hypotension, tachycardia, acute renal failure, and neurological toxicities such as aphasia, ataxia, headache, somnolence, and coma. Fever is usually the first toxicity to occur.

The protocol PI or their designees will be notified with the onset of CRS and with any change in grade in CRS.

CRS Grading: Grading will be based on the 2019 ASTCT CRS Consensus Guidelines (**Table 2:** CRS Grading as per ASTCT Consensus Guidelines with Generalized Management Approach)(54, 114); with more specific guidelines provided in **Table 3**.

Table 2: CRS Grading as per ASTCT Consensus Guidelines with Generalized Management Approach

CRS parameter	Grade 1	Grade 2	Grade 3	Grade 4^
Fever	≥ 38	≥ 38	≥ 38	≥ 38
With EITHER				
Hypotension	None	Not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
And/or				
Hypoxia	None	Requiring low-flow nasal cannula‡ or blow-by	Requiring high-flow nasal cannula‡, facemask, nonrebreather mask, or Venturi mask	Requiring positive pressure (eg, CPAP, BiPAP, intubation and mechanical ventilation)

⁎Fever is defined as temperature ≥38°C not attributable to any other cause. In participants who have CRS then receive antipyretics or anticytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.

†CRS grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a participant with temperature of 39.5°C, hypotension requiring 1 vasopressor, and hypoxia requiring low-flow nasal cannula is classified as grade 3 CRS. ‡Low-flow nasal cannula is defined as oxygen delivered at ≤6 L/minute. Low flow also includes blow-by oxygen delivery, sometimes used in pediatrics. High-flow nasal cannula is defined as oxygen delivered at >6 L/minute. ^Intubation may be indicated in participants who have a degree of neurotoxicity where there is concern for their ability to maintain a patent airway. This may occur either in the setting of CRS or after CRS has resolved. The decision for intubation should not be captured as a grade 4 CRS when the other criteria for such are not met. Intubation of a participant without hypoxia for the possible neurologic compromise of a patent airway alone or for a procedure is not, by definition, grade 4 CRS.

**The definition of "High dose" vasopressors requires ≥ 3 hours of any of the following: 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: vasopressin + norepinephrine equivalent of ≥ 10 mcg/kg/min; and if on combination vasopressors (excluding vasopressin) ≥ norepinephrine equivalent (cumulative) ≥ 20 mcg/kg/min

Treatment Guidelines: These guidelines aim to **prevent grade 3 CRS** in participants treated with CD19/22CART with exception of allowance of low-dose pressor support, which may be used to prevent excessive fluid bolus administration and volume overload, which can exacerbate pulmonary edema in the setting of capillary leak.

These guidelines may require modification based on each participant's specific clinical circumstances. Failure to follow these guidelines exactly is not considered a protocol deviation. Treatment will be based on optimal medical management and does not necessarily correlate with CRS grade.

The impact of tocilizumab, siltuximab, and/or steroids or other immunosuppressive agents have not been studied in this protocol, utilizing this CAR construct for this participant population. Thus, the indication for use of tocilizumab, siltuxuimab, or other interventions for CRS will be made by the PI or their designee after discussion with the clinical care team and assessment of participant status.

As of Amendment 5/08/2023, we will prioritize using siltuximab as first line CRS therapy for participants who require treatment for CRS see ***Figure 9****.*

Table 3: Guidelines for Monitoring and Treatment of CRS

	Symptom related to CRS	Suggested Intervention
General Management		• Daily participant weight assessment
Vital Sign Monitoring		• Vital signs (temperature, blood pressure, heart rate, respiratory rate, pulse oximetry) should be checked a minimum of every 4 hours during CRS • Strict I/Os q shift
Laboratory Monitoring		• With first onset of fevers, initiate twice daily: CBC, acute care, mineral and hepatic panel, and once daily: PT/PTT/fibrinogen/d-dimer, ferritin, LDH, and CRP and others as clinically indicated (e.g., CPK) or per protocol
Infectious Disease Monitoring		• Consider an infectious disease consult for all participants with a history of multi-drug resistant organisms or complex prior infectious disease history (prior to CAR infusion) • Expectant management for fever & neutropenia with antibiotic coverage • Imaging as clinically indicated
Electrolyte Abnormalities		• Allopurinol for prevention of hyperuricemia associated with tumor lysis (as clinically indicated) • Electrolyte abnormalities are common, particularly hypoMg/Ca/Phos, and should be recognized and treated

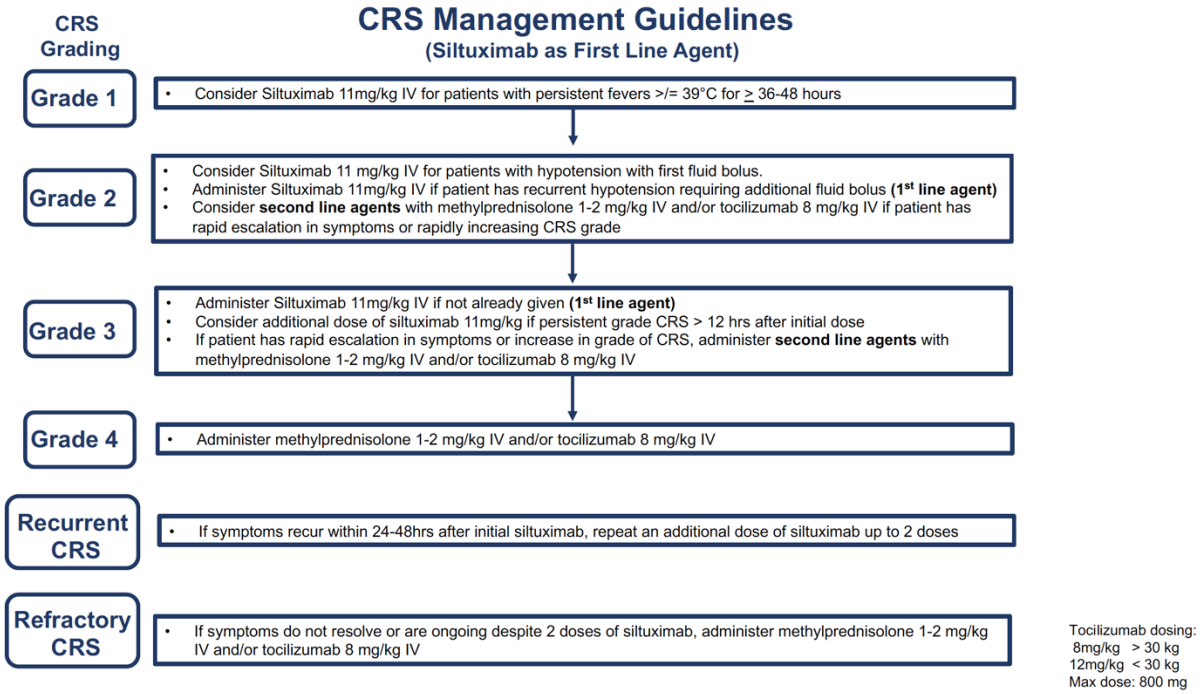
Fever management	Fever of ≥ 38 °C	• Acetaminophen PO/IV every 4-6 hours, supportive care (avoid NSAIDS and steroids)
	Persistent fever of ≥ 39°C for < 36 hours that is unresponsive to acetaminophen	• Optimize supportive care • See **Figure 9**
	Persistent fevers ≥ 39°C for ≥ 36-48 hours	• See **Figure 9**
Hypotension	Initial hypotension	• 10-20 cc/kg or 500-1000 cc Fluid bolus, target Hgb > 8.0 g/dL, • Echocardiogram as feasible and as clinically indicated • **Notify the ICU**
	Persistent/recurrent hypotension after initial fluid boluses (within 6 hours)	• Consider initiation of low-dose pressors for persistent hypotension after 1-2 fluid boluses. • See **Figure 9** • Fluid balance must be closely monitored to avoid fluid overload while optimizing circulatory status; aggressive fluid boluses may precipitate respiratory compromise
	Worsening hypotension	• Initiation of higher dose pressors or addition of a second pressor for hypotension (excluding vasopressin) • See **Figure 9**
Oxygen Requirement	Requirements of any oxygen supplementation	• CXR (additional imaging as clinically indicated) • Assess fluid status
	Signs of respiratory distress	• CXR (additional imaging as clinically indicated) • **Notify the ICU** • See **Figure 9**
	Increasing respiratory support with concern for impending intubation	• STAT CXR (CT as clinically indicated) • **Notify the ICU** • See **Figure 9**
Consider ICU Transfer		• Persistent hypotension with initiation of a second fluid bolus • Persistent tachycardia with a heart rate higher than 125 beats per minutes on at least 2 occasions separated by 4 hours (for ≥ age 16 years) or > 150 beats per minute for age < 16 years • Ejection fraction abnormalities (≥grade 3) • Worsening respiratory status • Neurotoxicity necessitating ICU level care (e.g., 1:1 nursing, seizures, frequent neurological checks)

Refractory CRS	Refractory or ongoing symptoms 24-48 hours after 2 doses of Siltuximab	See **Figure 9**
Recurrent CRS	Recurrent symptoms within 24-48hrs after initial siltuximab	• See **Figure 9**
DIC	Laboratory abnormalities, petechiae, nose bleeds, hematuria	• Cryoprecipitate to maintain Fibrinogen ≥ 150 units • Platelet transfusions to maintain platelet count ≥ 50,000/uL (or higher if suspected acquired platelet function defect) • PRBC transfusion to maintain hematocrit ≥ 25 • FFP to target PTT < 40 (as feasible) • Vitamin K to target INR < 1.3
HLH/MAS	Symptomatic*: presence of Ferritin > 10,000 mcg/L and at least two of the following criteria: LFTs ≥ grade 3, Creatinine ≥ grade 3, Pulmonary edema > grade 3, presence of hemophagocytosis	• Supportive care, optimize medical management. • Consider Methylprednisolone 1-2 mg/kg IV/PO up to every 6-12 hours with continued symptoms (First dose 2 mg/kg). Wean with clinical improvement. • Consider HLH dosing of dexamethasone 10 mg/m2/day for starting dose (or methylprednisolone equivalents for older subjects) Consider Anakinra (dosing based on rheumatology consult) but may consider starting at 5mg/kg/day divided BID to QID. • Rheumatology consult
	Asymptomatic: laboratory-based findings alone without clinical manifestations	• Supportive care, optimize medical management • Consider Anakinra (dosing based on rheumatology consult).
Neurotoxicity	Somnolence, seizure, blurry vision, hallucinations	• Neuro consult • See separate guidelines listed below

**HLH will be suspected by the presence of Ferritin > 100,000 mcg/L and at least one of the following criteria: LFTs ≥ grade 3, Creatinine ≥ grade 3, Pulmonary edema > grade 3, presence of hemophagocytosis* (115).

Medication Maximum Dosing:
**Steroid dosing: For participants above 70 kg or with obesity, consider maximum daily steroid dose no greater than 150 mg methylprednisolone equivalents (e.g., 30 mg dexamethasone/day).
- Tocilizumab: Maximum dose of 800 mg/dose

Figure 9


CRS Grading
CRS Management Guidelines
(Siltuximab as First Line Agent)
Grade 1
• Consider Siltuximab 11mg/kg IV for patients with persistent fevers >/= 39°C for ≥ 36-48 hours
Grade 2
• Consider Siltuximab 11 mg/kg IV for patients with hypotension with first fluid bolus.
• Administer Siltuximab 11mg/kg IV if patient has recurrent hypotension requiring additional fluid bolus (1st line agent)
• Consider second line agents with methylprednisolone 1-2 mg/kg IV and/or tocilizumab 8 mg/kg IV if patient has rapid escalation in symptoms or rapidly increasing CRS grade
Grade 3
• Administer Siltuximab 11mg/kg IV if not already given (1st line agent)
• Consider additional dose of siltuximab 11mg/kg if persistent grade CRS > 12 hrs after initial dose
• If patient has rapid escalation in symptoms or increase in grade of CRS, administer second line agents with methylprednisolone 1-2 mg/kg IV and/or tocilizumab 8 mg/kg IV
Grade 4
• Administer methylprednisolone 1-2 mg/kg IV and/or tocilizumab 8 mg/kg IV
Recurrent CRS
• If symptoms recur within 24-48hrs after initial siltuximab, repeat an additional dose of siltuximab up to 2 doses
Refractory CRS
• If symptoms do not resolve or are ongoing despite 2 doses of siltuximab, administer methylprednisolone 1-2 mg/kg IV and/or tocilizumab 8 mg/kg IV
Tocilizumab dosing:
8mg/kg > 30 kg
12mg/kg < 30 kg
Max dose: 800 mg

15.5 APPENDIX E: CALCULATION OF WEIGHT FOR CELL DOSE CALCULATION IN MORBIDLY OBESE PARTICIPANTS

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

1. **Definition** Obesity is defined as a BMI > 30. For the purposes of this protocol, dose adjustments using practical body weight will be considered for those who have class 2 obesity or a BMI >35. Dose cap will be implemented at 100kg, even if participants do not meet class 2 obesity definitions.

   BMI = wgt (kg) / [hgt (M)]2

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

Ideal body weight (IBW)	**Definitions**
IBW (adult male, age >20)	50 kg + 2.3 kg per inch over 5 feet
IBW (adult female), age >20)	45.5 kg + 2.3 kg per inch over 5 feet
IBW (pediatrics, age 2-20)	50th percentile weight based on CDC growth curves

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" that may be used in calculating the targeted cell dose for participants who have a BMI >35.

4. **Example**:

   Participant's actual weight = 120 kg.

   Participant' s actual height 173 cm = 69 in

   BMI = 40

   IBW formula = 50 + 2.3(9) = 70.7 kg

   Midway point between 70.0 and 120 = 95 kg.

   The weight we would use in targeting cell dose is 95 kg.

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

2 Formula

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

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

15.6 APPENDIX F: RESPONSE CRITERIA

For the purposes of this study, participants should be re-evaluated for response as outlined in section **6.3**.

15.6.1 Appendix F 1: Response for Lymphoma

In addition to a baseline scan, confirmatory scans should also be obtained at least 4 weeks following initial documentation of objective response.

Response Criteria Lymphoma (Adapted from: Cheson BD, et al., Recommendations for Initial Evaluation, Staging, and Response Assessment of Hodgkin and Non-Hodgkin Lyphoma. *J Clin Oncol 32:3059-3067.*

Table 3. Revised Criteria for Response Assessment

Response and Site	PET-CT–Based Response	CT-Based Response
Complete	Complete metabolic response	Complete radiologic response (all of the following)
Lymph nodes and extralymphatic sites	Score 1, 2, or 3* with or without a residual mass on 5PS†	Target nodes/nodal masses must regress to ≤ 1.5 cm in LDi
	It is recognized that in Waldeyer's ring or extranodal sites with high physiologic uptake or with activation within spleen or marrow (eg, with chemotherapy or myeloid colony-stimulating factors), uptake may be greater than normal mediastinum and/or liver. In this circumstance, complete metabolic response may be inferred if uptake at sites of initial involvement is no greater than surrounding normal tissue even if the tissue has high physiologic uptake	No extralymphatic sites of disease
Nonmeasured lesion	Not applicable	Absent
Organ enlargement	Not applicable	Regress to normal
New lesions	None	None
Bone marrow	No evidence of FDG-avid disease in marrow	Normal by morphology; if indeterminate, IHC negative
Partial	Partial metabolic response	Partial remission (all of the following)
Lymph nodes and extralymphatic sites	Score 4 or 5† with reduced uptake compared with baseline and residual mass(es) of any size	≥ 50% decrease in SPD of up to 6 target measurable nodes and extranodal sites
	At interim, these findings suggest responding disease	When a lesion is too small to measure on CT, assign 5 mm × 5 mm as the default value
	At end of treatment, these findings indicate residual disease	When no longer visible, 0 × 0 mm
		For a node > 5 mm × 5 mm, but smaller than normal, use actual measurement for calculation
Nonmeasured lesions	Not applicable	Absent/normal, regressed, but no increase
Organ enlargement	Not applicable	Spleen must have regressed by > 50% in length beyond normal
New lesions	None	None
Bone marrow	Residual uptake higher than uptake in normal marrow but reduced compared with baseline (diffuse uptake compatible with reactive changes from chemotherapy allowed). If there are persistent focal changes in the marrow in the context of a nodal response, consideration should be given to further evaluation with MRI or biopsy or an interval scan	Not applicable
No response or stable disease	No metabolic response	Stable disease
Target nodes/nodal masses, extranodal lesions	Score 4 or 5 with no significant change in FDG uptake from baseline at interim or end of treatment	< 50% decrease from baseline in SPD of up to 6 dominant, measurable nodes and extranodal sites; no criteria for progressive disease are met
Nonmeasured lesions	Not applicable	No increase consistent with progression
Organ enlargement	Not applicable	No increase consistent with progression
New lesions	None	None
Bone marrow	No change from baseline	Not applicable
Progressive disease	Progressive metabolic disease	Progressive disease requires at least 1 of the following
Individual target nodes/nodal masses	Score 4 or 5 with an increase in intensity of uptake from baseline and/or	PPD progression:
Extranodal lesions	New FDG-avid foci consistent with lymphoma at interim or end-of-treatment assessment	An individual node/lesion must be abnormal with: LDi > 1.5 cm and Increase by ≥ 50% from PPD nadir and An increase in LDi or SDi from nadir 0.5 cm for lesions ≤ 2 cm 1.0 cm for lesions > 2 cm In the setting of splenomegaly, the splenic length must increase by > 50% of the extent of its prior increase beyond baseline (eg, a 15-cm spleen must increase to > 16 cm). If no prior splenomegaly, must increase by at least 2 cm from baseline New or recurrent splenomegaly
Nonmeasured lesions	None	New or clear progression of preexisting nonmeasured lesions

(continued on following page)

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. J Clin Oncol 2003;21:4642-4649

Bone Marrow Classification

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

- Complete Response (CR)
    1. M1 marrow, absence of peripheral blasts (morphologic), absence of extramedullary sites of disease, peripheral blood neutrophil count ≥ 1,000/μL and platelet count ≥ 100,000/μL. 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 (CR_{cyto}): In addition to above CR criteria, reversion to normal karyotype for those with previously detected cytogenetic abnormality.
    4. Molecular CR (CR_{molec}): In addition to above CRc criteria, normalization of previously detected molecular cytogenetic abnormality.
- 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.
- 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
- Stable Disease (SD)
    1. Does not meet the criteria for CR, PR, HA, or PD

- 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
- 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 participant'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 participant 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

15.7 APPENDIX G: GUIDELINES FOR EVALUATION, GRADING AND TREATMENT OF CAR-T RELATED NEUROTOXICITY

15.7.1 Appendix G 1: Encephalopathy assessment tools for grading of Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS)

To determine the ICANS grade, complete the appropriate encephalopathy assessment below:

1. Immune effector Cell-associated Encephalopathy (ICE)

**For use with adolescents and adults ≥12 years of age.*

Field	Suggested Assessment	Points
Orientation	Orientation to year, month, city, hospital	4 points
Naming	Name 3 objects (e.g., point to clock, pen, button)	3 points
Following Commands	E.g. show me 2 fingers or close your eyes and stick out your tongue	1 point
Writing	Ability to write a standard sentence (e.g. It is a sunny day)	1 point
Attention	Count backwards from 100 by 10	1 point

Score	ICANS Grade
10	No Impairment
7-9	Grade 1 ICANS
3-6	Grade 2 ICANS
0-2	Grade 3 ICANS
Participant unarousable and unable to perform ICE	Grade 4 ICANS

2. Cornell Assessment of Pediatric Delirium (CAPD)

**For use with children <12 years of age.*

Answer the following based on interaction with the child over the course of the shift	**Never 4**	**Rarely 3**	**Sometimes 2**	**Often 1**	**Always 0**
1. Does the child make eye contact with the caregiver?					
2. Are the child's actions purposeful?					
3. Is the child aware of his/her surroundings?					
4. Does the child communicate needs and wants?					
	Never 0	**Rarely 1**	**Sometimes 2**	**Often 3**	**Always 4**
5. Is the child restless?					
6. Is the child inconsolable?					
7. Is the child underactive – very little movement while awake?					
8. Does it take the child a long time to respond to interactions?					

ASTCT Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS) Consensus Grading

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE Score* (≥12 years)	7-9	3-6	0-2	Participant unarousable and unable to perform ICE
CAPD score (<12 years)	<9	<9	≥9	Unable to perform CAPD
Depressed level of consciousness†	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Participant is unarousable or requires vigorous or repetitive tactile stimuli to arouse. Stupor or coma.
Seizure (any age)	N/A	N/A	Any clinical seizure focal or generalized that resolves rapidly; or non-convulsive seizures on EEG that resolve with intervention.	Life-threatening prolonged seizure (>5 min); or repetitive clinical or electrical seizures without return to baseline in between.
Motor weakness‡ (any age)	N/A	N/A	N/A	Deep focal motor weakness such as hemiparesis or paraparesis.
Raised ICP/Cerebral Edema (any age)	N/A	N/A	Focal/local edema on neuroimaging§	Decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad; or signs of diffuse cerebral edema on neuroimaging.

Note: ICANS grade is determined by the most severe event (ICE or CAPD score, level of consciousness, seizure, motor findings, raised ICP/cerebral edema) not attributable to any other cause.
*A participant with an ICE score of 0 may be classified as grade 3 ICANS if awake with global aphasia, but a participant with an ICE score of 0 may be classified as grade 4 ICANS if unarousable.
†Depressed level of consciousness should be attributable to no other cause (i.e., no sedating medications).
‡Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0, but they do not influence ICANS grading.
§Intracranial hemorrhage with or without associated edema is not considered a neurotoxicity feature and is excluded from ICANS grading. It may be graded according to CTCAE v5.0.

Neurotoxicity Treatment Guidelines (suggested guidelines only)

Neurotoxicity Treatment*	
Grade 1:	- Supportive care
Grade 2:	- Neurology Consult - Vigilant supportive care - Consider brain imaging with MRI - Consider corticosteroids first (*e.g.*, dexamethasone 10 mg IV q6hrs, methylprednisolone 1mg/kg BID)
Grade 3:	- As above with Grade 2 - Consider ICU transfer - Consider EEG and/or CSF evaluations as clinically indicated - Monitor with continuous cardiac telemetry and pulse oximetry - Consider IT dexamethasone or hydrocortisone.
Grade 4:	- As above with Grade 3 - Consider corticosteroids (*e.g.*, methylprednisolone 1g/day x 3 doses, followed by a rapid taper consisting of 250 mg BID x 2 days, 125 mg BID x 2 days and then 60 mg BID x 2 days)
**CRS should be treated as per the standard guidelines for treatment of systemic CRS, which may include utilization of tocilizumab, siltuximab, and/or steroids. Guidelines for treatment of neurotoxicity is intended to augment treatment of underlying systemic CRS or for additional treatment of neurotoxicity that extends beyond other systemic manifestations of CRS. Please note that signs and symptoms of neurotoxicity may be delayed from onset of initial signs of CRS and vigilant care is needed to identify and optimize treatment of neurotoxicity.*	

15.8 APPENDIX H: NEUROLOGIC AND COGNITIVE TESTING TOOLS

15.8.1 Appendix H 1: Neuropsychological Test Battery (CD19/CD22-CAR)

Domains	Measure	Age range (yrs)	Time (min)
Pre-baseline practice session (<2 min each of the 6 tasks)		5.0 – adult	10
Baseline and Day 28 follow-up (±7 days) assessment:			
Processing Speed	Cogstate Detection Task	5.0 - adult	3
Attention	Cogstate Identification Task	5.0 - adult	3
Working Memory	Cogstate One-Back Task	5.0 - adult	4
Visual Learning/Memory	Cogstate One Card Learning	5.0 - adult	6
Executive Function	Cogstate Groton Maze Task	5.0 – adult	7
PSI	Wechsler SS and Cancellation		
Verbal Fluency	DKEFS FAS and McCarthy category	5.0 – adult	2
Neurologic Symptoms	Neuro-Symptom Checklist (parent/observer report)	all	(2)
Background Information	Background Information Form (parent/observer report or adult self-report)	all	(5)

• This full test battery (cognitive tests and Neuro-Symptom Checklist) listed above will be administered at baseline and Day 28 post-infusion (± 7 days) to subjects who have isolated CNS disease, and who speak English and Spanish. It also will be administered at 3-months post-infusion if the participant returns for a follow-up evaluation.

• The Neuro-Symptom Checklist (NSC) also will be administered to a parent/observer at Day 10 (± 4 days) and then again between day 28 post-infusion (± 7 days) to assess for late-developing or resolving neurotoxicity only in subjects with isolated CNS disease. The same parent/observer should complete the checklist at each required time point when possible. If a parent/observer is not available at baseline or follow-up evaluation, the adult participant can complete the checklist at these time points, but a parent/observer should complete it at the Day 10 (± 4 days) time point if at all possible. An interpreter can be used to administer the NSC to subjects who do not speak English if needed.

• We estimate that this test battery takes < 1 hour to administer. It will take about 45 minutes to complete at baseline, which includes a 10-minute practice session of the Cogstate tasks for all participants prior to the baseline testing, and approximately 30-35 minutes for subsequent assessments, which do not include the practice tests. The parent or other observer familiar with the parent will be asked to complete the Neuro-symptom checklist (1-2 minutes) and background form (5 minutes) while the participant is being tested but an adult participant can complete these forms if no parent/observer is available.

• Some measures may not be administered if the participant does not appear to understand the task or becomes frustrated, is extremely fatigued, is ill (e.g., nauseous), or has physical (e.g., motor, vision) difficulties. This will not be considered a deviation.

CogState Computerized Test Battery (when available)

CogState is an approximately 20-minute computerizeda battery for children and adults ages 4 - 90 years, which includes tasks tapping processing speed, attention and vigilance, learning and memory, working memory and executive functions (Maruff et al., 2009; Pietrzak et al., 2009). This measure was designed for repeated assessments over a short time period with virtually no practice effects in adults and children due to multiple forms (Falleti et al., 2006; Mollica et al., 2005). Age-based standard scores (mean = 100, SD 10) are computed for each task based on a normative sample of several hundred individuals. CogState has strong construct validity, minimal practice effects, and good stability. It was designed to be language independent in order to ensure the validity of the tests in a cross-cultural setting (e.g., Bangirana et al., 2009; Boivin et al., 2010; Cairney, Clough, Jaragba & Maruff, 2007; Dingwall & Cairney, 2010; Dingwall, Maruff, Frederisckon & Cairney, 2011; Lewis et al., 2010). Further, CogState has been used in numerous pediatric, as well as adult, oncology clinical trials.

Detection Task: This subtest of the CogState takes 2 minutes to administer and measures psychomotor function and processing speed. It uses a well-validated simple reaction time paradigm using playing card stimuli. In this task, the playing cards are all red and black jokers. The subject is asked to press the **Yes** key as soon as the card in the center of the screen flips over.

Identification Task: This task takes 2 minutes to administer. It is a measure of visual attention and uses a well-validated choice reaction time paradigm using playing card stimuli. In this task, the playing cards are all either red or black jokers. The subject is asked whether the card currently being presented in the center of the screen is red. This subject responds by pressing the **Yes** key when the joker card is red and **No** when it is black.

One Back Task: The One Back Memory task takes approximately 2 minutes to administer and is a measure of working memory. It uses a well-validated n-back paradigm using playing card stimuli. In this task, the playing cards are identical to those found in a deck of playing cards with the exception of the joker. The subject is asked whether the card currently being presented is the same as the one presented immediately previously. The subject responds by pressing the **Yes** or **No** key.

One Card Learning Task: This task takes 5 minutes to administer. The One Card Learning task is a measure of visual recognition memory and uses a well-validated pattern separation paradigm using playing card stimuli to measure hippocampal functioning. In this task, the playing cards are identical to those found in a deck of playing cards with the exception of the joker. The subject is asked whether the card currently being presented in the center of the screen was seen previously in this task. The subject responds by pressing the **Yes** or **No** key.

Continuous Paired Associate Learning Task: The Continuous Paired Associate Learning task is a measure of visual associate memory, which takes approximately 5-7 minutes to

administer. It uses a well-validated paired associate learning paradigm that assesses hippocampal-dependent learning and memory by asking the subject to learn the locations of a number of amoeba-like shapes on the computer screen. This task consists of a single amoeboid shape displayed in the center of the screen surrounded by a number of blue-filled circles. Beneath all but two of the blue-filled circles are amoeboid shapes, one of which matches the central display; the two remaining circles are distractors. In the exposure phase of the task all of the to-be-remembered pattern-location associations are presented on the computer screen simultaneously. After a five-second delay, a pattern is shown in the central location and this signals that the subject should touch the location in the periphery that contains the same pattern. This process continues until the participant has acknowledged all of the pattern-location associations. The learning phase begins with the same task display presented during the exposure phase except that now all of the peripheral locations are shown as filled circles. One of the patterns presented in the exposure phase is presented in the center location. With the presentation of this pattern, the subject is required to select the peripheral location where an identical pattern is hidden beneath the circle. This process continues until the correct location of each pattern is found. Finding the correct location for all patterns in the set is defined as a learning trial. There are six learning trials. The software records each move as an error or as a correct move.

Groton Maze Learning Test: The Groton Maze Learning task is a measure of problem solving and reasoning and takes approximately 5-7 minutes to administer. It uses a well-validated maze-learning paradigm. In this task, the subject is shown a 10 x 10 grid of boxes on a computer screen. A 28-step pathway is hidden among these 100 possible locations. Each box represents move locations, and the grid refers to the box array (i.e., 10 x 10). Subjects are required to find the hidden pathway guided by four search rules. These are: do not move diagonally, do not move more than one box (i.e., do not jump), do not move back on the pathway, and return to the last correct location after an error. At each step only the most recently selected box is shown. Feedback is given with visual and auditory cues (green check marks and red crosses) to indicate whether the selected box is correct or incorrect. The head of path, or the last correct location, flashes with a green check when two errors are made in succession (failing to return errors). There are 20 well-matched alternate pathways available. The software records each move as an error or a correct move.

*All CogState descriptions are provided courtesy of CogState

Neuro-symptom checklist

The Neuro-symptom checklist, which consists of neurologic symptoms previously observed in participants experiencing cytokine release syndrome, was developed specifically for studies assessing neurotoxicity of CAR T cell therapy. It assesses the severity (mild, moderate, severe) and duration (<24 hours, 24-48 hours, and >48 hours) of symptoms, including fever, visual and auditory hallucinations, responsiveness to commands, disorientation, depressed mood, pain, and fatigue. The caregiver of the participant is asked to complete the checklist based on their observations in the past two weeks; if the caregiver is not available, the adult participant can complete the checklist. If possible, the same person(s) should complete the checklist at each evaluation.

Participant's Name: _____ Date: _____

Rater's Name: _____ Protocol: _____
(Please have the same rater at each evaluation if possible)

Neuro Symptom Checklist

(For parent/adult observer of participants 2.0 years and older)

For each item below, please check one box to describe the worst severity and one box to indicate the duration of each of these symptoms in the past week:

1) Visual hallucinations	❑No ❑Yes	If yes: a) Severity:	❑ Mild	❑ Moderate	❑ Severe
		b) Duration:	❑ < 24 hours	❑ 24-48 hours	❑ >48 hours
2) Auditory hallucinations	❑No ❑Yes	If yes: a) Severity:	❑ Mild	❑ Moderate	❑ Severe
		b) Duration:	❑ < 24 hours	❑ 24-48 hours	❑ >48 hours
3) Unresponsive to commands	❑No ❑Yes	If yes: a) Severity:	❑ Mild	❑ Moderate	❑ Severe
		b) Duration:	❑ < 24 hours	❑ 24-48 hours	❑ >48 hours
4) Disorientation/ confusion	❑No ❑Yes	If yes: a. Severity:	❑ Mild	❑ Moderate	❑ Severe
		b) Duration:	❑ < 24 hours	❑ 24-48 hours	❑ >48 hours

5) Depressed/sad mood ❑No ❑Yes	If yes: a. Severity:	❑ Mild	❑ Moderate	❑ Severe
	b) Duration:	❑ < 24 hours	❑ 24-48 hours	❑ >48 hours
6) Distressed/upset mood ❑No ❑Yes	If yes: a. Severity:	❑ Mild	❑ Moderate	❑ Severe
	b) Duration:	❑ < 24 hours	❑ 24-48 hours	❑ >48 hours
7) Drowsiness/ Sleepiness ❑No ❑Yes	If yes: a. Severity:	❑ Mild	❑ Moderate	❑ Severe
	b) Duration:	❑ < 24 hours	❑ 24-48 hours	❑ >48 hours
8) Difficulty speaking ❑No ❑Yes	If yes: a. Severity:	❑ Mild	❑ Moderate	❑ Severe
	b) Duration:	❑ < 24 hours	❑ 24-48 hours	❑ >48 hours
9) Pain ❑No ❑Yes	If yes: a. Severity:	❑ Mild	❑ Moderate	❑ Severe
	b) Duration:	❑ < 24 hours	❑ 24-48 hours	❑ >48 hours
10) Seizures ❑No ❑Yes	If yes: a. Severity:	❑ Mild	❑ Moderate	❑ Severe
	b) Duration:	❑ < 24 hours	❑ 24-48 hours	❑ >48 hours

11) Blurred vision	❑No ❑Yes	If yes: Severity:	❑ Mild	❑ Moderate	❑ Severe
		b) Duration:	❑ < 24 hours	❑ 24-48 hours	❑ >48 hours
12) Other (please specify): _____	❑No ❑Yes	If yes: Severity:	❑ Mild	❑ Moderate	❑ Severe
		b) Duration:	❑ < 24 hours	❑ 24-48 hours	❑ >48 hours

15.8.2 Appendix H 1: CAR Neurotoxicity Screen Checklist

This form serves as a general guideline, but is not required.

Examiner:_____

Date:_____

____Baseline Evaluation

____Follow-Up Evaluation

I. Mental status

	Yes	No
Is subject alert and oriented to time and place?		
Is subject able to perform 3-step command?		
Can subject name 3 objects?		
Comments:		

II. Cranial Nerves

Gross examination of cranial nerves:	Yes	No
Is cranial nerve II intact?		
Are cranial nerves III, IV, VI intact?		
Is cranial nerve V intact?		
Is cranial nerve VII intact?		
Is cranial nerve VIII intact?		
Is cranial nerve IX intact?		

Is cranial nerve X intact?		
Is cranial nerve XI intact?		
Is cranial nerve XII intact?		
Does subject have papilledema?		
Comments:		

III. Deep Tendon Reflexes

	Biceps	Triceps	Brachioradialis	Knee	Ankle	Babinski
Right						
Left						
Comments:						

IV. Motor

	Deltoid	Biceps	Triceps	Wrist flexors	Wrist extensors	Finger flexors	Iliopsoas	Quadriceps	Ankle dorsiflexion	Ankle plantar flexion	Great Toe extension
Right											
Left											
Comments:											

V. Sensory

Vibration	RUE	RLE	LUE	LLE	Does it affect ADLs
Decreased to ankle					
Decreased to knee					
Decreased in finger					
Decreased to wrist					
Comments:					

Temperature	RUE	RLE	LUE	LLE	Does it affect ADLs
Decreased to ankle					
Decreased to knee					
Decreased in finger					
Decreased to wrist					
Comments:					

Pinprick	RUE	RLE	LUE	LLE	Does it affect ADLs
Decreased to ankle					
Decreased to knee					
Decreased in finger					
Decreased to wrist					
Comments:					

VI. Coordination and Gait

	Yes	No
Is subject able to perform rapid alternating movements?		
Is subject able to perform finger to nose testing?		
Does subject have normal gait?		
Does subject have normal heel and toe walking?		
Comments:		

VII. Primary team to consult the following service at the request of the examiner:

Ophthalmology	
Neurology	

Tendon Reflex Grading Scale	
Grade	**Description**
0	Absent
1+	hypoactive
2+	Normal
3+	Hyperactive without clonus
4+	Hyperactive with clonus

Grading for Muscle Strength	
Grade	**Description**
0/5	No muscle movement
1/5	Visible muscle movement, but no movement at the join
2/5	Movement at the joint, but not against gravity
3/5	Movement against gravity, but not against added resistance
4/5	Movement against resistance, but less than normal

VIII. Handwriting Sample: To be performed if feasible depending on participant clinical condition and age

Baseline prior to chemotherapy:

Start of CRS +/-2 days:

CRS peak +/-2 days as feasible:

CRS resolution:

15.9 APPENDIX I: BIOSPECIMEN COLLECTION SCHEDULE AND FORMS

15.9.1 Appendix I 1: Correlative Sample Schedule

(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 Timepoints	Volume/type	Tube*	Notes
Cell Product Samples					
RCL (replication competent retrovirus)	p24 antigen + PERT Assay (product only)/ Indiana University	As per Center for Cellular Engineering (CCE) SOP	Minimum of 5 x 10^5 cells from the cellular product	In cryovial with 1mL freezing media (90% AB serum, 10% DMSO) Frozen at -80 C	Samples will be stored in CCE/ Frederick repository and tested only if clinically indicated. If clinical indication for testing exists, samples will be 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
	Rapid PCR analysis (product only) Location: DTM	As per Center for Cellular Engineering (CCE) SOP	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 CCE ***Results must be available prior to cell infusion***
CD4/8 selected Cell Product Storage for future research	Biospecimen Processing Core (BPC)	As per Center for Cellular Engineering (CCE) SOP	3 vials 2x10^7 CD4/8 enriched cells	In cyrovials in 1mL freezing media each (90% AB serum, 10% DMSO) Frozen at -80º C	Received from CCE BPC

Study	Test/Lab Location^	Sample Timepoints	Volume/type	Tube*	Notes
Cell product Storage for future research	Biospecimen Processing Core (BPC)	As per Center for Cellular Engineering (CCE) SOP	Residual cells that remain following selection of the starting material which will not be used for CAR T-cell manufacturing (e.g., the unselected fraction (discard) of non-T cells)	In cryovials with in 1mL freezing media each (90% AB serum, 10% DMSO) Frozen at -80º C	Received from CCE BPC
Manufactured Cell product Storage for future research and regulatory studies	Biospecimen Processing Core (BPC) or Leidos Biomedical Research	As per Center for Cellular Engineering (CCE) SOP	Up to 10 vials $2x10^7$ or unused cellular product unusable for participant treatment	In cryovials in 1mL freezing media each (90% AB serum, 10% DMSO) Frozen at -80 C	Received from CCE Post Infusion Product: BPC Unused cellular product: Courier to NCI-Frederick repository 301-846-5893 to schedule pick-up
Participant Testing					
Flow Cytometry: Bone marrow	ALL panel including hematagones and/or CAR detection Stetler-Stevenson Lab 10/3S240 301-402-1716	1. At screening (may be done at CLIA certified outside lab) 2. Day 28 (± 4 days) 3. All additional samples only as feasible: Monthly (± 2 weeks) for 2 months, then q3-6 months (± 1 month) until 1yr or CAR loss.	3-5 mL marrow aspirate	Collect 3-5 mL bone marrow aspirate into heparinized syringe and IMMEDIATELY remove syringe, MIX well inverting 5 or more times.	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

Study	Test/Lab Location^	Sample Timepoints	Volume/type	Tube*	Notes
Flow Cytometry: Peripheral Blood	ALL panel including hematogones and/or CAR detection Stetler-Stevenson Lab 10/3S240	1. Prior to chemo (within 14 days) if applicable to disease site. (may be done at CLIA certified outside lab). Samples obtained from screenin evaluation is acceptable if done within 14 days 2. Day 7, 14, 21, 28 (± 4 days) 3. All additional samples only as feasible: Monthly (± 2 weeks) for 2 months, then q3-6 months (± 1 month) until 1yr or CAR loss.	10-20 mL peripheral blood	Sodium Heparin tube a room temperature	Schedule with lab in advance using Microsoft Outlook calendar and order test in CRIS.
Flow Cytometry: CSF	Flow Cytometry for CAR T cells/ Stetler-Stevenson Lab 10/3S240 301-402-1716	1. At screening (if feasible). 2. Day 28 (± 4 days) 3. All additional samples only as feasible: Monthly (± 2 weeks) for 2 months, then q3-6 months (± 1 month) until 1yr or CAR loss.	3-8 mL CSF	Spinal fluid collection tube delivered immediately to the lab Flow media available on 3 NE North Procedure Area.	Repeat CSF studies will be done in participants with a history of CNS involvement.

Study	Test/Lab Location^	Sample Timepoints	Volume/type	Tube*	Notes
RCL (replication competent retrovirus)	High-sensitivity PCR analysis (participant samples)/ Indiana University	Prior to cell infusion (up to 14 days prior)	3-6 mL/ peripheral blood	Purple top (EDTA) or PAX DNA tube refrigerated at 4 C	Samples will be stored in CCE/ Frederick repository and tested only if clinically indicated. If clinical indication for testing exists, samples will be 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
DNA for CAR Peripheral Blood	PCR for CAR promoter from T cell genomic DNA/ Leidos Biomedical Research	1. Prior to cell infusion (within 1 day) 2. Day 1, 7, 14, 21, 28 (± 4 days) 3. If feasible: Repeat monthly (± 2 weeks) X 2 months, then q3-6 months (± 1 month) until CAR negative.	5 mL peripheral blood	PAX DNA tube per sample at room temperature	Samples will be stored and batched Courier to NCI-Frederick repository 301-846-5893 to schedule pick-up

Study	Test/Lab Location^	Sample Timepoints	Volume/type	Tube*	Notes
DNA for CAR Bone Marrow	PCR for CAR promoter from T cell genomic DNA/ Leidos Biomedical Research	2. Day 28 (± 4 days) 3. If feasible: Repeat monthly (± 2 weeks) X 2 months, then q3-6 months (± 1 month) until CAR negative.	3-8 mL bone marrow	PAX DNA tube per 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-7, and TNFα)	Multiplex cytokine panel Leidos Biomedical Research	1. Prior to cell infusion (within 1 day) 2. Day 1 and Day 3 3. Daily for days 5-21 (only while inpatient). While outpatient, to be collected at least two times weekly. 4. Day 28 (± 4 days). 5.Additional samples may be collected monthly (± 2 weeks), then q3-6 months (± 1 month) until 1 yr or CAR loss.	4 mL peripheral blood	For scheduled samples 4 ml Green Sodium heparin tube at room temperature. Cytokines that are done on evenings and weekends will be spun per NCI Frederick SOP and sent frozen.	At any time for participants suspected of developing cytokine storm, additional samples will be collected, stored in the refrigerator sent ASAP for analysis Courier to NCI-Frederick repository. Call 301-846-5893 to schedule sample pick-up.

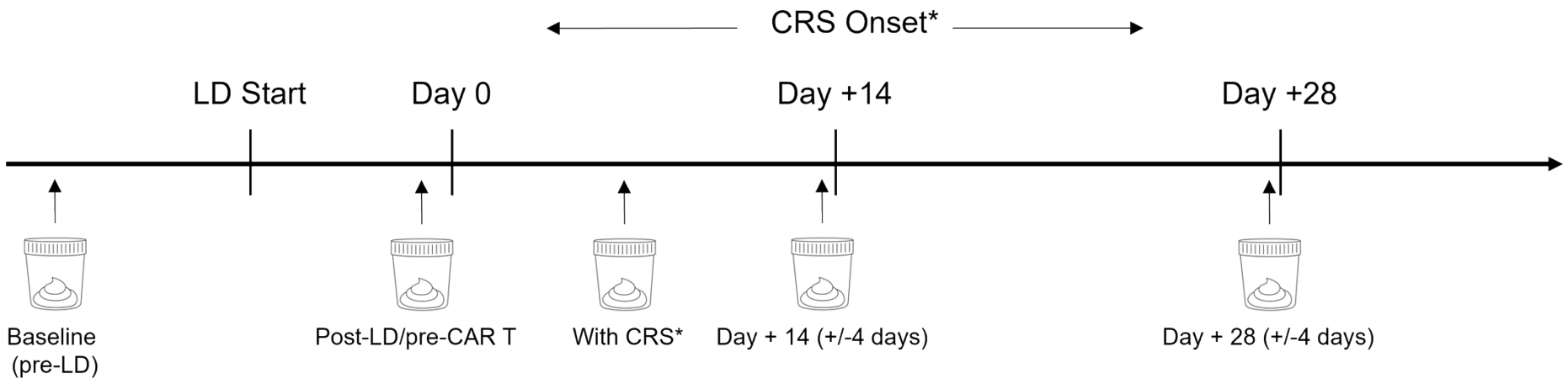
Study	Test/Lab Location^	Sample Timepoints	Volume/type	Tube*	Notes
Lymphocyte Phenotyping Panel	Lymphocyte Phenotyping PID Panel or TBNK NIH Clinical Lab	1. Prior to chemo (within 14 days). Samples obtained from screening evaluation is acceptable if done within 14 days. 2. Day 14, 28 (± 4 days) 3. If feasible: Monthly (± 2 weeks) for 2 months, then q3-6 months (± 1 month) until 1yr or CAR loss.	6 mL peripheral blood	Purple top tube	Schedule with clinical lab 301-496-4879 and order test in CRIS.
Sequence based detection of MRD (optional)	Adaptive Biotechnologies Corporation	1. At time of screening 2. Day 28 (± 4 days). 3. Month 2 and 3 (± 2 weeks) if feasible and applicable	$1X10^6$ PBMC in pellets (cryopreserved)	Bone marrow or peripheral blood	Samples will be stored in NCI-Frederick repository, call 301-846-5893 to schedule pick-up. Batched samples will be shipped on dry ice to Adaptive Biotechnologies: 1551 Eastlake Ave E, Suite 200 Seattle, WA 98102
Cytogenetics (Cyto)	Department of Transfusion Medicine	As clinically indicated, at PI's discretion.	3-5 ml of bone marrow	Marrow: 0.5 ml preservative-free sodium heparin in capped syringe	Order in CRIS as "Other Mayo Lab"

Study	Test/Lab Location^	Sample Timepoints	Volume/type	Tube*	Notes
Gut Microbiome (Optional)	Hourigan Lab (NIAID) Building 50, Rm 5512 Phone: 240-292-4552	See **Appendix** I 2: Gut Microbiome Sample Collection Timepoints (all will be considered optional) Collect sample with any clinically relevant event or if subject does not stool within designated time windows		Sterile container	Stool samples will be handled using standard contact precautions and stored at -80C as soon as possible after collection.
Metabolomic Analysis (optional) Peripheral Blood	Taylor Lab (POB) CRC/1-3940	1. At the time of apheresis 2. Prior to lymphodepletion Following CAR-T therapy: Day 0, 7, 14, 21, 28 (+/- 3 days).	5-10 ml peripheral blood	Sodium heparin tube at room temperature	
Metabolomic Analysis (optional) Urine	Taylor Lab (POB) CRC/1-3940	1. At the time of apheresis 2. Prior to lymphodepletion Following CAR-T therapy: Day 0, 7, 14, 21, 28 (+/- 3 days).	5 ml urine	Sterile container	The samples must be kept in a fridge (4C) – on ice and processed as soon as possible (<1-2 h) after blood draw

^ The location of specimen processing or analysis may be adjusted with the permission of the PI or laboratory investigator.

* Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.

15.9.2 Appendix I 2: Gut Microbiome Sample Collection Timepoints (all will be considered optional)



*Optional sample to be collected with CRS onset (+ 2 days)

15.9.3 Appendix I 3: CD19/CD22 Expression for FACS for Samples to be sent to NCI: Shipment and Consent Process

Section A

Samples

CD 19/22 Expression for FACS Analysis for Samples to be sent to NCI: Sample Preparation

- For subjects with malignant cells in the peripheral blood: collect approximately 5 cc of blood in green-top (Sodium Heparin) tube.

- For subjects with probable marrow involvement, but without malignant cells in the blood: approximately 3-5 cc of marrow, add 1 cc of heparin (1,000 μ/mL) to the syringe prior to aspirating the marrow, place in green-top (Sodium Heparin) tube.

- Label the tube with **subject's name, date of birth and draw date**. Sample should also be labeled as "bone marrow" or "peripheral blood."

Section B

Preparation of shipment

1. Schedule in advance with NIH, NCI, POB.
   Include the following email addresses:
   NCIPBLLBMT@mail.nih.gov
2. Send subject's or parents' contact information to the NIH, NCI, POB team so that the NIH team can obtain phone consent.
   Include the following email addresses:
   NCIPBLLBMT@mail.nih.gov
3. Obtain Consent signatures.
4. Collect blood or bone marrow per the directions in **Section A**
5. Label the sample with:
   a. **subject's name**
   b. **date of birth**
   c. **draw date.**
   d. **label as "bone marrow" or "peripheral blood"**
      - Fax to 301-480-5157.
6. Prior flow cytometry report from diagnosis or relapse.
7. CBC results from the day the sample was obtained.
8. Package according to FedEx regulations for Diagnostic/Clinical Specimen shipment.
   a. The samples should be kept at room temperature and should be shipped in containers without ice. Do not use ice. Do not refrigerate.

b. Samples are best shipped on a Monday, Tuesday, or Wednesday. Do not send samples on Friday, Saturday, or the day preceding a holiday. Tests are not run on weekends or holidays.

9. Include in Package with the sample:

   a. Original signed and witnessed consent.
   b. Prior flow cytometry report from diagnosis or relapse.
   c. CBC results from the day the sample was obtained.

10. Ship by **Federal Express First Overnight** (earliest next business day delivery) so that the sample is delivered by 10 am the next morning).

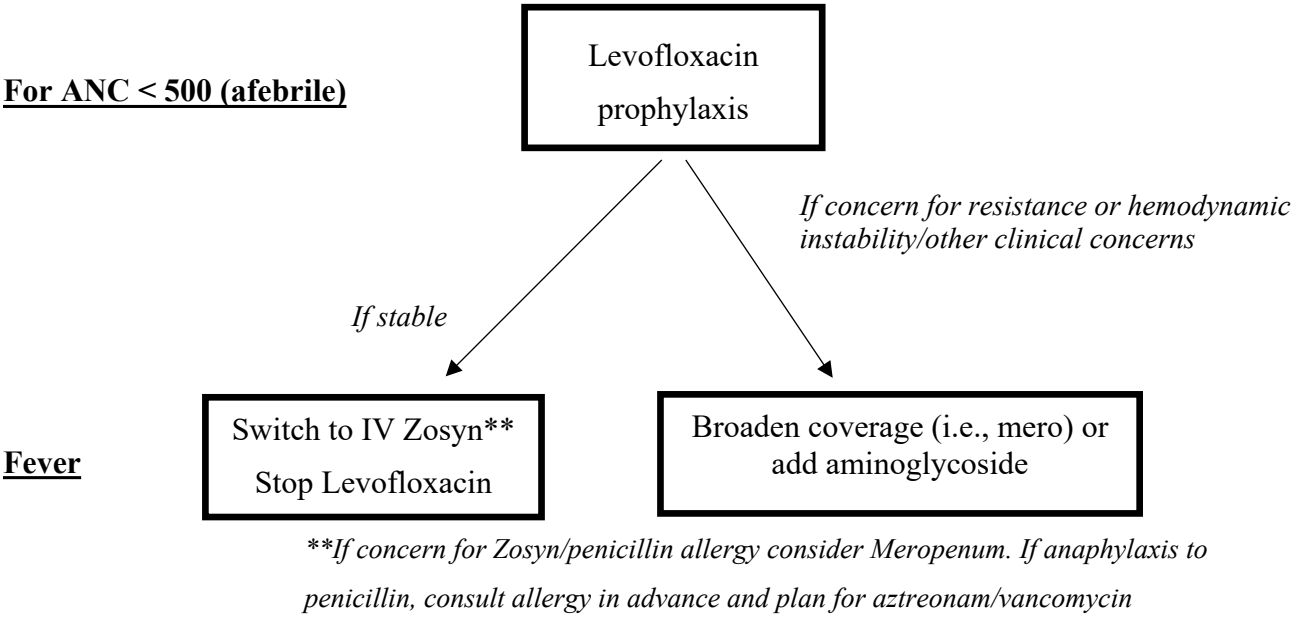
    *Note: Samples that arrive after 10 am may be cancelled by the NIH Flow Cytometry Lab due to poor viability. In that case the sample would be discarded. If the sample is viable, results may be delayed.

    Ship To:
    Attn: Robert Honec/ Flow Cytometry Lab
    Laboratory of Pathology, CCR, NCI, NIH
    10 Center Drive, Bldg 10, Room 3S240
    Bethesda, MD 20892
    Phone: 301-480-8074/8077

15.10 APPENDIX J: SUGGESTED INFECTION PROPHYLAXIS GUIDELINES

These are suggested guidelines only (not mandated) and should be applied only after discussion with the protocol and modified as needed based on individual needs.

CD19/CD22 CAR T Cell Antimicrobial Prophylaxis



Antibacterial Prophylaxis

1.) When ANC < 500, will initiate Levofloxacin prophylaxis
    a. >40KG, Dose 500 mg Q 24 hours
    b. 20-40KG, 250-375 mg Q24 hours
    c. < 20KG, Dose 10mg/kg Q 24 hours

2.) If participant has fever on Levofloxacin
    a. Routine surveillance blood cultures
    b. Change antibiotics to IV Zosyn (may be modified based on participant specific antimicrobial resistance or allergy)
        i. Adult Dosing: 4.5g Q 6 hrs
        ii. Pediatric Dosing (< 40 kg): 100 mg/kg of piperacillin component q6h
    c. Stop Levofloxacin
    d. In the setting of penicillin/Zosyn allergy, start with IV Meropenum
        i. Adult and pediatric dosing: 20 mg/kg Q 8, max dose of 1 gram Q 8 hrs
        ii. If history of true anaphylaxis to Penicillins, please consider aztreonam/vancomycin and discuss with allergy consult in advance of starting meropenum. For such participants, implementation of levofloxacin prophylaxis will be discussed on a case by case basis.

3.) In the setting of suspected resistance, hemodynamic instability or clinical exam findings
    a. Consider addition of an aminoglycoside or broadening coverage

4.) When participant defervesces and is clinically stable without an active infection, and has ANC < 500
    a. Consider stopping IV antibiotics
    b. Add prophylactic Levofloxacin

5.) Consider discontinuing Levofloxacin prophylaxis at Day 28 if participant is outpatient, well-appearing, and/or when ANC >250 AND participant is clinically stable without any evidence for ongoing inflammation or CRS. Continue Levofloxacin as clinically indicated if ANC<250 and trial a dose of filgrastim.

Antifungal Prophylaxis

1.) Micafungin
    a. >50 KG, Dose 50 mg IV daily
    b. < 50 KG, Dose 1 mg/kg IV daily
2.) Posaconazole
    a. 13 years and older: **Delayed release tablets:** Day 1: 300 mg tab BID, then 300 mg daily
    b. <13 yo **or** less than 40 kg: **Oral Suspension:** 4 mg/kg/dose TID, >13 years old: max 200 mg TID
    c. <13 yo **or** 25- 40 kg: **Delayed release tablets** 200 mg Q12 followed by 200 mg day

Antiviral Prophylaxis

1.) HSV prophylaxis with Acyclovir/Valtrex if + HSV titers
    a. Acyclovir: 20mg/kg PO BID (max dose 800 mg BID)

PCP Prophylaxis

1.) Bactrim → Pentamidine (300mg inhaled q month) prior to CAR T cell infusion
2.) For participants that cannot do inhaled pentamidine (e.g. younger participant): Pentamidine may be given at 4 mg/kg (max 300 mg) intravenously once a month.