University of Pennsylvania

Phase 2 Study of Humanized CD19-directed Chimeric Antigen Receptor-modified T cells (huCART19) for Very High-Risk Subsets of B cell Acute Lymphoblastic Leukemia (B-ALL)

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Correlative Laboratory Team:	Translational and Correlative Studies Laboratory (TCSL) University of Pennsylvania
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LIST OF ABBREVIATIONS

ALL	acute lymphoblastic leukemia
APC	antigen presenting cell
aAPC	artificial APC
AE	adverse event
B-ALL	B lineage acute leukemia
B-cell ALL	B cell acute lymphoblastic leukemia
CAR	chimeric antigen receptor
CART19 cells	CD19 redirected autologous T cells; also called CTL019
CCI	Center for Cellular Immunotherapies
CGTL	Cell and Gene Therapy Laboratory at CHOP
CHOP	Children's Hospital of Philadelphia
CFR	code of federal regulations
CLL	chronic lymphoblastic leukemia
CMV	Cytomegalovirus
CNS	central nervous system
COG	Children's Oncology Group
CR	complete remission
CRi	complete remission with incomplete blood count recovery
CRF	case report form
CRP	C-reactive protein
CRS	cytokine release syndrome
CSF	cerebral spinal fluid
CTCAE	common terminology criteria of adverse events
CTRC	clinical and translational research center
CT scan	computed tomography scan
CTL	cytotoxic T lymphocyte
CVPF	clinical cell and vaccine production facility
CTL	cytotoxic T lymphocyte
CD137	4-1BB costimulatory molecule
DFS	disease free survival
DOR	duration of response
DSMB	data and safety monitoring board
DSMC	data and safety monitoring committee
ECOG	Eastern Cooperative Oncology Group
EFS	event free survival
FACT	Foundation for the Accreditation of Cellular Therapy
FAS	full analysis set
FDA	food and drug administration
FISH	fluorescent in situ hybridization
GCP	good clinical practices
GMP	good manufacturing practices
GVHD	graft versus host disease
HAMA	human anti-murine antibody

HSCT	hematopoietic stem cell transplantation
huCART19 cells	humanized CD19 redirected autologous T cells
IBC	Institutional Biosafety Committee
IRB	Institutional Review Board
KM	Kaplan-Meier
LGL	large granular lymphocytes
MAS	macrophage activation syndrome
MRD	minimal residual disease
MRI	magnetic resonance imaging
NCCN	National Comprehensive Cancer Network
ORR	overall remission rate
OS	overall survival
PBMC	peripheral blood mononuclear cells
PD	progressive disease
PK	pharmacokinetics
PR	partial remission
QoL	quality of life
RAC	NIH Office of Biotechnology Recombinant DNA Advisory Committee
RCR/L	replication competent lentivirus
RFS	relapse free survival
SAE	serious adverse event
scFv	single chain Fv fragment
SCT	stem cell transplant
TCR	T cell receptor
TCSL	Translational and Correlative Studies Laboratory
TLS	tumor lysis syndrome
UPenn	University of Pennsylvania
Vβ	a rearranged T cell specific gene that can be used to determine clonality of a T cell population
VHR	very high risk
VSV-G	Vesicular Stomatitis Virus, Glycoprotein
WBC	white blood cell

STUDY SUMMARY

Title	Phase 2 Study of Humanized CD19-directed Chimeric Antigen Receptor-modified T cells (huCART19) for Very High-Risk Subsets of B cell Acute Lymphoblastic Leukemia (B-ALL)
Short Title	Phase 2 study of huCART19 in Pediatric ALL
Protocol Numbers	18CT014; CHOP IRB# 18-015566 Penn IRB #831916
Phase	Phase 2
Methodology	This is a two cohort, open-label, phase 2 study to determine the efficacy of huCART19 in pediatric and young adult patients with CD19 expressing relapsed and refractory B-cell acute lymphoblastic leukemia.
Study Duration	The protocol will require approximately 36 months to complete enrollment. Each subject will be followed for approximately 1 year post-infusion as part of this study.
Study Center(s)	Children's Hospital of Philadelphia (CHOP)
Objectives	**Primary Objective:** 1. To determine efficacy of huCART19 **Secondary Objective:** 1. To describe additional efficacy endpoints 2. To further evaluate the safety of huCART19 in pediatric ALL Please refer to protocol **Section 2** for corresponding study endpoints.
Number of Patients	A total of 100 infused subjects is targeted. Approximately 52 subjects will be infused in Cohort A (up to 62 subjects enrolled) and approximately 48 subjects will be infused in Cohort B (up to 54 subjects enrolled).
Diagnosis and Main Inclusion Criteria	Inclusion criteria are designed to include pediatric and young adult patients aged 3 months -29 years with CD19 expressing relapsed/refractory B-cell acute lymphoblastic leukemia (ALL).
Investigational Product, Dose, Route, Regimen	huCART19 cells transduced with a lentiviral vector to express humanized anti-CD19 scFv:41-BB:TCRζ, administered by IV injection with a planned dose of $5x10^6$ huCART19 cells/kg on day 0 with possible subsequent doses
Duration of administration	The duration of CART19 administration will be based on the total volume to be infused and the recommended infusion rate of 10-20mL per minute. The transduced T cells will be administered by slow IV push.
Reference therapy	None. This protocol will be given to subjects with unmet medical needs for which there are no current curative therapeutic options.

Statistical Methodology	The primary endpoint is 1-year Event-Free Survival (EFS), with time to event or censoring defined as the time from infusion to the first of any of the following events: no response, relapse, death due to any cause, or to the last day of follow up if no event occurs. All analyses will be performed for cohort A and B separately. Kaplan-Meier (KM) curves will be generated and one-sample Log-rank test will be used to test the 1-year EFS against the historical rate. One-year EFS and its 95% confidence interval (CI) will also be estimated based on the KM method. For cohort A, the null hypothesis is 40% 1-year EFS and the alternative hypothesis is 60% 1-year EFS. The sample size of 52 provides 95% power to test this hypothesis, using a one-sided one-sample Log-rank test with 10% type I error, assuming 1 year of follow up for each subject. For cohort B, the null hypothesis is 15% 1-year EFS and the alternative hypothesis is 30% 1-year EFS. The sample size of 48 provides 91% power to test this hypothesis, using a one-sided one-sample Log-rank test with 10% type I error, assuming 1 year of follow up for each subject. We expect that we could infuse approximately 85% of enrolled subjects, so we will enroll approximately 62 subjects for cohort A and approximately 54 subjects for cohort B to obtain the targeted number of infused subjects.

1. INTRODUCTION

While survival rates in children with de novo B-ALL approach 90%,[2] subgroups of patients at very high risk (VHR) of relapse are in need of novel therapeutic approaches. Many of these patients can be identified early in therapy by poor response to therapy. Refractory ALL poses a therapeutic challenge, with long-term survival close to 30% for patients who do not achieve remission with induction chemotherapy (induction failure).[3] Survival rates for children who experience early medullary relapse of B-ALL, defined as a marrow relapse within 36 months of diagnosis, are equally poor, with EFS rates <30% reported.[4] In COG trials, survival rates for these patients have not improved measurably in 30 years.[5,6] Even for patients who achieve a second remission, those remissions are frequently not sustained,[4,7] and with each subsequent relapse, achieving remission is harder, and long-term survival is extremely poor.[4]

Intensive chemotherapy followed by hematopoietic stem cell transplant (HSCT) [8] has been considered a standard treatment approach for patients with poor outcomes similar to these subsets.[9,10] However, even with these intensified approaches, these subgroups have demonstrated suboptimal outcomes; therefore, we hypothesize that these leukemias may be chemo-refractory.

Adoptive transfer of T cells engineered to express a chimeric antigen receptor (CAR) has emerged as a powerful technology producing dramatic responses in patients with highly refractory malignancies. Our group and others have shown complete remission (CR) rates of 70-93% in children and adults with relapsed/refractory acute lymphoblastic leukemia (ALL)[1,11-15,16,17]. Outcomes using autologous T cells expressing a murine CAR directed at CD19 (CART19) in a large multicenter trial of 75 patients (median survival not reached) led to the 2017 FDA approval of this agent in pediatric and young adult patients with r/r disease[17]. Remission for some patients with CTL019 have been durable even without a consolidative HSCT [16,17]. However, a subset of patients do not respond or relapse due to poor CAR T cell expansion and persistence. Approximately 20% of patients demonstrate early recovery of CD19-expressing B cells,[18] a surrogate marker of CD19-directed CAR T cell loss, within 6 months of CART19 infusion. Without further therapy, early loss of CAR T cells confers an increased risk of CD19+ relapse. Options for patients who do not respond or relapse after prior engineered cell therapy are extremely limited.

We have assessed a humanized version of CART19, called huCART19, in a Phase 1 trial at the Children's Hospital of Philadelphia (CHOP). The 13BT022 trial evaluated huCART19 in CAR-naïve subjects as well as subjects who had previously received anti-CD19 CARs, either CTL019 at CHOP or another anti-CD19 CAR elsewhere. Clinical success was seen on this trial in both patient subsets.

Based on our previous success and lack of clinical options in patients with very high risk (VHR) ALL, we propose to test huCART19 as an alternative treatment approach, in newly diagnosed B-ALL patients predicted to have an exceedingly poor outcome with conventional chemotherapy, in high-risk first relapse, and in second or greater relapse in this phase 2 trial (Cohort A). In addition, a second cohort (Cohort B) will test the efficacy of huCART19 in patients with poor response to prior B cell directed engineered cell therapy.

1.1. Background

1.1.1. Engineered T cells with redirected specificity: chimeric antigen receptors (CAR)

CAR T cells are patient-derived lymphocytes transfected with a gene encoding a chimeric transmembrane receptor that enables recognition of target cell-surface antigens with high specificity in a non-MHC restricted manner, coupling antigen-binding with intracellular signals that promote T cell effector functions[19,20]. The extracellular component of CARs typically consists of the antigen-binding domains from the heavy and light chains of an antibody configured as a single-chain (i.e., a single-chain variable fragment, or scFv). The intracellular component typically consists of signaling domains that mediates T cell activation (as reviewed[21,22]). The CAR T cells used in this study are so-called second-generation CARs that incorporate the TCRζ and 4-1BB signaling domains and are transduced via lentiviral vector.
The clinical efficacy of CAR T cells was first reported by Porter et al. using CART19/CTL019 in patients with advanced, treatment-refractory CLL[23,24]. This was followed by additional reports with CART19/CTL019 in ALL[16,17] and non-Hodgkin's lymphoma[25], along with reports from other centers evaluating anti-CD19 CAR T cells (as reviewed [26,27]).

Response rates and duration of response to CAR T cell therapies vary considerably, however, even among the various CD19+ malignancies treated with anti-CD19 CARs. Therefore, the design of this study is informed by clinical and correlative data from ALL patients treated with CART22 and CART19 in previous studies.

1.1.2. CD19 as a therapeutic target for B cell leukemia and lymphoma

CD19 is a 95kDa glycoprotein present on B cells from early development until differentiation into plasma cells[28-30]. It is a member of the immunoglobulin (Ig) superfamily and a component of a cell surface signal transduction complex that regulates signal transduction through the B cell receptor[30-32]. Mice lacking CD19 have a decreased number of B cells in peripheral lymphoid tissues, a decreased B cell response to oral vaccines and mitogens, and decreased serum Ig levels[30,33]. Expression of CD19 is restricted to B lineage cells and is not expressed by pluripotent blood stem cells[29]. CD19 is also expressed by most B cell lymphomas, mantle cell lymphoma, ALLs, CLLs, hairy cell leukemias, and a subset of acute myelogenous leukemias[28,34,35]. CD19 thus represents a highly attractive target for immunotherapy[29]. Furthermore, CD19 is not present on most normal tissues, other than normal B cells, including pluripotent blood stem cells[29], which makes CD19 a relatively safe target presenting a minimal risk of autoimmune disease or irreversible myelotoxicity. Anti-CD19 antibodies and scFvs either native or conjugated to radioisotopes or toxins are currently being developed and have demonstrated promise in both mouse models[36-40] and human and non-human primates[22,41-50].

1.1.3. Lymphodepleting chemotherapy

Adoptive immunotherapy are designed to capitalize on homeostatic T cell proliferation[51,52], the phenomenon whereby naive T cells proliferate and differentiate into memory-like T cells when the total numbers of naive T cells in the host are reduced to low levels[51,53]. This lymphodepletion also eliminates regulatory T-cells and other competing elements of the immune system that act as "cytokine sinks", enhancing the availability of cytokines such as IL-7 and IL-15 that are important for T cell proliferation and survival[54]. This concept was initially demonstrated clinically using ex-vivo-expanded tumor-infiltrating lymphocytes in patients with metastatic melanoma; in these patients, pre-treatment with

cyclophosphamide (60mg/kg x 2 days) and fludarabine (25 mg/m^2 x 5 days) enabled *in vivo* engraftment and clinical activity[55].

Lymphodepleting chemotherapy has become standard for CAR T cell treatment. While initial studies allowed investigators to choose among chemotherapy regimens based on patients' prior treatment histories, more recent studies have been more prescriptive with the recognition that the lymphodepletion regimen affects clinical activity and toxicity. For example, Turtle et al. compared results obtained in sequential cohorts of non-Hodgkin lymphoma patients treated with a CD28-based second-generation anti-CD19 CAR following cyclophosphamide with or without fludarabine (Cy vs Cy/Flu)[56]. Subjects receiving Cy/Flu were more likely to have a complete response (50% vs 8%) and exhibited more robust *in vivo* proliferation and more durable *in vivo* persistence. As might be expected with increased proliferation, Cy/Flu was also associated with higher frequency of severe CRS and neurotoxicity, though this toxicity agent differential was only apparent in the highest cell-dose cohort. These data suggest that fludarabine contributes to *in vivo* CAR T cell expansion and persistence, leading to increased efficacy but also perhaps increased toxicity. However, fludarabine is likely just one of many factors (e.g., cell dose, CAR design specifications, target antigen expression, disease burden, pre-manufacturing T cell phenotype) contributing to efficacy and toxicity profile of CAR T cell treatment regimens. The optimal lymphodepletion regimen likely varies by CAR T cell product and disease and will need to be determined empirically through clinical investigation.

The "flu/cy" regimen has developed into one of the standard regimens for lymphodepletion prior to CAR T cell administration. In fact, the prescribed lymphodepleting chemotherapy regimen for use with the FDA-approved CTL019 therapy in ALL consists of fludarabine (30 mg/m^2/day x 4 days) and cyclophosphamide (500 mg/m^2/day x 2 days, starting with fludarabine).

1.1.4. Phase 1 trial of huCART19 in pediatric ALL patients – 13BT022

In a Phase 1 trial in children and young adults with relapsed/refractory B-ALL at CHOP (13BT022, NCT02374333), 62 subjects have been infused with huCART19 as of April 2018. Infused subjects received a single dose of huCART19 (median 6x10^6 huCART19 cells/kg; range 5.03x10^5 – 1.38x10^7 huCART19 cells/kg) at Day 0, post-lymphodepleting chemotherapy. The huCART19 cells have been well tolerated in this population as grade 1-2 cytokine release syndrome have been the most commonly observed related adverse events (57%). We observed that huCART19 can induce durable remissions without further therapy in this clinical setting. An interval data analysis showed a 100% CR rate (22 out of 22 subjects) in patients with no prior CAR T cell exposure, with a 12 month RFS of 82%. In addition, in patients with a prior poor or transient response to murine CD19-directed CAR T cells, 56% (9 out of 16 subjects) achieved CR with B cell aplasia. A 56% RFS at 12 months was observed in the retreatment cohort[57]. The safety and efficacy on this trial encouraged us to expand our experience with huCART19 in pediatric ALL patients in a Phase 2 study that will enroll patients with VHR ALL (Cohort A) and patients with poor response to prior B cell-directed cell therapy (Cohort B).

1.2. Investigational Agent

The investigational agent in this protocol is humanized CART19 cells (huCART19). The huCART19 cells will be manufactured in the CVPF at UPenn. huCART19 cells will be administered as a single infusion of 2 - 5 x 10^6 huCART19 cells/kg.

See the huCART19 Investigator Brochure for additional details.

1.2.1. Drug interactions

huCART19 cells are expected to retain many of the properties of natural T cells. As such, they will be expected to be susceptible to immunosuppressive agents such as corticosteroids, immunophilins such as cyclosporine and tacrolimus, methotrexate, mycophenolate mofetil, mTOR inhibitors such as rapamycin, alemtuzumab, daclizumab, ontak. Lymphocytes are especially susceptible to cytotoxic and chemotherapeutic agents that are commonly administered for hematologic malignancies such as cyclophosphamide and fludarabine.

1.3. Preclinical Data

The preclinical assessment and evaluation of the huCART19 is detailed in the huCART19 Investigator Brochure.

Briefly, huCART19 was evaluated for equivalency to murine CART19 in vitro and in vivo. Based on all studies performed, huCART19 was determined to be equivalent to murine CART19 in all tests performed including killing assays, T cell proliferation assays, cytokine induction and potency assays, as well as in an NSG mouse model.

Therefore, based on these preclinical data, huCART19 was believed to be equally potent and safe when compared to murine CART19 in a clinical environment. To date, the clinical data supports this theory as no significant safety concerns related to viral transduction or ex vivo expansion have emerged in more than 300 pediatric and adult subjects treated with various CARs [mostly murine CART19 but also huCART19] at CHOP and Penn.

1.4. Previous Clinical Data

Prior clinical experience and the safety data collected from prior studies are available in the current version of the huCART19 Investigator Brochure.

1.5. Inclusion of children in the research

Broadly speaking, we feel inclusion of pediatric patients in this pilot protocol where we have initial feasibility data is appropriate. This is the approach that is generally pursued in early phase cancer trials for three reasons: i) to get clinical experience in pediatric populations, ii) to learn about issues with dosing or administration that might be specific to younger patients, and iii) allow pediatric access to novel therapies earlier in the development process. The population of pediatric relapsed/refractory leukemia patients targeted for enrollment in this study have no curative options for treatment. New and highly innovative approaches are desperately needed for this group of pediatric patients. Our initial pediatric experience with huCART19 cells demonstrates a powerful signal for potential efficacy, with a 100% CR rate and 82% RFS at 1 year in children with relapsed/refractory B-ALL and a 56% CR rate with B cell aplasia and 56% RFS at 1 year in children with poor response to prior B cell directed engineered cell therapy[57]. With this, we believe there exist both compelling reasons to include children and the prospect of benefit in patients with no curative options.

1.6. Dose Rationale and Risk/Benefits

1.6.1. Dose Rationale

The huCART19 transduced cell dose will be targeted at $5x10^6$ huCART19 cells/kg (acceptable dose range $2x10^5$/kg – $5x10^5$/kg) with a maximum total huCART19 cell dose of $2.5x10^8$ huCART19 cells. These doses are based on the global phase 2 trial of CTL019 (NCT02228096). Because there are about 1×10^{12} T cells in a healthy adult (equivalent to $2x10^{10}$ T cells/kg), assuming a transduction efficiency of 10%, the total cell dose is equivalent to about 0.25% of the total body mass of T cells[85,86]. Therefore, we expect the initial frequency of cells to be present at about 0.25% at baseline following infusion. In addition, in our animal models, we find that a dose of 5 million cells per animal causes a robust antitumor response. The dose given to animals, when scaled, is similar to $5x10^9$ cells in humans.

1.6.2. Potential Risks

Please refer to the current version of the huCART19 Investigator Brochure for expected adverse events.

1.6.3. Potential benefits

Outcome remains poor for pediatric patients with r/r B cell ALL. Treatment options include further treatment with salvage chemotherapy, allogeneic hematopoietic stem cell transplantation (HSCT) or supportive care.

Based on inclusion criteria, patients have poor outcomes with standard available therapies. Furthermore, most patients will not be eligible for allogeneic transplant. For ALL patients, there is little benefit from allogeneic SCT with relapsed and active disease and pediatric centers do not offer SCT to patients in this situation because of futility. Therefore, any benefit that may be seen with CART19 cells will have a major impact for patients.

For patients who relapse after allogeneic transplant, treatment options are even more limited and outcomes dismal. Conventional chemotherapy is not curative and often highly toxic and ineffective. Second allogeneic transplant is associated with extensive morbidity, mortality, high relapse rate, and is ineffective for the majority of patients. Donor lymphocyte infusions result in response rates between 0-13% and there are very few long-term survivors.

Ongoing clinical trials with murine and humanized CART19 cells expressing the second generation CAR with an anti-CD19 scFv and 4-1BB and TCR signaling domains, in patients with B-cell ALL and CLL (both CD19 expressing B cell malignancies) described above, show that CART19 therapy has potent anti-tumor activity in pediatric and adult ALL patients[97, 98, 101] (described in detail in **Section 1.4**). Therefore, the risk benefit ratio for huCART19 potential to induce remission in the ALL populations to be studied here is quite favorable.

2. STUDY OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
• To determine efficacy of huCART19	• 1-year Event-Free Survival in patients with newly diagnosed VHR B-ALL or high-risk relapse of B-ALL • 1-year Event-Free Survival in patients with poor response to prior B cell directed engineered cell therapy
Secondary	
• To describe additional efficacy endpoints • To further evaluate the safety of huCART19 in pediatric ALL	• Overall remission rate as determined by the response at day 28, computed as the proportion of subjects with CR or Cri, in patients with newly diagnosed VHR B-ALL or high-risk relapse of B-ALL (cohort A) and in patients with poor response to prior B cell directed engineered cell therapy (cohort B). • 2-year Event-Free Survival in patients with newly diagnosed VHR B-ALL or high-risk relapse of B-ALL (cohort A) and in patients with poor response to prior B cell directed engineered cell therapy (cohort B) • 2-year Relapse-Free Survival in patients with newly diagnosed VHR B-ALL or high-risk relapse of B-ALL (cohort A) and in patients with poor response to prior B cell directed engineered cell therapy (cohort B) • Frequency and severity of adverse events
Exploratory	
• Characterize the pharmacokinetic (PK) profile of huCART19 • Evaluate bioactivity of huCART19 cells • Describe checkpoint pathways and markers of T cell exhaustion	• Rate of huCART19 expansion and persistence (change over time as absolute values or fold change) • Systemic soluble immune and inflammatory factors pre- and post-huCART19 infusion • Measure anti-CAR antibodies and cellular immunogenicity • Measure checkpoint pathways (ie PD-1, CTLA-4, Tim-3, LAG-3) at timepoints before and after infusion

3. STUDY DESIGN

3.1. General Design

The study will consist of three sequential phases: 1) a screening phase, 2) a manufacturing and pre-treatment phase, consisting of apheresis (if applicable) and chemotherapy (if applicable), and 3) a treatment phase, consisting of a huCART19 transfused cell infusion and follow up evaluations. The evaluations and infusion schedule are included in **Appendix 1**. The general protocol schema is displayed in **Figure 3-1**.

After informed consent is obtained, patients will undergo screening tests and procedures to determine eligibility. Once patient eligibility is confirmed, patients who do not have apheresis product suitable for manufacturing will have cells collected by leukapheresis to obtain peripheral blood mononuclear cells

(PBMC) for this purpose. As is currently our practice, cells will be transduced with the anti-CD19 TCRζ/4-1BB lentiviral vector, expanded *in vitro* and then frozen for future administration. Cryopreserved historical apheresis products collected from the patient prior to study entry are usable for huCART19 manufacturing if collected at an appropriately certified apheresis center and the product meets adequate mononuclear cell yields. If a historical apheresis product is not available, an apheresis procedure will be scheduled for cell procurement after study entry.

Unless contraindicated and medically not advisable based on previous chemotherapy, patients will be given conditioning chemotherapy prior to huCART19 cell infusion with the intent of lymphodepletion. Additionally, if the patient's white blood cell (WBC) count is ≤1,000 /µL, conditioning/lymphodepleting chemotherapy may be held at investigator discretion. The chemotherapy will be planned so that the last dose is completed 2-5 days BEFORE the planned infusion of huCART19 cells. The chemotherapy start date will vary based on the duration of the selected chemotherapy regimen. If the period from chemotherapy to huCART19 infusion is delayed for 4 weeks or more, the patient will need to be re-treated with lymphodepleting chemotherapy prior to huCART19 infusion.

Two study cohorts are planned:

1. **Cohort A**: Patients with newly diagnosed VHR B-ALL or high-risk relapse of B-ALL
2. **Cohort B:** Patients with poor response to prior B cell directed engineered cell therapy

Regardless of Cohort assignment, subjects will be followed according to the same schedule.

Additional huCART19 infusions may also be administered in accordance with **Section 6.13**.

After completing follow-up on this study, subjects will be offered participation on a separate long-term protocol for fifteen years post-receipt of huCART19 cells to monitor for safety assessments per the FDA guidelines.

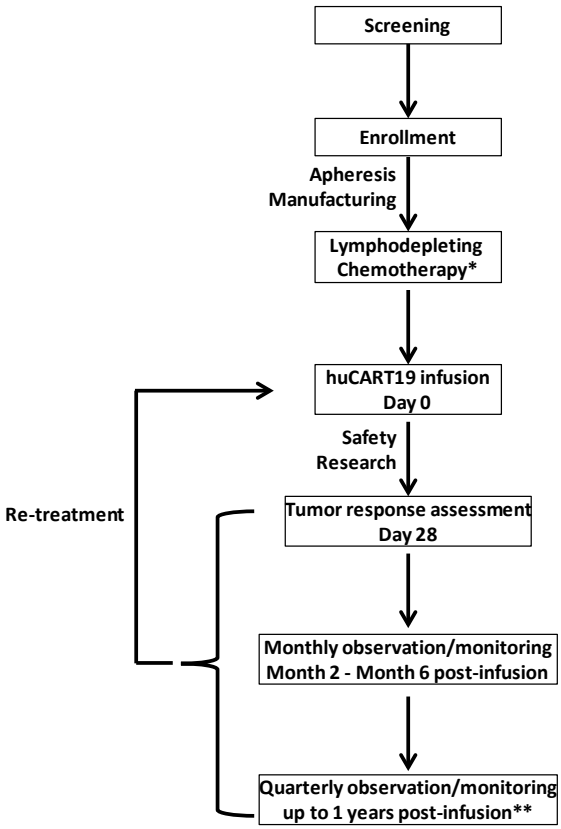

Figure 3-1: Study Schema

4. PATIENT SELECTION AND WITHDRAWAL

4.1. Inclusion Criteria

1. Signed informed consent form must be obtained.
2. Relapsed or refractory B-cell ALL:
   a. Cohort A: Patients with newly diagnosed VHR B-ALL or high-risk relapse of B-ALL who meet one of the following criteria:
      i. Newly diagnosed NCI HR B-ALL with induction failure: M3 marrow (>25% blasts) at end of induction OR
      ii. First marrow relapse of B-ALL at < 36 months from diagnosis OR
      iii. 2nd or greater relapse OR
      iv. Any relapse after allogeneic HSCT and ≥ 4 months from SCT at enrollment OR

v. Refractory disease defined as having not achieved an MRD-negative and/or CSF-negative CR after ≥ 2 chemotherapy regimens/cycles of frontline therapy or 1 cycle of reinduction therapy for patients in first relapse OR
vi. Ineligible for allogeneic SCT because of:
    1. Comorbid disease
    2. Other contraindications to allogeneic SCT conditioning regimen
    3. Lack of suitable donor
    4. Prior SCT
    5. Declines allogeneic SCT as the therapeutic option after documented discussion, with expected outcomes, about the role of SCT with a BMT physician not part of the study team

b. Cohort B: Patients previously treated with B cell directed engineered cell therapy who meet one of the following criteria:
    i. partial response or no response to prior cell therapy
    ii. CD19+ relapse after prior cell therapy
    iii. demonstrated early (≤6 months from infusion) B cell recovery suggesting loss of engineered cells
c. Patients with prior or current history of CNS3 disease will be eligible if CNS disease is responsive to therapy (at infusion, must meet criteria in **Section 5.3**)

3. Documentation of CD19 tumor expression in bone marrow, peripheral blood, CSF, or tumor tissue by flow cytometry at relapse (or a recent sample in the case of refractory disease). If the patient has received CD19-directed therapy, then the flow cytometry should be obtained after this therapy to show CD19 expression.
4. Adequate organ function defined as:
    a. A serum creatinine based on age/gender as follows:

	Maximum Serum Creatinine (mg/dL)	
Age	**Male**	**Female**
3 months to < 2 years	0.6	0.6
2 to < 6 years	0.8	0.8
6 to < 10 years	1.0	1.0
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

    b. ALT≤ 500 U/L
    c. Bilirubin ≤2.0 mg/dl
    d. Must have a minimum level of pulmonary reserve defined as ≤ Grade 1 dyspnea, < Grade 3 hypoxia; DLCO ≥ 40% (corrected for anemia) if PFTs are clinically appropriate as determined by the treating investigator
    e. Left Ventricular Shortening Fraction (LVSF) ≥ 28% or Ejection Fraction (LVEF) ≥ 45% confirmed by ECHO, or adequate ventricular function documented by a scan or a cardiologist.
5. Age 3 months to 29 years.
6. Adequate performance status (Lansky or Karnofsky score ≥50).
7. Subjects of reproductive potential must agree to use acceptable birth control methods, as described in protocol **Section 4.3**.

4.2. Exclusion Criteria

1. Active hepatitis B or active hepatitis C.
2. HIV Infection.
3. Active acute or chronic graft-versus-host disease (GVHD) requiring systemic therapy.
4. Concurrent use of systemic steroids or immunosuppression at the time of cell infusion or cell collection, or a condition, in the treating physician's opinion, that is likely to require steroid therapy or immunosuppression during collection or after infusion. Steroids for disease treatment at times other than cell collection or at the time of infusion are permitted. Use of physiologic replacement hydrocortisone or inhaled steroids is permitted as well.
5. CNS3 disease that is progressive on therapy, or with CNS parenchymal lesions that might increase the risk of CNS toxicity.
6. Pregnant or nursing (lactating) women.
7. Uncontrolled active infection.
8. Active medical disorder that, in the opinion of the investigator, would substantially increase the risk of uncontrollable CRS or neurotoxicity.

Please refer to the Concomitant Therapy Section 5.7 for windows related to apheresis and huCART19 infusion.

4.3. Patient Recruitment and Screening

Patients will be identified through the clinical practices of the investigator or sub-investigators and through referrals from outside hospitals and physicians. The study will be posted on clinicaltrials.gov.

Female patients of reproductive potential (women who have reached menarche and have not experienced treatment-related premature ovarian failure) must have negative serum pregnancy test performed at the time of screening and a negative urine pregnancy test within 48 hours of T cell infusion.

Due to the unknown risks of the CAR T cells with respect to pregnancy, as well as risks associated with lymphodepleting chemotherapy, it is recommended that all subjects of reproductive potential use at least one medically acceptable form of contraception for at least 1 year after their last infusion of CAR T cells. Investigators shall counsel subjects on the importance of pregnancy prevention and the implications of an unexpected pregnancy.
Medically acceptable birth control includes one of the following methods:

- Condoms (male or female) with or without a spermicidal agent
- Diaphragm or cervical cap with spermicide
- Intrauterine device (IUD)
- Hormonal-based contraception

Patients who are not of reproductive potential (females who are pre-menarchal or have experienced treatment-related premature ovarian failure or males who have documented azoospermia) do not require the use of contraception. Acceptable documentation of sterilization, azoospermia, and premature ovarian failure is specified below:

Written documentation by clinician or clinician's staff through one of the following:

1. Tanner I or age <11
2. Physician report/letter
3. Operative report or other source documentation in the patient record (a laboratory report of azoospermia is required to document successful vasectomy)
4. Discharge summary of the sterilization procedure or hysterectomy, and/or salpingectomy, oophorectomy
5. Laboratory report of azoospermia
6. Follicle stimulating hormone measurement elevated into the menopausal range

4.4. Withdrawal of Subjects

4.4.1. When and How to Withdraw Patients

Subjects who enroll but do not receive huCART19 cells will be prematurely discontinued from the study, will not be followed, and will be replaced in the study.

Reasons for premature discontinuation prior to receipt of CART19 cells include, but are not limited to, the following:

1. The subject is lost to follow-up.
2. The judgment of the principal investigator.
3. Patient non-compliance with the study therapy and/or clinic appointments.
4. Pregnancy: Withdraw subject if pregnancy occurs prior to the huCART19 infusion. If pregnancy occurs after the subject has received huCART19 cells, they will be kept active in the study for safety and pregnancy follow-up and outcome. No subsequent huCART19 infusions will be administered.
5. Voluntary withdrawal; a subject may remove himself/herself from the study at any time without prejudice. A subject may withdraw from the study at any time they wish to withdraw consent.
6. Progression of malignancy requiring alternative medical, radiation or surgical intervention.
7. A serious adverse event that requires the subject be withdrawn from the trial if the SAE occurs prior to the huCART19 T cell infusion.
8. Failure to produce a product that meets the release criteria (other than dose).
9. Termination of the study

Reasons for discontinuation of subjects after receipt of huCART19 cells include, but are not limited to, the below. Subjects may not be discontinued from primary follow-up prior to the Day 28 safety follow-up visit for reasons other than subject withdrawal of study consent or death.

1. The subject is lost to follow-up.
2. Voluntary withdrawal: a subject may remove himself/herself from the study at any time.
3. Disease progression of targeted malignancy
4. Receipt of alternative treatment for their targeted disease
5. Completion of study follow-up
6. Termination of the study

Subjects who do not complete the study protocol will be considered to have prematurely discontinued the study. The reasons for discontinuation (for example, voluntary withdrawal, toxicity, death) must be recorded on the case report form. Final study evaluations will be completed at the time of discontinuation.

4.4.2. Data Collection and Follow-up

Follow-up data collection after gene modified cell therapy clinical trials is specified by the FDA.

In the event that a subject cannot return to the study site for follow-up visits because of subject preference or geographical concerns, the subject's primary care physician and/or local oncologist will be asked to provide information from the subject's medical record to the study team at protocol defined time points (including the results of any routine care examinations and/or laboratory assessments), and assist in the collection of protocol required blood samples (if applicable) which will be sent for protocol required analyses.

Every effort will be made to contact patients who appear to be lost to follow-up in order to obtain survival data at minimum. In the event a patient fails to complete the follow-up requirements, documentation of all attempts to contact the patient includes at least 3 telephone contacts (on different days and at different times of the day), and a certified letter.

Subjects who complete follow-up as part of this protocol or discontinue participation early for any reason (per **Section 4.4.1** above), will be encouraged to enroll in a separate 15 year long term follow-up protocol to further evaluate long term adverse events related to the investigational product and survival.

4.4.3. Replacement of Subjects

All subjects who receive huCART19 cells as part of this study will be considered evaluable. Subjects who enroll but do not receive a huCART19 cell infusion will be removed from the study and replaced.

5. INVESTIGATIONAL PRODUCT

5.1. Description

Humanized CART19 (huCART19) cells are autologous T cells (collected from the patient) that have been engineered to express an extracellular single chain antibody (scFv) with specificity for CD19 linked to an intracellular signaling molecule comprised of a tandem signaling domain of the 4-1BB and TCRζ signaling modules.

Possible toxicities associated with the administration of huCART19 cells include transient fever, chills, nausea, and rigors. In order to minimize these events, subjects will receive premedication as instructed below in **Section 5.5**. Later toxicities (7-21 days post infusion) are likely to be related to CRS, or possibly tumor lysis. Toxicities that could potentially occur but are unprecedented are primarily related to the gene transfer are discussed in greater detail in the huCART19 Investigator Brochure. In addition, management of such toxicities is described in **Section 8.5**.

5.2. Cohort Assignment

Cohort assignment will occur at enrollment:

- **Cohort A**: Patients with newly diagnosed VHR B-ALL or high-risk relapse of B-ALL
- **Cohort B:** Patients with poor response to prior B cell directed engineered cell therapy

5.3. Subject Eligibility to Receive huCART19 Transduced Cells

For Day 0 Infusion:

1. Disease response: No evidence of high/accelerating disease burden that would, in the opinion of the treating physician, put the subject at significant potential risk for uncontrollable CRS or neurotoxicity.
2. All subjects must undergo influenza testing within 10 days prior to the first planned huCART19 infusion during the October-May influenza season. The test does not need to be repeated prior to subsequent huCART19 infusions. If the subject is positive for influenza, oseltamivir phosphate (Tamiflu®) or equivalent should be administered per package insert. The subject must complete treatment prior to receiving the huCART19 infusion. The test does not need to be repeated after treatment, however if influenza signs and symptoms are present, the huCART19 infusion should be delayed until the subject is asymptomatic.
3. Ability to maintain performance status as indicated in initial eligibility criteria.
4. If s/p allogeneic transplant, > 6 months from transplant.
5. At least 2 weeks from other investigational treatments.
6. Subjects experiencing toxicities prior to huCART19 infusion will have their infusion schedule delayed until these toxicities have resolved. The specific toxicities warranting delay of T cell infusions include:
    a. Pulmonary: Grade 3 or greater hypoxia or presence of radiographic abnormalities that are progressive
    b. Cardiac: New cardiac arrhythmia not controlled with medical management
    c. Hypotension requiring pressor support
    d. Neurologic: acute/ongoing neurologic toxicity > Grade 1 with the exception of controlled seizures or fixed neurologic deficits that have been stable/improving over the prior 3 months
    e. Uncontrolled active Infection

Subjects with CNS3 Disease - Additional Infusion Criteria:

1. Disease status:
    a. If CNS3 by spinal fluid involvement, stable/responding disease as indicated by:
        i. stable or decreasing CSF WBC, and
        ii. total CSF WBC < 100 in a sample obtained within 5 days of huCART19 infusion.
    b. If CNS3 by MRI findings, there must be interval stability or improvement on MRI within 2 weeks of infusion
    c. If CNS3 by cranial nerve findings, there must be stability or improvement of these cranial nerve findings on exam post intervention
2. Subjects with CNS3 disease requiring radiation therapy must be at least 8 weeks post cranial radiation at huCART19 infusion
3. Subjects must have no acute/ongoing neurologic toxicity > Grade 1 with the exception of a history of controlled seizures or fixed neurologic deficits that have been stable/improving over the past 3 months

Reinfusion:

1. Subjects should not experience a significant change in performance or clinical status compared to their previous study visit that would, in the opinion of the treating physician, increase the risk of experimental cell infusion. Fever may delay or preclude infusion.

2. Subjects experiencing new laboratory abnormalities that in the opinion of the treating investigator or PI feels may impact subject safety or the subjects' ability to receive huCART19 T-cells, may have their infusion delayed until both the treating investigator and PI determine it is clinically appropriate to proceed with the infusion.

Retreatment Infusion:

1. Subjects should not experience a significant change in performance or clinical status compared to their previous study visit that would, in the opinion of the treating physician, increase the risk of experimental cell infusion. Fever may delay or preclude infusion.
2. Subjects experiencing new laboratory abnormalities that in the opinion of the treating investigator or PI feels may impact subject safety or the subjects' ability to receive huCART19 T-cells, may have their infusion delayed until both the treating investigator and PI determine it is clinically appropriate to proceed with the infusion.
3. All subjects must undergo influenza testing within 10 days prior to their huCART19 retreatment infusion during the October-May influenza season. The test does not need to be repeated prior to subsequent huCART19 infusions. If the subject is positive for influenza, oseltamivir phosphate (Tamiflu®) or equivalent should be administered per package insert. The subject must complete treatment prior to receiving their huCART19 infusion. The test does not need to be repeated after treatment, however if influenza signs and symptoms are present, the huCART19 infusion should be delayed until the subject is asymptomatic.

5.4. huCART19 Treatment Regimen

Subjects will receive a single dose of huCART19 cells on Study Day 0. A cell dose of $5x10^6$ huCART19 cells/kg will be targeted. The acceptable target dose range is $2x10^5$ – $5x10^6$ huCART19 cells/kg, with the maximum single dose of huCART19 cells to be administered is $2.5x10^8$. We will use the following schedule:

If the minimum target dose is not achieved in manufacturing, a product that meets all release criteria may be infused at the investigator's discretion.

Timing of additional huCART19 infusions is described in **Section 6.13**. The same dosing parameters listed above for huCART19 will apply for reinfusion/retreatment doses.

5.5. Preparation and Administration of huCART19

In addition to the language below, please see the Investigational Product Handling Manual for further details on product thawing, transport, and labeling.

Manufacturing

huCART19 cell manufacturing is performed at the University of Pennsylvania Clinical Cell and Vaccine Production Facility (CVPF) and transported to the Cell and Gene Therapy Laboratory (CGTL) at CHOP.

Transport and CHOP Cell and Gene Therapy Laboratory Activities

The investigational product is transported in dry ice from the CVPF to the CGTL at CHOP. The frozen cells will be thawed at the CGTL at CHOP, the bag will be washed to recover all remaining volume with 5% HSA, the resulting volume will be drawn into a syringe with a new label affixed that includes the investigational product name, participant (denoted as DONOR or RECEIPIENT) ID number, expiration date and time,

"Caution: New Drug-Limited by Federal Law to Investigational Use", "For Autologous Use Only", and "Not Evaluated for Infectious Substances".

Additionally, an aliquot of the reconstituted product is sent for additional sterility testing. Sterility testing is not available in real-time prior to subject administration. If a positive sterility test is reported, the CGTL at CHOP will notify the Sponsor Medical Director and PI immediately.

The syringe is then transported to the subject's bedside for infusion. The final volume transported to subject's bedside will be recorded at the CHOP CGTL. The investigator must notify the Sponsor of any damaged or unusable cell products that were supplied to the investigator's site by the CGTL at CHOP.

Premedication
Side effects following T cell infusions include transient fever, chills, and/or nausea. It is recommended that the subject be pre-medicated with acetaminophen and an antihistamine prior to each CART19 cell infusion. Subjects should not receive systemic corticosteroids such as hydrocortisone, prednisone, methylprednisolone or dexamethasone at any time, except physiologic doses of hydrocortisone for adrenal support or in the case of a life-threatening emergency, since this may have an adverse effect on CART19 cell expansion and function.

Additional Safety Procedures Prior to Administration
Emergency medical equipment must be available during the infusion in case the subject has an allergic reaction, or severe hypotensive crisis, or any other reaction to the infusion. Vital signs (temperature, respiration rate, pulse, and blood pressure) will be taken before infusion.

5.6. huCART19 Product Infusions

Prior to each infusion, two individuals will independently verify all unique identifier information in the presence of the subject and confirm that the information on the product label is correctly matched to the participant. The product will then be checked to the subject per hospital standards.

Trained study staff will administer the huCART19 product by slow IV push via i.v. or central line, prior to the expiration date/time identified on the product label. **A leukoreduction filter must not be used for the infusion of the CART cell product**. The duration of CART19 administration will be based on the total volume to be infused and the recommended infusion rate of 10-20mL per minute.

Vital signs (temperature, respiration rate, pulse, blood pressure, and oxygen saturation as clinically indicated) will be measured within 10 minutes prior to the infusion and within 15 minutes after the infusion. Vital signs will also be measured 15 (+/- 5) minutes, 30 (+/- 5) minutes, 45 (+/- 5) minutes, and 60 (+/- 5) minutes after the infusion. If the subject's vital signs are not satisfactory and stable one hour post-CART19 infusion, vital signs will continue to be monitored as clinically indicated until stable. The subject will be discharged when medically stable and in accordance with hospital policy.

Febrile reaction
In the event of febrile reaction, an evaluation for infection should be initiated, and subjects managed appropriately with antibiotics, fluids and other supportive care as medically indicated and determined by the treating physician. In the event that the subject develops sepsis or systemic bacteremia following the CAR T cell infusion, appropriate cultures and medical management should be initiated. If a contaminated

huCART19 T cell product is suspected, the product can be retested for sterility using archived samples that are stored in the CVPF. Consideration of CRS as the most likely etiology should be given.

5.7. Concomitant Therapy

All prescription and nonprescription medication, vitamins, herbal and nutritional supplements, taken by the patient during the 30 days prior to screening/enrollment will be recorded. At every visit following the huCART19 infusions and until the subject has completed or has been discontinued from participation in the study, concomitant medications will be recorded in the medical record and on the appropriate CRF. Any additions, deletions, or changes of these medications will be documented. The following guidelines must be adhered to during the study:

- Granulocyte macrophage colony stimulating factor (GM-CSF) should be avoided due to potential to worsen CRS symptoms. G-CSF would be the preferred myeloid growth factor over GM-CSF, if medically indicated. The effects of G-CSF on CRS symptoms are unknown and can be used at the physician's discretion.
- Steroids or other immunosuppressant drugs should NOT be used within 48-72 hours (preferably 7 days) prior to the apheresis procedure.
- Steroids or other immunosuppressant drugs should NOT be used within 48 hours prior to or at any time following CART19 infusion unless under life threatening circumstances or at the physicians' discretion for CRS management.
- Recent or current use of inhaled steroids or physiologic replacement with hydrocortisone is allowed. Therapeutic doses of steroids must be stopped >48 hours prior to CART19 infusion. The following physiological replacement doses of steroids are allowed: 6-12 mg/m^2/day hydrocortisone or equivalent.
- Subjects with severe signs and symptoms attributable to CRS should be managed with the administration of tocilizumab or other anti-cytokine therapies **(Section 8.5.2)** for administration details.
- Neutropenic subjects will be administered broad-spectrum antibiotics at the start of fever and managed as per institutional SOPs.

6. STUDY PROCEDURES

Overview

The schedule of evaluations and study procedures are described in the Schedule of Evaluations located in **Appendix 1**. Also, refer to Sections **6.1** to **6.13** for further details of the schedule of each assessment, analysis and processing/handling of samples.

6.1. Screening (~Week -12 to Week -4)

Informed consent must be obtained before the patient can undergo any research-related procedures. Screening/enrollment assessments are described in this section and in the Schedule of Evaluations **(Appendix 1).** Values obtained in routine clinical care prior to informed consent can also be used for screening purposes.

- Verification of inclusion and exclusion criteria
- Demography including date of birth, sex, race, and ethnicity
- Documentation of medical history including prior and current medical conditions, and childbearing status
- Documentation of historical and concomitant medications and significant non-drug therapies

- Review of prior antineoplastic medications
- Physical exam and measurement of vital signs (height, weight, blood pressure, body temperature, respiration rate, and heart rate, and oxygen saturation via pulse oximetry as clinically indicated)
- Lansky or Karnofsky performance status
- ECHO
- Blood will be taken for Hematology, Coagulation, and Biochemistry analysis. Viral serologies (HIV, Hepatitis B/C). If the HCV antibody is positive, a screening HCV RNA by any RT-PCR or bDNA assay must be performed. Eligibility will be determined based on the screening value. The test is not required if documentation of a negative result of a HCV RNA test performed within 60 days prior to screening is provided.
- Serum pregnancy test for females of child bearing potential
- Serum IgG level
- Pulmonary function Test (DLCO)- if clinically appropriate as determined by the treating investigator
- BCR-ABL PCR (Ph+ ALL patients only; as clinically indicated)
- Bone marrow aspirate for disease as described in **Appendix 1**. If the results of recent testing (obtained at the time of the patient's most recent relapse) are available, these do not need to be repeated for enrollment.
- CNS evaluation- If CNS symptoms are present at Screening/Enrollment, then a lumbar puncture and brain imaging by MRI/CT will be performed to assess CNS leukemic involvement. If the results of a historical lumbar puncture (obtained at the time of the patient's most recent relapse) are available, this does not need to be repeated for enrollment.

In the event that the time between the screening visit and the infusion of huCART19 T cells exceeds the 12 week Screening/Enrollment Window the following will be repeated: Physical Examination, Performance Status Assessment, Complete Blood Count with differential and Platelet Count, Chemistry Panel, Pregnancy test. An ECHO must be performed within 8 weeks prior to the huCART19 infusion if not performed earlier.

6.2. Subject Enrollment

Assignment of subject numbers will occur at consent, will be in ascending order (18CT014-01, 18CT014-02, etc.) and no numbers will be omitted. Subject numbers will be used on all study documentation. Once assigned, the Subject Number must not be reused for any other subject and the Subject Number for that individual must not be changed, even if the subject is re-screened.

At the time a subject consents to participate in this study, a Consent Notification Form should be completed. Once required screening tests have been completed and the subject has been determined eligible by the physician-investigator, provide the documents listed below to:

Protocol Monitor and Sponsor Project Manager
Center for Cellular Immunotherapies (CCI)

Documents required:

1. Completed Enrollment Form
2. Redacted copy of signed patient consent and HIPAA authorization

3. Redacted source documentation to confirm enrollment/eligibility (including patient past medical history, laboratory, radiological reports, physical exam, concomitant medications and any other documentation to support subject met eligibility criteria and has completed all required screening assessments).

Upon informed consent completion and receipt of screening and eligibility documentation, a Sponsor Monitor will review and provide documentation that monitoring of eligibility has been completed. This documentation must be received prior to cell product manufacturing.

6.3. Apheresis (~Week -4 to -3)

After the monitoring visit for eligibility has been completed, a single large volume apheresis procedure may be carried out at a FACT-accredited apheresis center. PBMC are obtained for huCART19 manufacturing during this procedure. From a single leukapheresis, the intention is to harvest up to 50 x 10^9 (recommended 2-10x10^8/kg) cells to manufacture huCART19 T cells. Baseline samples for FDA look-back requirements and for research are also obtained and cryopreserved. The cell product is expected to be ready for release at least 4 weeks after beginning manufacturing.

Historical Apheresis Sample
Cryopreserved historical apheresis products collected from the patient prior to study entry are usable for huCART19 manufacturing if collected at an appropriately certified apheresis center and the product meets adequate mononuclear cell yields. If a historical apheresis product is not available, an apheresis procedure (as described above) will be performed for cell procurement after study eligibility has been confirmed.

Recommended criteria for apheresis product acceptance to initiate processing for clinical manufacturing to meet the dosing requirements includes the following specifications:
A CBC with automated differential on the apheresis product following completion of collection that reports absolute lymphocyte count (ALC) ≥ 500/µL. If the ALC <500/µL in the apheresis product, it is recommended that the CD3 cell count should be ≥ 150/µl for acceptance to begin processing for clinical manufacturing to achieve the target dose.

6.4. Assessment Types

6.4.1. Demographics, Eligibility Verification, Medical History, Historical and Concomitant Medications

Patient demographics will be recorded on the demography source documents. A physician-investigator will review inclusion/exclusion criteria to verify eligibility. A detailed medical history will be taken and recorded on the medical history CRF as well as current and prior (within 30 days of enrollment) concomitant medications.

6.4.2. Physical Exam

A complete physical examination will be performed according to **Appendix 1**. Physical examination will also be used to assess evidence of disease in the liver, spleen, and lymph node, skin, gum infiltration, and testicular involvement in males. Statements of “normal or WNL” will indicate lack of involvement. Height will be measured in centimeters and weight will be measured in kilograms.

Significant findings that are present prior to receipt of the investigational product must be included on

the Medical History eCRF. All findings made after receipt of the investigational product which meet the definition of an Adverse Event must be recorded on the Adverse Event CRF.

6.4.3. Vital Signs

Blood pressure, body temperature, oxygen saturation by pulse oximetry as indicated, respiration rate and heart rate will be measured as indicated in **Appendix 1** and will be recorded on source documents, and transcribed into the appropriate CRF pages. Vital signs (temperature, respiration rate, pulse, and blood pressure, and oxygen saturation as clinically indicated) will be measured within 10 minutes prior to the infusion and within 15 minutes after the infusion. Vital signs will also be measured at 30 (+/- 5) minutes, 45 (+/- 5) minutes, and 60 (+/- 5) minutes after the infusion. If the subject's vital signs are not satisfactory and stable one hour post-huCART19 infusion, vital signs will continue to be monitored as clinically indicated until stable. The subject will be discharged when medically stable and in accordance with hospital policy.

6.4.4. Performance Status

At Visits according to **Appendix 1**, the Karnofsky/Lansky performance scale will be used to evaluate the performance status of subjects.

Table 6-1: Karnofsky/Lansky Performance Scale

Karnofsky Scale (age ≥ 16 years)		Lansky Scale (age < 16 years)	
Able to carry on normal activity and to work; no special care needed.		**Able to carry on normal activity; no special care is needed.**	
100	Normal no complaints; no evidence of disease	**100**	Fully active
90	Able to carry on normal activity; minor signs or symptoms of disease	**90**	Minor restriction in physically strenuous play
80	Normal activity with effort; some signs or symptoms of disease	**80**	Restricted in strenuous play, tires more easily, otherwise active
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.		**Mild to moderate restriction**	
70	Cares for self; unable to carry on normal activity or to do active work	**70**	Both greater restrictions of, and less time spent in active play
60	Requires occasional assistance, but is able to care for most of his personal needs	**60**	Ambulatory up to 50% of the time, limited active play with assistance/supervision
50	Requires considerable assistance and frequent medical care	**50**	Considerable assistance required for any active play, fully able to engage in quiet play
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.		**Moderate to severe restriction**	
40	Disabled; requires special care and assistance	**40**	Able to initiate quiet activities

Karnofsky Scale (age ≥ 16 years)		Lansky Scale (age < 16 years)	
30	Severely disabled; hospital admission is indicated although death not imminent	30	Needs considerable assistance for quiet activity
20	Very sick; hospital admission necessary; active supportive treatment necessary	20	Limited to very passive activity initiated by others (e.g. television)
10	Moribund; fatal processes progressing rapidly	10	Completely disabled, not even passive play
0	Dead	0	Unresponsive

6.4.5. Cardiac Assessment: ECHO

An ECHO test is required to confirm protocol eligibility and should be repeated prior to infusion if more than 8 weeks prior to the huCART19 infusion or if clinically indicated.

6.4.6. Local Clinical Laboratory Evaluations

Screening/enrollment and other laboratory assessments will be performed accordingly to **Appendix 1**. Note: Additional assessments should be performed between visits as clinically required to follow all AEs. For all laboratory assessments that occur on Infusion Days, these should be performed **prior** to huCART19 infusion unless indicated otherwise.

The Investigator will evaluate the clinical significance of each applicable laboratory value outside of the reference range. This decision shall be based upon the nature and degree of the observed abnormality. Values which are considered clinically significant and/or related to huCART19 will be noted. The Investigator may choose to repeat any abnormal result once, in order to rule out laboratory error. Further details on recording abnormal laboratory values as AEs are described in **Section 8.1**.

Table 6-2: Local Clinical Laboratory Parameters Collection Plan

Test Category	Test Name
Hematology	Hematocrit, Hemoglobin, Platelets, White blood cells with a complete differential, including lymphoblasts
Chemistry	Glucose, BUN, Creatinine, Sodium, Potassium, Calcium, Total Protein, Albumin, Total Bilirubin, Alkaline phosphatase, ALT, AST, Phosphate, Magnesium
Additional chemistry, HLH/MAS and CRS screen – repeated if clinically indicated	Uric acid, LDH, Ferritin, CRP
Coagulation	Prothrombin time (PT), International normalized ratio (INR), Partial thromboplastin time (PTT), fibrinogen, D-dimer
Serology	HIV Ag/Ab combo, HBsAg, HCV antibody, HCV RNA-PCR (if applicable)
Influenza	Influenza A, Influenza B
B cells and T-Cell Subsets	CD19, CD4, CD3, CD8
Additional Assessments	Serum IgG levels, Serum or Urine Pregnancy Test

6.4.7. Additional Assessment of Laboratory Parameters for CRS

Hematology, coagulation and chemistry safety assessments will be performed at study visits according to **Appendix 1**.

As noted, side effects following CART19 cell infusions can induce high fevers and should be expected. If fevers are observed following huCART19 infusion, it is recommended that ferritin and CRP levels be monitored daily until resolution of the fever. Other chemistry parameters should be monitored per **Appendix 1** or as clinically indicated if CRS is suspected.

6.4.8. Viral Serology

Blood will be taken for HIV, Hepatitis B, and Hepatitis C at baseline. If the HCV antibody is positive, a screening HCV RNA by any RT-PCR or bDNA assay must be performed. Eligibility will be determined based on the screening value. The test is not required if documentation of a negative result of a HCV RNA test performed within 60 days prior to screening is provided.

6.4.9. Serum Immunoglobulin Levels

Peripheral blood will be sampled at screening and according to **Appendix 1**, for analysis of serum immunoglobulin.

6.4.10. Pregnancy Testing

Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, must use an effective contraception (see **Section 4.3** for details). For women of childbearing potential, a serum pregnancy test (β-HCG) will be performed at screening according to **Appendix 1**. During treatment, an additional pregnancy test will be performed within 48 hours prior to the first huCART19 infusion. Subjects should be instructed to inform their doctor of any positive urine pregnancy results not conducted at the clinic. Repeat serum pregnancy testing will be performed for confirmation of a positive urine pregnancy test. In case of pregnancy prior to huCART19 T cell infusion, subjects must be withdrawn from the study.

6.4.11. Research Assessments to Assess Engraftment, Persistence and Bioactivity

The following assessments will be performed according to **Appendix 1** and will be analyzed at the Translational and Correlative Studies Laboratory (TCSL). These tests may include:

- Serum Cytokines
- DNA qPCR for huCART19 persistence
- Flow cytometry for huCART19

RCL VSV-G testing will not be routinely performed as of Protocol V2. Blood samples will be collected and banked at pre-infusion and post-infusion at Months 3, 6, and 12. These samples may be used for future RCL VSV-G testing if indicated.

<u>Sample Handling</u>
Samples will be delivered, processed, and frozen as per SOP to the TCSL at the University of Pennsylvania. Samples will be stored for banking and bulk analyses. Documentation for sample receipt, processing, and storage and primary data from the research analyses will be collected and stored.

Translational and Correlative Studies Laboratory,
University of Pennsylvania
[redacted]

Unscheduled Research Sample Collections

Beyond the research sample collections scheduled for specific time points, up to 45 mL (3 tablespoons) of additional peripheral blood may be drawn twice per week to better characterize correlates of clinical events such as cytokine release syndrome. Marrow/LN collections would not exceed more than one procedure per month at physician's discretion. In addition, samples obtained during procedures performed for standard clinical indications (e.g., bone marrow samples, etc.) may be diverted for research use if such diversion does not substantially increase the risk of the procedure or compromise standard clinical diagnostic studies.

6.5. Cytoreductive/Lymphodepleting Chemotherapy

Prior to huCART19 cell infusion, an additional chemotherapy cycle is planned. While the choice of chemotherapy will be at the investigator's discretion depending on the patient's underlying disease and prior therapies, fludarabine (30 mg/m^2/day x 4 days) and cyclophosphamide (500 mg/m^2/day x 2 days) are the preferred agents, as there is the most experience with the use of these agents in facilitating adoptive immunotherapy on completed and ongoing pediatric CART19 trials. In addition, this is the lymphodepleting chemotherapy regimen prescribed with the FDA-approved CART19 therapy in ALL, Tisangenlecleucel (Kymriah™).

Note, the lymphodepleting chemotherapy prior to huCART19 cell infusion is **NOT required if patient's WBC ≤ 1,000 /µL. Additionally, if the period between chemotherapy and huCART19 infusion is delayed 4 or more weeks, the patient may need to be re-treated with lymphodepleting chemotherapy prior to huCART19 infusion.

The chemotherapy will be planned so that the last dose is completed approximately 2-5 days BEFORE the planned infusion of huCART19 cells. The purpose of the chemotherapy is to induce lymphopenia in order to facilitate the engraftment and homeostatic expansion of huCART19 cells. In addition, chemotherapy is intended to control the ALL. The chemotherapy is not investigational and may be given by a patient's local oncologist within the specified time frame.

All patients must undergo influenza testing within 10 days prior to the first planned huCART19 infusion during the October-May flu season. If the patient is positive for influenza, oseltamivir phosphate or equivalent should be administered per package insert. The patient must complete this course of treatment prior to receiving huCART19. The test does not need to be repeated after treatment, however if influenza signs and symptoms are present, the huCART19 infusions should be delayed until the patient is asymptomatic.

6.6. Pre-Infusion Evaluation (Day -1)

Subjects will undergo a Pre-Infusion Evaluation on Day -1 prior to their huCART19 infusion as outlined in the Schedule of Evaluations in **Appendix 1**.

6.7. huCART19 Infusion (Day 0)

Subject infusions are to begin 2 to 5 days after completion of chemotherapy as indicated in **Section 6.5**. Subjects will undergo tests and procedures in accordance with the Schedule of Evaluations in **Appendix 1**. This includes a CBC with differential prior to each infusion, as well as an assessment of CD3, CD4 and CD8 counts prior to the 1st infusion since chemotherapy is given in part to induce lymphopenia. Subjects will be pre-medicated as described in **Section 5.5**. huCART19 cells will be infused as described in **Section 5.4**.

6.8. Post-Infusion Follow-up Visits

Subjects will return to the clinic for safety follow-up and to have blood drawn for evaluation of the safety endpoints and secondary endpoints as outlined in the Schedule of Evaluations in **Appendix 1**.

Additional blood work for research evaluation may be requested at any time at the investigators' discretion, and is especially encouraged whenever there is a clinical concern for a potential toxicity related to CAR T cells.

6.9. Day 28: Follow Up

At the Day 28 visit, subjects will undergo tests and procedures in accordance with the Schedule of Evaluations in **Appendix 1**. Tumor response assessments will be done according to National Comprehensive Cancer Network (NCCN) v1 2013 guidelines **(Section 6.15)**.

6.10. Monthly Evaluations 2 to 6 Months Post Infusion

Subjects will follow-up with a study doctor or their primary oncologist on a monthly basis during months 2 to 6 post huCART19 cell infusion. At these study visits, subjects will undergo tests and procedures in accordance with the Schedule of Evaluations in **Appendix 1**. Tumor response will be measured according to **Sections 6.14** and **6.15** at Months 3 and Month 6.

6.11. Quarterly Evaluations for Up to 1 Year Post Infusion

Thereafter, subjects will be evaluated on a quarterly basis until 1 years post infusion. At these study visits, subjects will undergo tests and procedures in accordance with the Schedule of Evaluations in **Appendix 1**. Tumor response will be measured according to **Sections 6.14** and **6.15** at Months 9 and 12.

6.12. Long-term Follow-up

All subjects who receive CAR T cells will be encouraged to enroll in a separate 15 year long term follow-up protocol to further evaluate long term adverse events related to the investigational product at the time of discontinuation from the parent study and disease status/overall survival. Long-term follow-up includes evaluations that will be performed for up to 15 years on all subjects as recommended by the FDA for protocols utilizing integrating viral vectors.

6.13. Additional huCART19 Infusions

Timing and doses of additional CART19 infusions

For patients who have had i) evidence of brief B cell aplasia with subsequent B cell recovery (suggesting rapid CAR clearance), or ii) fever and other reversible toxicities without evidence of CAR expansion/LGLs, or iii) a partial or temporary response to the initial infusion, it may be that the initial dose of cells was not

adequate to produce a full therapeutic effect, or the cells may not have persisted long enough to produce longer-term disease control. In these cases, it may be appropriate to give more huCART19 cells. Additional huCART19 cells may be given no earlier than Day 14 and no less than 14 days from a prior infusion. Additional infusions will be administered as outlined below.

6.13.1. Reinfusions

Subjects in continued remission may receive additional huCART19 infusion(s) at the physician-investigator's discretion. Subjects must be evaluated by a physician-investigator for eligibility to receive additional huCART19 infusions according to criteria in **Section 5.3**. If performed, these additional infusions will be defined as "reinfusions'. For the purposes of study timepoint identification and reporting, each reinfusion will be considered a new treatment number (i.e. the first huCART19 infusion on Day 0 will be Treatment #1, the first reinfusion post Day 0 will be Treatment #2, the second reinfusion post Day 0 will be Treatment #3, etc).

If reinfusions are administered, additional safety follow-up visits are required. While all study visits will continue to be calculated based on the date of the initial huCART19 cell infusion (Day 0), subjects will also enter into a Reinfusion Section of the Schedule of Evaluations at the time reinfusion of huCART19 cells is initiated. Where Reinfusion Study Visits may overlap with existing study timepoints (i.e. Reinfusion Day 0 may overlap with the Month 3 study timepoint), all study tests/procedures required for both visits should be completed per protocol requirements but will not be duplicated. When post-reinfusion follow-up is completed per protocol. The subject will resume the study follow-up timepoints per the Primary Schedule of Evaluations. Additional follow-up post reinfusion may also be performed per physician-investigator discretion.

Both study visit identifiers will be used for data collection/reporting purposes (i.e. continuous study visit day and reinfusion visit timepoint). Please refer to the Schedule of Evaluations in **Appendix 1**.

6.13.2. Retreatment

Subjects who relapse post huCART19 infusion may also receive additional huCART19 infusions post-relapse at the physician-investigator's discretion. Subjects must be evaluated by a physician-investigator for eligibility to receive additional huCART19 infusions according to criteria in **Section 5.3**. If performed, additional infusions administered post-relapse will be defined as "retreatment".

If a decision is made to retreat a subject post-relapse, the subject will officially discontinue the Primary Schedule of Evaluations (**Appendix 1**) at the time of their first retreatment study visit/procedure (i.e. lymphodepleting chemotherapy) and enter the Retreatment Schedule of Evaluations (**Appendix 2**). Retreatment study visit timepoints will be distinguished from the primary Schedule of Evaluations by a "-R" identifier (i.e. Day 0-R, Day 1-R, etc). Follow-up will continue under the Retreatment Schedule of Evaluations for up to 12 months post retreatment.

Subjects in continued remission post-retreatment may also receive additional huCART19 infusions at the physician-investigator's discretion. Subjects must be evaluated by a physician-investigator for eligibility to receive additional CART19 cell infusions according to criteria in **Section 5.3**. If performed, these additional infusions post-retreatment will be defined as "reinfusions". For the purposes of study timepoint identification and reporting, each reinfusion post-retreatment will be considered a new treatment number under the Retreatment Schedule of Evaluations (i.e. the first huCART19 retreatment infusion on

Day 0-R will be Treatment #1, the first reinfusion post Day 0-R will be Treatment #2, the second reinfusion post Day 0-R will be Treatment #3, etc).

If reinfusions are administered, additional safety follow-up visits are required. While all study visits will continue to be calculated based on the date of the retreatment CART19 cell infusion (Day 0-R), subjects will also enter into a Reinfusion Section of the Retreatment Schedule of Evaluations at the time reinfusion of CART19 cells is initiated. Where Reinfusion Study Visits may overlap with existing study timepoints (i.e. Reinfusion Day 0 may overlap with the Month 3-R study timepoint), all study tests/procedures required for both visits should be completed per protocol requirements but will not be duplicated. When post-reinfusion follow-up is completed per protocol, the subject will resume the study follow-up timepoints per the Retreatment Schedule of Evaluations. Additional follow-up post reinfusion may also be performed per physician-investigator discretion.

Both study visit identifiers will be used for data collection/reporting purposes (i.e. continuous retreatment study visit day and reinfusion visit timepoint). Please refer to the Retreatment Schedule of Evaluations in **Appendix 2** for additional information.

6.14. Efficacy Assessments

Tumor response assessments will be done at baseline (prior to huCART19 infusion) and then at Day 28 and Months 3, 6, 9 and 12 after huCART19 cell infusion or until the patient requires alternative therapy for their disease. Assessments will be made as clinically indicated by physical exam, chest x-ray (if clinically indicated), CSF evaluation, hematology blood panel, and bone marrow biopsy and aspirate.

Disease assessment collection plan is detailed in **Table 6-3**.

Table 6-3: Disease Assessment Collection Plan (assessments are all standard of care)

Procedure	Pre-Infusion Assessments	Post Infusion Assessments
Bone marrow aspirate and biopsy for blast cell counts	~Day -5 to Day -1 (and after start of LD chemotherapy)	Day 28, Months 3, 6, 9 and 12
Peripheral Blood for blast, neutrophil and platelet cell counts	Mandated	Day 28, Months 3, 6, 9 and 12
CNS Evaluation[2]	If clinically indicated at Screening/Enrollment[1]; Mandated at Day -1	Mandated at Day 28; then as clinically indicated at subsequent evaluations
Physical Exam for extramedullary disease	~Day -5 to Day -1 (and after start of LD chemotherapy)	If clinically indicated
MRD assessment of bone marrow by flow cytometry (every patient)	~Day -5 to Day -1 (and after start of LD chemotherapy)	Day 28, Months 3, 6, 9 and 12
BCR-ABL assay of blood and bone marrow aspirate for patients with Ph+ ALL	As clinically indicated	Day 28, Months 3, 6, 9 and 12- as clinically indicated

**Lymph node aspirate performed as clinically indicated.

1- If CNS symptoms are present at Screening/Enrollment, then a lumbar puncture and brain imaging by MRI/CT will be performed to assess CNS leukemic involvement. If the results of a historical lumbar puncture (obtained at the time of the patient's most recent relapse) are available, this does not need to be repeated for enrollment.
2- If CNS symptoms are present, additional testing may be required as per clinical discretion. This may include a lumbar puncture and brain imaging by MRI/CT.

6.14.1. Physical Exam

A physical examination will be used to assess evidence of extramedullary disease in the liver, spleen, lymph node, skin, gum infiltration, testicular involvement and other sites if applicable. The scope of assessments performed as part of this evaluation is based on the physician's clinical discretion. Extramedullary involvement is to be assessed pre-infusion and will be followed as clinically appropriate.

6.14.2. Bone Marrow Aspirate/Biopsy and Peripheral Blood

Bone marrow biopsies and aspirate will be measured for tumor evaluations and efficacy analysis per **Appendix 1**.

6.14.3. Cerebrospinal Fluid (CSF) Assessment

If CNS symptoms are present at Screening/Enrollment, a lumbar puncture will be performed to assess CNS leukemic involvement. CNS evaluations will also be performed at baseline (Day -1) and on Day 28. If CNS symptoms are identified at these timepoints, additional testing may be performed as per clinical discretion. Subsequent CNS evaluations and CSF assessments will be performed as clinically indicated (i.e. if CSF involvement previously confirmed or by the presence of neurologic symptoms). Additionally, CSF may be assessed as clinically indicated during the height of CRS.

CSF will be analyzed for cell count and differential, cytology, and for the presence of huCART19 cells.

6.14.4. Extramedullary Disease

If extramedullary disease is present prior to treatment, this will be followed as clinically appropriate.

6.14.5. Minimal Residual Disease (MRD)

All patients will have multiparameter flow cytometry on bone marrow aspirate for MRD status at each time point a bone marrow aspirate is performed **(Appendices 1 + 2)**.

6.14.6. Quantitative BCR-ABL: Ph+ ALL Patients

If clinically indicated, bone marrow aspirates sampled at the time points for tumor assessments will additionally be analyzed for quantitative BCR-ABL levels for Ph positive ALL patients only.

6.15. ALL Response Criteria

The response criteria will be evaluated accordingly to **Table 6-4**. The definitions are primarily based on the standardized response criteria defined by National Comprehensive Cancer Network (NCCN) Guidelines (NCCN, 2013 v.1) and further supported by the workshop report from American Society of Hematology (ASH) and the International Working Group (IWG) guideline for acute myeloid leukemia (AML). The Cheson IWG guideline and Appelbaum ASH report were used in recent drug approvals (e.g. Marqibo) in ALL, prior to the NCCN guideline availability. The NCCN guidance is a more recently published updated US based guideline for ALL.

Efficacy assessments (**Section 6.14**) will be performed based on bone marrow and blood morphologic criteria, physical examination findings, and imaging (as clinically indicated), along with laboratory assessments of CSF and bone MRD assessment. The overall disease response is determined at a given evaluation using the criteria described in **Table 6-4**.

Table 6-4: Overall Disease Response Classification at a Given Evaluation Time

Response category	Definition
Complete remission (CR)	All the following criteria are met: **Bone marrow** Trilineage Hematopoiesis (TLH) and < 5% leukemic blasts by morphology (confirmed by flow cytometry) **Peripheral blood** Neutrophils > 1.0×10^9/L, and Platelets > 100×10^9/L, and Circulating leukemic blasts < 1% by morphology (if blasts present, must be confirmed by flow cytometry) **Extramedullary disease** No evidence of extramedullary disease (no CNS disease, mediastinal disease, no other extramedullary sites involvement) **Transfusion independency** No platelet and/or neutrophil transfusions within 1 week before peripheral blood sample for disease assessment
Complete remission with incomplete blood count recovery (CRi)	All criteria for CR as defined above are met, except that the following exist: Neutrophils ≤ 1.0×10^9/L, or Platelets ≤ 100×10^9/L, or Platelet and/or neutrophil transfusions within week before peripheral blood sample for disease assessment
No response (Treatment failure)	Failure to attain the criteria needed for any response categories
Relapsed Disease	Only in patients with a CR or CRi: Reappearance of leukemic blasts in the blood (≥ 1% by morphology, must be confirmed by flow cytometry), or Reappearance of leukemic blasts in bone marrow (≥ 5% by morphology, confirmed by flow cytometry), or (Re-)appearance of any extramedullary disease after CR

Note:

The NCCN guidance has defined a progressive disease (PD) category. In this document, PD is considered the same as "No response" or "Treatment failure", which is consistent with the Cheson et al. (2003)[94] guideline. The difference between PD and "No response" in ALL is not believed to be clinical meaningful.

B cell aplasia status: In addition to disease response defined above, all patients will be evaluated for B cell aplasia status at pre-infusion and each response assessment time point thereafter. In particular, achievement of B cell aplasia will be the key biologic response endpoint for subjects who are enrolled in continued remission but have demonstrated B cell recovery after prior therapy with engineered T cells. B cell aplasia is defined as less than 3% of peripheral blood lymphocytes are CD19+ or an absolute CD19+ lymphocyte count of less than 50/µL.

7. STATISTICAL PLAN

7.1. Design Overview

This is a two cohort, open-label, phase 2 study to determine the efficacy of huCART19 in pediatric and young adult patients with CD19-expressing relapsed and refractory B-cell acute lymphoblastic leukemia.

7.2. Sample Size Justification

This is a phase 2 study of huCART19 to determine the efficacy of huCART19 in relapsed/refractory B-ALL. The primary endpoint of 1-year EFS will be determined in 2 cohorts based on exposure to prior B cell directed engineered cell therapy.

A total of 100 infused subjects is targeted. Cohort A will enroll up to approximately 62 patients with newly diagnosed VHR B-ALL or high-risk relapse of B-ALL to achieve a sample size of 52 infused patients for the primary endpoint; Cohort B will enroll up to approximately 54 patients with poor response to prior B cell directed engineered cell therapy to achieve a samples size of 48 infused patients. For cohort A, the null hypothesis is 40% 1-year EFS and the alternative hypothesis is 60% 1-year EFS. The sample size of 52 provides 95% power to test this hypothesis, using a one-sided one-sample Log-rank test with 10% type I error, assuming 1 year of follow up for each patient[95]. For cohort B, the null hypothesis is 15% 1-year EFS and the alternative hypothesis is 30% 1-year EFS. The sample size of 48 provides 91% power to test this hypothesis, using a one-sided one-sample Log-rank test with 10% type I error, assuming 1 year of follow up for each patient. We expect that we could infuse approximately 85% of enrolled patients, so we will enroll approximately 62 patients for cohort A and approximately 54 patients for cohort B to obtain the targeted number of infused patients.

7.3. Analysis Sets

- The **Enrolled Set** comprises all subjects who sign an informed consent form and are confirmed eligible for the study (i.e. excluding screen failure patients).
- The **Full Analysis Set (FAS)** comprises all subjects who received the huCART19 cells. The FAS will be used for all the analyses, including the primary, secondary, and exploratory analyses.

Definitions relevant to the Analysis Sets:

1) **Screen failure -** Any patient who fails to meet the inclusion/exclusion criteria specified by the protocol.

2) **Manufacturing failure** – Any patient who has manufactured huCART19 cells that do not meet the manufacturing release criteria.

7.4. Analysis of Primary Objective

The primary endpoint is the 1-year Event-Free Survival in patients with newly diagnosed VHR B-ALL or high-risk relapse of B-ALL (cohort A) and in patients with poor response to prior B cell directed engineered cell therapy (cohort B). Time to event or censoring is defined as the time from infusion to the first of any of the following events: no response, relapse, death due to any cause, or to the last day of follow up if no event occurs. All analyses will be performed for cohort A and B separately. Kaplan-Meier (KM) curves will be generated and one-sample Log-rank test will be used to test the 1-year EFS against the historical rate. One-year EFS and its 95% confidence interval (CI) will also be estimated based on the KM method.

7.5. Analysis of Secondary Objectives

- Overall remission rate is determined by the response at day 28, computed as the proportion of subjects with CR or CRi according to the response criterion described in **Section 6.15**. The proportion and its 95% CI will be calculated.
- 2-year EFS with 95% CI will estimated based on the KM method.
- Relapse-free Survival (RFS) is defined as the time from achievement of remission (CR or Cri) to the date of relapse or death due to ALL, or censored at the last day of follow-up. Relapse-free Survival will be assessed only in subjects with the best overall response of CR or CRi. KM curves will be generated and 2-year relapse-free survival with its 95% CI will be estimated.

All adverse events, CRS, and neurotoxicity will be summarized based on the incidence rate as the main source to characterize the safety profile of huCART19 administration. After completing post-infusion follow-up on this study, subjects will be asked to participate in a separate destination long-term follow-up study for up to 15 years after their first huCART19 infusion. Response data from this study may be used to support analysis of the 2-year EFS and RFS secondary objectives above.

7.6. Analysis of Exploratory Objectives

A nonlinear mixed effect model describing huCART19 expansion followed by biphasic decline will be fit to the qPCR cellular kinetic data. Descriptive statistics will be calculated for change over time as absolute values or fold change. Summary statistics will also be provided for measures of anti-CAR antibodies and cellular immunogenicity, and measures of checkpoint pathways (e.g. PD-1, CTLA-4, Tim-3, LAG-3) at time points before and after infusion.

8. SAFETY AND ADVERSE EVENTS

8.1. Definitions

Adverse Event

An ***adverse event*** (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Intercurrent illnesses or injuries should be regarded as adverse events.

Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- leads to a persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly or birth defect
- an important medical event

Note that hospitalizations that meet the following criteria should not be reported as serious adverse events:

- Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, such as preplanned study visits and preplanned hospitalizations for study procedures or treatment administration
- Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
- Social reasons and respite care in the absence of any deterioration in the patient's general condition

Note: Treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above is not a serious adverse event.

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the patient, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result-in patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Unexpected adverse events

An adverse event is considered unexpected if the event, and severity and/or frequency of the event, is not consistent with the risk information described in the investigator brochure or protocol. Please refer to the huCART19 Investigator Brochure for complete details.

Related adverse events

An adverse event is considered related to participation in the research if there is a reasonable possibility that an event was caused by an investigational product, intervention, or research-required procedures. For the purposes of this study, "reasonable possibility" means there is evidence to suggest a causal relationship. Related adverse events will be classified as possibly related, probably related, and definitely related:

- **Possibly Related**: There is some evidence to suggest a causal relationship, however other factors may have contributed to the event.
- **Probably Related:** There is evidence to suggest a causal relationship, and the influence of other factors is unlikely.

- **Definitely Related:** There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.

Adverse Event Reporting Period

Collection of adverse events will begin at the time of apheresis and will continue until the subject is off-study. For subjects who do not undergo apheresis on this study (i.e. historical apheresis product is available), adverse event reporting will begin on Day 0 (from the start of the first huCART19 infusion) until the subject is off-study.

If a subject is taken off study within 30 days of the T-cell infusion, all SAEs experienced within 30 days after the T-cell infusion should be reported to the sponsor. Any SAEs experienced after this 30 day period should be reported to the sponsor if the investigator suspects a causal relationship to the study treatment.

Preexisting Condition/General Physical Examination Findings

A preexisting condition is one that is present at the start of the Adverse Event Reporting Period. All clinically significant abnormalities should be recorded as a preexisting condition on the medical history eCRF. During the course of the study, a preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens. Preexisting conditions that improve should also be recorded appropriately.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Laboratory abnormalities that meet the criteria for Adverse Events should be followed until they have returned to normal or an adequate explanation of the abnormality is found. When an abnormal laboratory or test result corresponds to a sign/symptom of an already reported adverse event, it is not necessary to separately record the lab/test result as an additional event. Laboratory abnormalities, that do not meet the definition of an adverse event, should not be reported as adverse events. A Grade 3 or 4 event (severe) as per CTCAE does not automatically indicate a SAE unless it meets the definition of serious as defined above and/or as per investigator's discretion. Whenever possible, a diagnosis, rather than a symptom should be provided (i.e. anemia instead of low hemoglobin).

8.2. Recording of Adverse Events

Safety will be assessed by monitoring and recording potential adverse effects of the treatment using the Common Terminology Criteria version 5.0 (CTCAE v5.0) at each study visit. Patients will be monitored by medical histories, physical examinations, and blood studies to detect potential toxicities from the treatment. If CTCAE grading does not exist for an adverse event, the severity of mild, moderate, severe, life-threatening, and death, corresponding to Grades 1-5, will be used whenever possible.

At each contact with the subject, the investigator must seek information on adverse events by non-directive questioning and, as appropriate, by examination. Adverse events also may be detected when

they are volunteered by the subject during the screening process or between visits, or through physical examination, laboratory test, or other assessments. Information on all adverse events should be recorded in the source documentation. All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis. To the extent possible, adverse events should be recorded as a diagnosis and symptoms used to make the diagnosis recorded within the diagnosis event. Do not list symptoms separately if a diagnosis can be assigned. The safety team may require events be reported separately if they occur as SAEs (or in the context of a SAE) even if they can also be considered a constituent of another AE such as CRS.

All adverse events occurring during the adverse event reporting period (defined in **Section 8.1** above) must be recorded.

As much as possible, each adverse event should be evaluated to determine:

1. The severity grade (CTCAE Grade 1-5)
2. Its duration (Start and end dates)
3. Its relationship to the study treatment- [Reasonable possibility that AE is related: No (unrelated/unsuspected) or Yes (a suspected adverse reaction)]. If yes (suspected) - is the event possibly, probably or definitely related to the investigational treatment?
4. Expectedness to study treatment- [Unexpected- if the event severity and/or frequency is not described in the investigator brochure and protocol (in the absence of an investigator brochure)].
5. Action taken with respect to study or investigational treatment (none, dose adjusted, temporarily interrupted, permanently discontinued, unknown, not applicable)
6. Whether medication or therapy taken (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
7. Whether it is serious, where a serious adverse event (SAE) is defined as in **Section 8.1**.

All adverse events should be treated appropriately. If a concomitant medication or non-drug therapy is given, this action should be recorded. Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study treatment, the interventions required to treat it, and the outcome.

Progression of malignancy, documented appropriately in the medical records, should not be reported as a serious adverse event. Adverse events that occur concurrently with the progression of malignancy but that are not related to disease progression (i.e. deep vein thrombosis or hemoptysis) will be reported as an adverse event as described above. Progression of malignancy resulting in death should be reported as a serious adverse event.

Serious adverse events that are still ongoing at the end of the adverse event reporting period must be followed to determine the final outcome. Any serious adverse event that occurs after the adverse event reporting period and is considered to be possibly related to the study treatment or study participation, should be recorded and reported.

<u>Grading System of Cytokine Release Syndrome (CRS)</u>

A protocol specific grading system (**Table 8-1**) has been developed to capture cytokine release syndrome in CAR T-cell protocols. Please refer to the current version of the huCART19 Investigator Brochure for additional detail on CRS in CAR T-cell therapy.

For the purposes of reporting and grading on clinical trials using CART19 cells, we will use the following grading for CRS Toxicity. The start date of CRS is a retrospective assessment of the date of onset of persistent fevers and/or myalgia consistent with CRS and not explained by other events (i.e. sepsis). The stop date of CRS is defined as the date when the patient has been afebrile for 24 hours and off vasopressors for 24 hours. For the purposes of defining the CRS start date, a fever is defined as a temperature of 38.0° C (100.4° F).

Table 8-1: CRS Grading Criteria

CRS Toxicity Grade (Modified)				
1	**2**	**3**	**4**	**5**
Mild reaction: Treated with supportive care such as anti-pyretics, anti-emetics	Moderate reaction requiring IV fluids or parenteral nutrition; some signs of organ dysfunction (i.e. grade 2 creatinine or grade 3 liver function tests [LFTs] related to CRS and not attributable to any other condition). Hospitalization for management of CRS related symptoms including fevers with associated neutropenia.	More severe reaction: Hospitalization required for management of symptoms related to organ dysfunction including grade 4 LFTs or grade 3 creatinine related to CRS and not attributable to any other conditions. This excludes management of fever or myalgias. Includes hypotension treated with IVFs* or low-dose pressors, coagulopathy requiring fresh frozen plasma (FFP) or cryoprecipitate, and hypoxia requiring supplemental oxygen (nasal cannula oxygen, high flow oxygen, Continuous Positive Airway Pressure [CPAP] or Bilateral Positive Airway Pressure [BiPAP]. Patients admitted for management of suspected infection due to fevers and/or neutropenia may have grade 2 CRS.	Life-threatening complications such as hypotension requiring high dose pressors (see Table 8-2), or hypoxia requiring mechanical ventilation	Death

*CRS Grade 3 language clarification: "hypotension treated with intravenous fluids" is further defined as hypotension requiring multiple fluid boluses for blood pressure support.

Table 8-2: High Dose Vasopressor Use

Definition of "High-Dose" Vasopressors	
Vasopressor	Dose for ≥ 3 hours
Norepinephrine monotherapy	≥ 0.2 mcg/kg/min
Dopamine monotherapy	≥ 10 mcg/kg/min
Phenylephrine monotherapy	≥ 200 mcg/min
Epinephrine monotherapy	≥ 0.1 mcg/kg/min
If on vasopressin	High-dose if vaso + Norepinephrine Equivalent (NE) of ≥0.1 mcg/min (using Vasopressin and Septic Shock Trial (VASST) formula)
If on combination vasopressors [96]	Norepinephrine equivalent of ≥ 20 mcg/min (using VASST formula)
Vasopressin and Septic Shock Trial (VASST) Equivalent Equation: Norepinephrine equivalent dose = [norepinephrine (mcg/min)] + [dopamine (mcg/kg/min) ÷ 2] + [epinephrine (mcg/min)] + [phenylephrine (mcg/min) ÷10] Criteria from Russell et al, 2008[96]. **Note:** Pediatric weight adjustments should be taken into consideration.	

Grading System for Neurotoxicity

As described in the current version of the huCART19 Investigator Brochure, neurotoxicity has been observed with CAR T cell products. Since the myriad manifestations of CAR-related neurotoxicity may not fall cleanly under a single CTCAE category, this study will utilize the grading system in Table 8-3 to categorize adverse events that are judged by the investigator to constitute neurotoxicity related to CAR T cells. To align with CTCAE V5.0 reporting criteria, for qualifying events, the CTCAE Term "Nervous System Disorders – Other, Specify" will be utilized in accordance with the corresponding grades below, with additional toxicity detail reported as "CAR Neurotoxicity". Constituent adverse events of neurotoxicity will contribute to an evaluation of the overall neurotoxicity grade as described in Table 8-3. Specific component neurotoxicity adverse events will also be reported separately if they include events of seizures, cerebral edema, and papilledema.

Table 8-3: Neurotoxicity Grading System

Neurologic Event Grade	Event Descriptions
Grade 1	• Mild impairment or confusion • No objective evidence of increased intracranial pressure

Table 8-3: Neurotoxicity Grading System

Neurologic Event Grade	Event Descriptions
Grade 2	• Moderate impairment or confusion • No objective evidence of increased intracranial pressure (or not assessed) • Symptoms limiting instrumental ADLs • Aphasia- receptive or expressive characteristics; ability to read, write or communicate intelligibly is not impaired or only mildly impaired

Grade 3	• Severe Impairment
	  ○ Grade 3 confusion (severe disorientation)
	  ○ Grade 3 somnolence (obtundation or stupor)
	  ○ Grade 3 encephalopathy (severe symptoms)
	• Stage 1-2[#] Papilledema (if assessed); CSF opening pressure <20 mmHg (if assessed)
	• Seizure
	• Symptoms limiting self-care ADLs
	• Aphasia- Severe receptive or expressive characteristics, impairing ability to read, write or communicate intelligibly

Grade 4	• Life-threatening consequences
	  ○ Grade 4 confusion (life-threatening consequences; urgent intervention indicated)
	  ○ Grade 4 somnolence (obtundation or stupor)
	  ○ Grade 4 encephalopathy (life-threatening consequences)
	• Unable to participate in CARTOX-10* assessment
	• Stage 3-5 papilledema[#] (if assessed); CSF opening pressure ≥20 mmHg (if assessed); evidence of cerebral edema on brain imaging.
	• Status epilepticus
	• New, focal and sustained motor weakness
	• Requirement for mechanical ventilation due to neurologic symptoms

[#]Papilledema is staged according to the modified Frisen scale.

8.3. Reporting of Serious Adverse Events

Every SAE, **regardless of suspected causality**, occurring during the adverse event reporting period defined in **Section 8.1** must be reported to the sponsor within 24 hours of learning of its occurrence. The original SAE notification may take place by email to meet the 24 hour reporting window.

Within 3 business days of initial knowledge of the event, the investigator must submit a complete SAE form to the Sponsor along with any other diagnostic information that will assist the understanding of the event. The Investigator will keep a copy of this SAE Form on file at the study site.

New or follow-up information for SAEs should be promptly reported as updates become available.

At a minimum follow-up SAE Forms should be submitted:

- Within 1 week of ICU admission or any life-threatening event
- Within 2 weeks of hospital discharge

Follow-up information should be submitted as an amendment to the initial SAE form, and should include both the follow-up number and report date. The follow-up information should describe whether the event has resolved or continues, if there are any changes in assessment, if and how it was treated, and whether the patient continued or withdrew from study participation.

Report serious adverse events by email to:

Attention: Clinical Safety Manager or designee
Center for Cellular Immunotherapies (CCI)
University of Pennsylvania

At the time of the initial notification, the following information should be provided:

- Study identifier
- Subject number
- A description of the event
- Date of onset
- Current subject status
- Whether study treatment was discontinued
- The reason the event is classified as serious
- Investigator assessment of the association between the event and study treatment
- Expectedness relative to investigational product(s)

8.3.1. Investigator Reporting: Notifying Local Regulatory Committees

Notify the local site regulatory review committees as per institutional requirements.

8.4. Pregnancies

To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to protocol sponsor within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. If a pregnancy occurs on study, this will be reported as an SAE using the SAE Report Form.

Pregnancy outcomes must be collected for the female partners of any males who took study treatment in this study. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

8.5. Toxicity Management, Stopping Rules and Study Termination

It is expected that AEs will occur frequently in this population based on the underlying advanced hematologic malignancy and that these can be SAEs. Therefore, there is no specific occurrence of SAEs that define a stopping rule, but the review of SAEs will form the basis for potential early stopping of the study. Only unexpected SAEs that are related to the huCART19 cells would define a stopping rule. The review of these adverse events, and any decision to prematurely stop subject enrollment, will be determined by the Sponsor.

In addition to the above, premature termination of the clinical trial may occur because of a regulatory authority decision, change in opinion of the DSMB or local regulatory review committees, determination that there are problems in the cell product generation, as a result of safety concerns, or at the discretion of the Sponsor or study investigators. Additionally, recruitment may be stopped for reasons of particularly low recruitment, protocol violations, or inadequate data recording.

8.5.1. Criteria for Stopping or Pausing Treatment on the Study

The study will be stopped if:

- Any patient develops uncontrolled T cell proliferation that does not respond to management.
- Grade 5 CRS is observed in 2 out of the first 3 subjects, 3 out of the first 8 subjects, 4 out of the first 14 and 5 at any time.
- Grade 4 neurotoxicity that does not resolve to Grade 1 within two weeks, observed in 2 out of the first 3 subjects, 3 out of the first 8 subjects, 4 out of the first 14, and 5 at any time.
- Grade 4 GVHD is observed in 2 out of the first 3 subjects, 3 out of the first 8 subjects, 4 out of the first 14 and 5 at any time.
- Premature study termination may occur if the Investigator, Sponsor, DSMB, ACC DSMC, or any appropriate independent review board or regulatory body decides for any reason that patient safety may be compromised by continuing the study.
- Premature study termination may occur if the Sponsor decides to discontinue the development of the intervention to be used in this study.

The stopping rules for CRS, neurotoxicity and GVHD were calculated based on the lower limit of the 90% confidence interval exceeding 10%.

8.5.2. General Toxicity Management Considerations

Replication-competent lentivirus (RCL).

RCL may in theory be generated during the CAR T cell manufacturing phase or subsequently after introduction of vector transduced cells into the subject. However, an RCL resulting from the production phase is highly unlikely since elements are incorporated in the design of the vector system that minimize vector recombination and generation of RCL. Furthermore, the vector used to transduce the product undergoes sensitive assays to confirm that the vector is RCL negative before it can be released for use in product manufacture. Though theoretical, development of RCL could pose a risk to both the subject and their close contact(s), and therefore, samples will be archived during the course of the trial. In the event of a suspected RCL, measures to detect RCL will be followed as per the recent FDA guidance, *Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up*.

Clonality and insertional oncogenesis

Monitoring for T cell clonal outgrowth will be performed by qPCR for huCART19 and by CBC count. huCART19 levels that continue to rise in a manner inconsistent with observed kinetics (i.e. initial expansion after infusion) will be examined to determine if these are expected due to subject's clinical course (reappearance of disease) or other cause. If clonal expansion is suspected, the patient's T cells will be evaluated for the pattern of vector insertion.

If integration site analysis reveals mono- or oligoclonality and/or integration at or near an oncogenic locus, a monitoring plan, including follow-up molecular analyses, will be developed in collaboration between the PI and Regulatory Sponsor of the original study that is specific for the health care risks that are anticipated given the nature of the integration site and vector target cell type.

If integration site analysis reveals mono- or oligoclonality and/or integration at or near an oncogenic locus, a monitoring plan, including follow-up molecular analyses, will be developed in collaboration between the PI and Regulatory Sponsor of the original study that is specific for the health care risks that are anticipated given the nature of the integration site and vector target cell type.

Uncontrolled T cell proliferation

Toxicity associated with allogeneic or autologous T cell infusions has been managed with a course of pharmacologic immunosuppression. CAR T cell associated toxicity has been reported to respond to systemic corticosteroids[97]. If uncontrolled T cell proliferation occurs (grade 3 or 4 toxicity related to CAR T cells), subjects may be treated with corticosteroids. Subjects will be treated with pulse methylprednisolone (2mg/kg i.v. divided q12 hr x 2 days), followed by a rapid taper.

B cell depletion

In the event of clinically significant hypogammaglobulinemia (i.e. systemic infections), patients may be given intravenous immunoglobulin (IVIG) by established clinical dosing guidelines to restore normal levels of serum immunoglobulin levels.

Infusion reaction

Acetaminophen and an antihistamine may be repeated every 6 hours as needed. It is recommended that patients not receive treatment-dose corticosteroids at any time, except as per the CRS algorithm, since this may have an adverse effect on huCART19 cells.

Febrile reaction

In the event of febrile reaction, an evaluation for infection should be initiated, and patients managed appropriately with antibiotics, fluids and other supportive care as medically indicated and determined by the treating physician. In the event that the patient develops sepsis or systemic bacteremia following CAR T cell infusion, appropriate cultures and medical management should be initiated. If a contaminated huCART19 T cell product is suspected, the product can be retested for sterility using archived samples that are stored in the CVPF. Consideration of CRS (see below) should be given.

Cytokine Release Syndrome (CRS)/Macrophage Activation Syndrome (MAS)

Tocilizumab should be used as a single, weight-based dose of 8-12 mg/kg at the time of hemodynamic instability (as per Tocilizumab package insert). This management approach is designed to avoid life-threatening toxicities, so the timing of the tocilizumab should be individualized, in close consultation with the study team. Steroids have not always been effective in this setting and may not be necessary given the rapid response to tocilizumab. Because steroids will interfere with huCART19 function and efficacy, if used, they should be rapidly tapered.

Upon developing the prodrome of high-persistent fevers following huCART19 infusion, patients should then be followed closely. Infection and tumor lysis syndrome work up should be immediately undertaken. The pharmacy should be notified of the potential need for tocilizumab. Patient management in an intensive care unit may be required and the timing is dependent upon local institutional practice. In addition to supportive care, tocilizumab may be administered in cases of moderate to severe CRS, especially if the patient exhibits any of the following:

- Hemodynamic instability despite intravenous fluid challenges and moderate stable vasopressor support
- Worsening respiratory distress, including pulmonary infiltrates, increasing oxygen requirement including high-flow O_2, and/or need for mechanical ventilation.
- Any other signs or symptoms of rapid deterioration despite medical management

Not all Grade 4 CRS reactions following CD19-targeting CAR T cells have been immediately treated with tocilizumab and decisions are, in part, based upon the rapidity of the syndrome onset and underlying patient reserve.

Please refer to **Table 8-4** below for the CRS Treatment Algorithm.

CRS has been associated with biochemical and physiologic abnormalities consistent with MAS. Moderate to extreme elevations in serum C-reactive protein (CRP) and ferritin have been seen with CART19 associated CRS, however the magnitude and kinetics vary greatly between individual patients. CRS management decisions should be based upon clinical signs and symptoms and response to interventions, not these laboratory values *per se*.

CTCAE grading of CRS relates to its occurrence with acute infusional toxicities, whereas the CRS associated with CART19 therapy is not acute, but rather delayed. Refer to **Section 8.2** and **Table 8-1** for modified definitions of grading of huCART19 delayed CRS events.

Table 8-4: CRS Treatment Algorithm

Pretreatment
Acetaminophen/paracetamol and diphenhydramine /H1 anti-histamine Prophylaxis for complications of TLS as appropriate
CART19 infusion **Prodromal syndrome:** low grade fevers, fatigue, anorexia (hours to days)
Observation, rule out infection (surveillance cultures) Antibiotics per local guidelines (febrile neutropenia) Symptomatic support
Symptom progression: High fevers, hypoxia, mild hypotension **1st Line Management:** Oxygen, fluids, low dose vasopressor support, antipyretics Monitor/manage complications of TLS
Further symptom progression: • Hemodynamic instability despite intravenous fluids and moderate to “high dose” vasopressor support OR • Worsening respiratory distress, including pulmonary infiltrates increasing oxygen requirement including high-flow Oxygen (O2) and/or need for mechanical ventilation OR • Rapid clinical deterioration **2nd Line Management:**
Tocilizumab: IV infusion over 1 hour • Patient weight < 30 kg: 12 mg/kg i.v. • Patient weight ≥ 30 kg: 8 mg/kg i.v. (max dose 800 mg) Hemodynamic and respiratory support
Lack of clinical improvement while awaiting tocilizumab response **3rd Line Management:**
Consider other diagnosis causing clinical deterioration (i.e. sepsis, adrenal insufficiency) If no improvement with 1st dose of tocilizumab within 12 to 18 hours, consider steroids (plan rapid taper after hemodynamic normalization): 2 mg/kg methylprednisolone as an initial dose, then 2 mg/kg per day. As steroids are tapered quickly, monitor for adrenal insufficiency and need for hydrocortisone replacement If no response to steroids within 24 hours, consider 2nd dose of Tocilizumab (dosed as above) Hemodynamic and respiratory support

Lack of clinical improvement while awaiting response to 3rd line management **4th Line Management:**
Consider other diagnosis causing clinical deterioration (i.e. sepsis, adrenal insufficiency) If no response to steroids and 2nd dose of tocilizumab within 24 hours or further clinical deterioration, consider siltuximab 11 mg/kg IV over 1 hour Hemodynamic and respiratory support
Lack of clinical improvement while awaiting response to 4th line management **5th Line Management:**
Consider other diagnosis causing clinical deterioration (i.e. sepsis, adrenal insufficiency) In ongoing CRS despite prior therapy, consider anti-T cell therapies such as cyclophosphamide, anti-thymocyte globulin, or alemtuzumab Hemodynamic and respiratory support

Neurologic Toxicity

Guidelines for grading and management of neurologic toxicity are described in **Table 8-3** and **Table 8-5,** respectively. The mechanism underlying neurotoxicity after CAR T cell administration is poorly understood. Anecdotal evidence suggests that anti-IL6 therapies, which are effective for CRS, do not prevent or reverse neurotoxicity. Neurotoxicity can be asynchronous with cytokine release syndrome, often reaching its maximum intensity later than CRS or even arising after resolution of CRS. The following principles guide the recommended management of neurotoxicity:

- Most instances of neurologic toxicity are self-limited, but life-threatening complications such as cerebral edema develop in some cases of patients treated with other CAR products, such as JCAR015[98] . This has not been seen in children treated with huCART19 or murine CART19 (CTL019) at CHOP or on the multisite trials. Corticosteroid therapy has not been necessary in any pediatric patient with moderate (grade 2-3) neurotoxicity. The efficacy of corticosteroids is unproven, and corticosteroids may adversely affect CAR T cell efficacy, so a risk-benefit determination must be made by the CAR team and treating physician. In exceptional circumstances, cytotoxic chemotherapy could be considered in grade 4 cases based on a report that cerebral edema improved promptly after cyclophosphamide chemotherapy in one case[99].
- Consultation with a neurologist is helpful for properly documenting and tracking neurologic abnormalities, evaluating for other potential etiologies of neurologic abnormalities, and managing neurologic emergencies such as seizure or elevated intracranial pressure.
- Coagulopathy and thrombocytopenia should be aggressively managed. Both coagulopathy and thrombocytopenia often develop with CRS. Intracranial hemorrhage has been observed in conjunction with severe neurotoxicity. In addition, a recent report suggests that thrombocytopenia is an independent risk factor for CAR-related neurotoxicity, and platelets may serve as a source of mediators that stabilize the endothelium and counteract destabilizing effects of cytokines elaborated during CAR T cell proliferation[93].

CRS should be managed concurrently with neurotoxicity according to guidelines enumerated above. It is difficult to distinguish delirium secondary to CRS from mild/early CAR-related neurotoxicity (and these phenomena may not be distinct pathophysiologically).

Table 8-5: Neurotoxicity Management

Neurologic Event Grade	Toxicity Management Guidelines - If subject experiencing concurrent CRS, follow CRS management guidelines in parallel.
Grade 1	Consider non-sedating antiseizure medicines (eg levetiracetam) for seizure prophylaxis in patients with history of neurotoxicity, seizure, or focal MRI findings.
Grade 2	Consider neurology consultation. Consider non-sedating antiseizure medicines (eg.levetiracetam) for seizure prophylaxis in patients with history of neurotoxicity, seizure, or focal MRI findings.
Grade 3	Consider neurology consultation. Consider head imaging, preferably MRI; consider lumbar puncture and/or funduscopic exam. Consider EEG monitoring. Administer antiseizure medicines for clinical or subclinical seizures in consultation with neurology. Recommend starting with non-sedating antiseizure medicines (eg.levetiracetam). Administer platelet transfusion if platelet count <30000/µl; monitor for coagulopathy- if fibrinogen < 150 mg/dl give cryoprecpitate. If the patient is worsening rapidly and especially if concern for cerebral edema exists, consider the administration of dexamethasone up to 0.2mg/kg every 6 hours (maximum 10mg/dose, 16mg/day).
Grade 4	Consider neurology consultation. Perform head imaging, preferably MRI; consider lumbar puncture and/or funduscopic exam. Consider EEG monitoring. Administer antiseizure medicines for clinical or subclinical seizures in consultation with neurology. Recommend starting with non-sedating antiseizure medicines (eg.levetiracetam). If the patient is worsening rapidly and especially if concern for cerebral edema exists, consider the administration of dexamethasone up to 0.2mg/kg every 6 hours (maximum 10mg/dose, 16mg/day). If no response in severe cases, consider the administration of methylprednisone 1000 mg intravenous per day for a total of 3 days then taper as indicated. Administer platelet transfusion if platelet count <30000/µl; monitor for coagulopathy- if fibrinogen < 150 mg/dl give cryoprecipitate. Consider cytotoxic chemotherapy (e.g., cyclophosphamide 1.5 g/m2) for especially severe cases, especially where the patient is not improving.

Tumor lysis syndrome

TLS resulting in renal insufficiency, or rapidly rising uric acid, or evidence of organ dysfunction will be managed with fluids, allopurinol, and/or rasburicase as clinically indicated and determined by the treating physicians.

GVHD

Patients treated with huCART19 post allogeneic SCT will be monitored for GVHD.

8.6. Protocol Exceptions and Deviations

Exception:

A one time, **intentional** action or process that departs from the approved study protocol, intended for **one** occurrence. If the action disrupts the study progress, such that the study design or outcome (endpoints) may be compromised, or the action compromises the safety and welfare of study subjects, **advance** documented approval from the Regulatory Sponsor and local regulatory review committees per institutional guidelines is required. Approval from the Regulatory Sponsor must be received prior to submission to the IRB and local regulatory review committees for approval.

Deviation:

A one time, **unintentional** action or process that departs from the approved study protocol, involving one incident and **identified retrospectively**, after the event occurred. If the impact on the protocol disrupts the study design, may affect the outcome (endpoints) or compromises the safety and welfare of the subjects, the deviation must be reported to the Regulatory Sponsor within 10 business days of PI knowledge, and to local regulatory review committees per institutional guidelines. Acknowledgement from the Regulatory Sponsor must be received prior to submission to local regulatory review committees.

Other deviations should be appropriately documented (such as a subject missing a visit unless critical/important treatment or procedure was missed and must have been done at that specific time) per site policies/procedures.

Include the following information on the Sponsor supplied exception/deviation form: protocol number, subject study number, comprehensive description of the exception/deviation from the protocol, rationale, and corrective and preventative action plan (deviations only). Ensure all completed exception/deviation forms are signed by the Principal Investigator (or physician sub-investigator) and submitted to the Sponsor Project Manager for review.

Attention: Sponsor Project Manager
Center for Cellular Immunotherapies (CCI)
University of Pennsylvania

Once approval of the exception request or acknowledgement of the deviation has been granted by the Regulatory Sponsor, the exception or deviation will be submitted to all applicable committees for review and approval/acknowledgement as per institutional guidelines.

8.7. Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.8. Independent Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) comprised of four individuals including physicians with experience in oncology and/or gene transfer therapy will be assembled and will work under a charter

developed for safety oversight of this study. The DSMB will provide guidance/advice to the Regulatory Sponsor and Clinical Investigators. The DSMB will evaluate subject safety as specified in the DSMB Charter.

A DSMB meeting will occur approximately every 6 months. If necessary, additional meetings of the DSMB may be held if safety issues arise in between scheduled meetings.

It is envisioned that the DSMB may make four types of recommendations, namely:

- No safety or efficacy issues, ethical to continue the study as planned.
- Serious safety concerns precluding further study treatment, regardless of efficacy.
- Overwhelming evidence for futility, recommend stopping the study.
- Recommendation to continue the study but proposing an amendment to the protocol (e.g., incorporate an additional safety assessments).

A sponsor representative will share the outcome of the DSMB meeting with the PI via email, for submission to local regulatory review committees as required per institutional policy.

9. DATA HANDLING AND RECORDKEEPING

9.1. Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from patients in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research patient to revoke their authorization for use of their PHI.

In the event that a patient revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of patient authorization. For patients that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the patient is alive) at the end of their scheduled study period.

9.2. Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, patients' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, patient files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

The investigator must maintain source documents for each patient in the study, consisting of case and visit notes (hospital or clinical medical records) containing demographic and medical information, laboratory data, electrocardiograms and the results of any other tests or assessments. All information

recorded on the eCRFs must be traceable to source documents in the patient's file. The investigator must also keep the original signed informed consent form, and a signed copy must be given to the patient and/or legally acceptable surrogate.

9.3. Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All entries will be entered into an electronic data capture system (EDC). The Principal Investigator is responsible for assuring that the data entered into eCRF is complete, accurate, and that entry and updates are performed in a timely manner.

9.4. Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

10. STUDY MONITORING, AUDITING, AND INSPECTING

10.1. Study Monitoring Plan

This study will be monitored according to the Sponsor Data and Safety Monitoring Plan.

Interim Monitoring Visits will be conducted during the course of the study. The Monitors will assure that submitted data are accurate and in agreement with source documentation; verify that investigational products are properly stored and accounted for; verify that subject consent for study participation has been properly obtained and documented; confirm that research subjects entered into the study meet inclusion and exclusion criteria; and assure that all essential documentation required by Good Clinical Practices (GCP) guidelines are appropriately filed. At the end of the study, Monitors will conduct a close-out visit and will advise on storage of study records and disposition of unused investigational products.

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2. Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory bodies. The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance offices.

The Principal Investigator must notify the Sponsor in real-time if an audit/inspection notification is received.

11. ETHICAL CONSIDERATIONS

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

Per local requirements, all subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator obtaining the consent.

The protocol is listed on clinicaltrials.gov.

12. STUDY FINANCES

12.1. Funding Source

This study will be funded through CHOP internal funds.

12.2. Conflict of Interest

All Investigators will follow their Institutional Policy on Conflicts of Interest Related to Research.

12.3. Patient Stipends or Payments

There is no patient stipend/payment for participation in this protocol.

12.4. Study Discontinuation

The study may be discontinued at any time by the IRB, the Sponsor, the FDA, or other government agencies as part of their duties to ensure that research subjects are protected.

13. PUBLICATION PLAN

Publication of the results of this trial will be governed by University of Pennsylvania policies. Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

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APPENDIX 1: Schedule of Evaluations

	Screening and Enrollment	Apheresis	Lymphodepleting Chemotherapy	Pre-Infusion	huCART19 Infusion	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up	Follow-Up	Monthly Follow-Up	Quarterly Follow-Up	REINFUSIONS[25]		
															Prior to Reinfusion	huCART19 Reinfusion Day 0	+3 (+/- 1d), +7 (+/- 1d), +10 (+/-1d), +14 (+/- 1d) Post-Reinfusion[31]
	~-12W to -4W	~-4W to -3W	~ -1W	~ -1D	Day 0	D1 (+1d)	D3 (+/-1d)	D7 (+/-2d)	D10 (+/-2d)	D14 (+/-2d)	D21 (+/-3d)	D28 (+/-3d)	M2, M3, M4, M5, M6 (+/-14d)	M9, M12 (+/-14d)	Pre-Reinfusion	Re-Infusion	Safety Follow-up
Informed Consent	X																
Interventions																	
Apheresis[6]		X															
Lymphodepleting Chemotherapy[4]			X												X[30]		
huCART19 Infusion[23]					X											X[25]	
Patient History/Clinical Assessments																	
Demography	X																
Inclusion/exclusion criteria	X																
Relevant medical history/current medical conditions	X																
Diagnosis and extent of cancer	X																
Prior antineoplastic therapy	X[7]																

	Screening and Enrollment	Apheresis	Lymphodepleting Chemotherapy	Pre-Infusion	huCART19 Infusion	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up	Follow-Up	Monthly Follow-Up	Quarterly Follow-Up	REINFUSIONS[25]		
															Prior to Reinfusion	huCART19 Reinfusion Day 0	+3 (+/- 1d), +7 (+/- 1d), +10 (+/-1d), +14 (+/- 1d) Post-Reinfusion[31]
	~-12W to -4W	~-4W to -3W	~ -1W	~ -1D	Day 0	D1 (+1d)	D3 (+/-1d)	D7 (+/-2d)	D10 (+/-2d)	D14 (+/-2d)	D21 (+/-3d)	D28 (+/-3d)	M2, M3, M4, M5, M6 (+/-14d)	M9, M12 (+/-14d)	Pre-Reinfusion	Re-Infusion	Safety Follow-up
Prior/concomitant medications	X		X----------											----------X	X----------		----------X
Physical examination	X			X	X	X	X	X	X	X	X	X	X	X		X	X
Performance status (Karnofsky or Lansky)	X			X	X	X	X	X	X	X	X	X	X	X		X	X
Height	X																
Weight	X				X												
Vital signs[24]	X			X	X[12]	X	X	X	X	X	X	X	X	X		X[12]	X
Laboratory assessments																	
Hematology (5 ml lavender top, EDTA)	X			X	X	X	X	X	X	X	X	X	X	X		X	X
Chemistry (3 ml SST)	X			X	X	X	X	X	X	X	X	X	X	X		X	X
Coagulation [PT, PTT, INR, fibrinogen, D-dimer] (4.5 ml blue top citrate)	X			X	X	X	X[13]	X	X[13]	X	X	X				X	X[13]
Urine or Serum Pregnancy Test[11] (1 ml SST)	X			X													
HIV Test (1ml SST)	X																
Viral Serology (Hepatitis B and C) (5ml red top, serum)	X																

	Screening and Enrollment	Apheresis	Lymphodepleting Chemotherapy	Pre-Infusion	huCART19 Infusion	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up	Follow-Up	Monthly Follow-Up	Quarterly Follow-Up	REINFUSIONS[25]		
															Prior to Reinfusion	huCART19 Reinfusion Day 0	+3 (+/- 1d), +7 (+/- 1d), +10 (+/-1d), +14 (+/- 1d) Post-Reinfusion[31]
	~-12W to -4W	~-4W to -3W	~ -1W	~ -1D	Day 0	D1 (+1d)	D3 (+/-1d)	D7 (+/-2d)	D10 (+/-2d)	D14 (+/-2d)	D21 (+/-3d)	D28 (+/-3d)	M2, M3, M4, M5, M6 (+/-14d)	M9, M12 (+/-14d)	Pre-Reinfusion	Re-Infusion	Safety Follow-up
Serum Immunoglobulin levels (1ml SST)- IgG only	X											X	X	X			
HLH/MAS and CRS Labs (Ferritin and CRP)[8]					X[8]	X	X	X	X	X	X	X				X	X
Influenza A + B			X[18]														
CD19					X							X	X	X			
T cell Subsets (CD3, CD4, CD8)					X							X					
Research Analyses[2, 3]																	
Serum ~5cc (Red top)				X	X[19]	X	X	X	X	X	X	X	X	X		X[32]	X
Exploratory analyses (i.e. Cytokines)				X	X	X	X	X	X	X	X	X					
PBMC ~25cc (Lavender, EDTA)				X				X	X	X	X	X	X	X		X[32]	X
DNA (qPCR CART19 persistence)[22]				X				X	X	X	X	X	X	X		X	X[29]

	Screening and Enrollment	Apheresis	Lymphodepleting Chemotherapy	Pre-Infusion	huCART19 Infusion	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up	Follow-Up	Monthly Follow-Up	Quarterly Follow-Up	REINFUSIONS[25]		
															Prior to Reinfusion	huCART19 Reinfusion Day 0	+3 (+/- 1d), +7 (+/- 1d), +10 (+/-1d), +14 (+/- 1d) Post-Reinfusion[31]
	~-12W to -4W	~-4W to -3W	~ -1W	~ -1D	Day 0	D1 (+1d)	D3 (+/-1d)	D7 (+/-2d)	D10 (+/-2d)	D14 (+/-2d)	D21 (+/-3d)	D28 (+/-3d)	M2, M3, M4, M5, M6 (+/-14d)	M9, M12 (+/-14d)	Pre-Reinfusion	Re-Infusion	Safety Follow-up
Exploratory analyses (i.e. multiparametric flow cytometry)				X				X	X	X	X	X					
Bone Marrow/LN aspirate[27] (~5 cc lavender top, EDTA)	X			X[14]								X[1]	X[1]	X[1]			
Exploratory analyses (i.e. qPCR for huCART19 homing)	X			X								X[1]	X[1]	X[1]			
Marrow Serum (~2 cc red top)	X			X								X[1]	X[1]	X[1]			
Exploratory analyses (i.e. cytokines)	X			X								X[1]					
Disease Monitoring[20]																	
Tumor response assessments				X								X[1]	X[1]	X[1]	X[28]		
Physical exam (extramedullary disease)[16]	X			X													

	Screening and Enrollment	Apheresis	Lymphodepleting Chemotherapy	Pre-Infusion	huCART19 Infusion	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up	Follow-Up	Monthly Follow-Up	Quarterly Follow-Up	REINFUSIONS[25]		
															Prior to Reinfusion	huCART19 Reinfusion Day 0	+3 (+/- 1d), +7 (+/- 1d), +10 (+/-1d), +14 (+/- 1d) Post-Reinfusion[31]
	~-12W to -4W	~-4W to -3W	~ -1W	~ -1D	Day 0	D1 (+1d)	D3 (+/-1d)	D7 (+/-2d)	D10 (+/-2d)	D14 (+/-2d)	D21 (+/-3d)	D28 (+/-3d)	M2, M3, M4, M5, M6 (+/-14d)	M9, M12 (+/-14d)	Pre-Reinfusion	Re-Infusion	Safety Follow-up
Bone marrow aspirate/biopsy (cytogenetics/FISH if appropriate)	X[5]			X[14]								X[1]	X[1]	X[1]			
CNS evaluation[9]	X			X								X	As clinically indicated				
Mediastinal disease assessment (Chest X-ray)[16]	X																
MRD by flow cytometry	X			X								X	X[1]	X[1]			
BCR-ABL (Ph+ patients only)[27]	X											X	X[1]	X[1]			
Safety																	
Adverse events[26]		X----------X													X----------X		
ECHO[21]	X																
Pulmonary Function Test (DLCO)/ Pulmonary Reserve[15]	X																
Total clinical blood draw (mL)	27	0	0	19	12.5	12.5	12.5	12.5	12.5	12.5	12.5	16.5	16.5	16.5	0	12.5	12.5

	Screening and Enrollment	Apheresis	Lymphodepleting Chemotherapy	Pre-Infusion	huCART19 Infusion	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up	Follow-Up	Monthly Follow-Up	Quarterly Follow-Up	REINFUSIONS[25]		
															Prior to Reinfusion	huCART19 Reinfusion Day 0	+3 (+/- 1d), +7 (+/- 1d), +10 (+/-1d), +14 (+/- 1d) Post-Reinfusion[31]
	~-12W to -4W	~-4W to -3W	~ -1W	~ -1D	Day 0	D1 (+1d)	D3 (+/-1d)	D7 (+/-2d)	D10 (+/-2d)	D14 (+/-2d)	D21 (+/-3d)	D28 (+/-3d)	M2, M3, M4, M5, M6 (+/-14d)	M9, M12 (+/-14d)	Pre-Reinfusion	Re-Infusion	Safety Follow-up
Total research blood draw (mL)	0	0	0	30	5	0	0	30	30	30	30	30	30	30	0	30	30
Total blood draw (mL)	27.0	0	0	49	17.5	12.5	12.5	42.5	42.5	42.5	42.5	46.5	46.5	46.5	0	42.5	42.5
Total blood draw (Tbsp.; approximately)	2	0	0	3	1	1	1	3	3	3	3	3	3	3	0	3	3

Note on outpatient visits: the Oncology clinic is not open on holidays or weekends. Visits that fall in these days will be rescheduled as soon as is practical.

[1] Tumor response assessments will be performed at Day 28, Months 3, 6, 9 and 12 after initial huCART19 cell infusion (Refer to Section 6.14 for further details and frequency).

[2] TCSL has requested lab samples for research be sent to TCSL as soon as collected. If required to keep research labs after hours, please keep red tops upright, lavender tubes should be room temperature on rotating platforms. In the event that something unexpected occurs, additional research sample collection may be done as necessary. Blood collections are not to exceed 3 tablespoons of blood twice in one week time window. Marrow/LN collections would not exceed more than one procedure per month. This would be at the PI's discretion.

[3] RCL VSV-G testing will not be routinely performed as of Protocol V2. Blood samples will be collected and banked at pre-infusion and post-infusion at Months 3, 6, and 12. These samples will be used for future RCL VSV-G testing if indicated.

[4] Lymphodepleting chemotherapy prior to huCART19 cell infusion is NOT required if WBC ≤ 1,000/µL. The chemotherapy will be planned so that the last dose is completed approximately 2-5 days BEFORE the planned infusion of huCART19 cells. Please refer to Section 6.5 for complete details.

[5] Bone marrow performed for disease and MRD assessment. If the results of a historical marrow or tissue biopsy (obtained at the time of the patient's most recent relapse) are available, this does not need to be repeated for enrollment.

[6] Apheresis will be performed to obtain a target of 50 x 10^9 (10^9/kg) white blood cells to manufacture huCART19 T cells. Apheresis can occur any time after the monitoring visit for eligibility is completed. Cryopreserved historical apheresis products collected from the patient prior to study entry are usable for huCART19 manufacturing if collected at an appropriately certified apheresis center and the product meets adequate mononuclear cell yields.

[7] Prior antineoplastic therapy to be collected up through the lymphodepleting chemotherapy prior to huCART19 cell infusion.

[8] Repeated as clinically indicated or if HLH/MAS or CRS is suspected. If fevers are observed following huCART19 infusion, every attempt will be made to monitor additional Ferritin and CRP levels **daily** at fever onset and until resolution of the fever.

[9] If CNS symptoms are present at Screening/Enrollment then a lumbar puncture and brain imaging by MRI/CT will be performed to assess CNS leukemic involvement. If the results of a historical lumbar puncture (obtained at the time of the patient's most recent relapse) are available, this does not need to be repeated for enrollment. CNS evaluations will also be performed at Day -1 and Day 28. If CNS symptoms are identified at these timepoints, additional testing may be performed as per clinical discretion. If CSF involvement was confirmed at Enrollment, then lumbar punctures will be repeated thereafter as clinically indicated. Please refer to **Section 6.14.3** for complete details.

[10] Months 3, 6, 9 and 12

[11] Pregnancy test for females of childbearing potential only. Pre-infusion pregnancy test to be performed within 48 hours prior to the huCART19 infusion.

[12] Vital signs will be measured within 10 minutes prior to the infusion and within 15 minutes after the infusion. Vital signs will also be measured at 30 (+/- 5) minutes, 45 (+/- 5) minutes, and 60 (+/- 5) minutes after the infusion. If the subject's vital signs are not satisfactory and stable one hour post-huCART19 infusion, vital signs will continue to be monitored as clinically indicated until stable.

[13] D-dimer required on Days 3 and 10.

[14] Bone marrow biopsy/aspirate to be performed between initiation of LD chemo and huCART19 T-cell infusion.

[15] DLCO $\geq$ 40% (if PFTs are clinically appropriate as determined by the treating investigator); Pulse Oxygen > 92% on room air

[16] A chest x-ray for mediastinal disease will be performed at Screening/Enrollment if clinically indicated. If extramedullary disease is present prior to treatment, this will be followed as clinically appropriate.

[17] Visit windows have been incorporated to allow for flexibility in scheduling however these visits cannot overlap and all individual follow-up visits must be performed.

[18] All subjects must undergo influenza testing within 10 days prior to the first planned huCART19 infusion during the October-May flu season. If the subject is positive for influenza, oseltamivir phosphate (Tamiflu®) or equivalent should be administered per package insert. The subject must complete this course treatment prior to receiving huCART19. The test does not need to be repeated prior to the initial huCART19 infusion, however if influenza symptoms are present, the huCART19 infusions should be delayed until the subject is asymptomatic.

[19] Research blood (5 cc red top) to be taken between 20-120 minutes post-huCART19 infusion.

[20] Please refer to **Table 6-3** for details regarding Disease Monitoring requirements.

[21] ECHO must be performed within 8 weeks prior to the huCART19 infusion.

[22] Testing for huCART19 persistence by qPCR will continue until any 2 sequential tests are negative documenting loss of CAR T cells.

[23] See **Section 5.3** regarding criteria to proceed with huCART19 infusion.

[24] Vital sign assessments include blood pressure, body temperature, respiration rate, and heart rate, and oxygen saturation via pulse oximetry as clinically indicated.

[25] Subjects in continued remission may receive additional huCART19 infusions at the physician-investigator's discretion. Subjects must be evaluated by a physician-investigator for eligibility to receive additional huCART19 infusions according to criteria in **Section 5.3**. If performed, these additional infusions will be defined as "reinfusions". If reinfusions are administered, additional safety follow-up visits are required. While all study visits will continue to be calculated based on the date of the initial huCART19 infusion (Day 0), subjects will also enter into a Reinfusion Section of the Schedule of Evaluations at the time reinfusion of huCART19 cells is initiated. When Reinfusion Study Visits may overlap with existing study timepoints (i.e. Reinfusion Day 0 may overlap with the Month 3 study timepoint), all study tests/procedures required for both visits should still be completed per protocol requirements but will not be duplicated. When post-reinfusion follow-up is completed per protocol, the subject will resume the study follow-up per the primary Schedule of Evaluations. Additional follow-up post reinfusion may also be performed per physician-investigator discretion. Please refer to **Section 6.13** for additional information. Please refer to the Retreatment Schedule of Evaluations (**Appendix 2**) for retreatment with huCART19 post-relapse.

[26] Collection of adverse events will begin at the time of apheresis and will continue until the subject is off-study. For subjects who do not undergo apheresis on this study (i.e. historical apheresis product is available), adverse event reporting will begin on Day 0 (from the start of the first huCART19 infusion) until the subject is off-study.

[27] If LN aspirate performed as per clinical discretion.

[28] Disease assessments may be performed prior to reinfusion of huCART19 cells per clinical discretion. If a bone marrow aspirate is performed, a portion of this sample may also be used for research purposes.

[29] qPCR persistence testing performed post reinfusion on Days +7, +10, and +14 only.

[30] Lymphodepleting chemotherapy may be repeated prior to huCART19 reinfusions per clinical discretion. Please refer to **Section 6.5** for additional information.

[31] Additional follow-up post-reinfusion may also be performed per physician-investigator discretion.

[32] Research samples to be collected pre-infusion.

APPENDIX 2: Retreatment Schedule of Evaluations[24]

	Lymphodepleting Chemotherapy	Pre-Infusion	huCART19 Infusion	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up	Follow-Up	Monthly Follow-Up	Quarterly Follow-Up	REINFUSIONS[21]		
													Prior to Reinfusion	huCART19 Reinfusion Day 0	+3 (+/- 1d), +7 (+/- 1d), +10 (+/-1d), +14 (+/- 1d) Post-Reinfusion[28]
	~ -1W-R	~ -1D-R	D0-R	D1-R (+1d)	D3-R (+/-1d)	D7-R (+/-2d)	D10-R (+/-2d)	D14-R (+/-2d)	D21-R (+/-3d)	D28-R (+/-3d)	M2-R, M3-R, M4-R, M5-R, M6-R (+/-14d)	M9-R, M12-R (+/-14d)	Pre-Reinfusion	Reinfusion Day 0	Safety Follow-up
Interventions															
Lymphodepleting Chemotherapy[4]	X												X[25]		
huCART19 Infusion[15]			X											X[21]	
Patient History/Clinical Assessments															
Prior/concomitant medications	X----------											----------X	X----------		----------X
Physical examination		X	X	X	X	X	X	X	X	X	X	X		X	X
Performance status (Karnofsky or Lansky)		X	X	X	X	X	X	X	X	X	X	X		X	X
Weight			X												
Vital signs[7]		X	X[12]	X	X	X	X	X	X	X	X	X		X[12]	X
Laboratory assessments															
Hematology (5 ml lavender top, EDTA)		X	X	X	X	X	X	X	X	X	X	X		X	X
Chemistry (3 ml SST)		X	X	X	X	X	X	X	X	X	X	X		X	X

	Lymphodepleting Chemotherapy	Pre-Infusion	huCART19 Infusion	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up	Follow-Up	Monthly Follow-Up	Quarterly Follow-Up	REINFUSIONS[21]		
													Prior to Reinfusion	huCART19 Reinfusion Day 0	+3 (+/- 1d), +7 (+/- 1d), +10 (+/-1d), +14 (+/- 1d) Post-Reinfusion[28]
	~ -1W-R	~ -1D-R	D0-R	D1-R (+1d)	D3-R (+/-1d)	D7-R (+/-2d)	D10-R (+/-2d)	D14-R (+/-2d)	D21-R (+/-3d)	D28-R (+/-3d)	M2-R, M3-R, M4-R, M5-R, M6-R (+/-14d)	M9-R, M12-R (+/-14d)	Pre-Reinfusion	Reinfusion Day 0	Safety Follow-up
Coagulation [PT, PTT, INR, fibrinogen, D-dimer] (4.5 ml blue top citrate)		X	X	X	X[13]	X	X[13]	X	X	X				X	X[13]
Urine or Serum Pregnancy Test[11] (1 ml SST)		X													
Serum Immunoglobulin levels (1 ml SST)- IgG only										X	X	X			
HLH/MAS and CRS Labs (Ferritin and CRP)			X[8]	X	X	X	X	X	X	X				X	X
Influenza A + B	X[18]														
CD19			X							X	X	X			
T cell Subsets (CD3, CD4, CD8)			X							X					
Research Analyses[2, 3]															
Serum ~5cc (Red top)		X	X[19]	X	X	X	X	X	X	X	X	X		X[29]	X
Exploratory analyses (i.e. Cytokines)		X	X	X	X	X	X	X	X	X					
PBMC ~25cc (Lavender, EDTA)		X				X	X	X	X	X	X	X		X[29]	X

	Lymphodepleting Chemotherapy	Pre-Infusion	huCART19 Infusion	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up	Follow-Up	Monthly Follow-Up	Quarterly Follow-Up	REINFUSIONS[21]		
													Prior to Reinfusion	huCART19 Reinfusion Day 0	+3 (+/- 1d), +7 (+/- 1d), +10 (+/-1d), +14 (+/- 1d) Post-Reinfusion[28]
	~ -1W-R	~ -1D-R	D0-R	D1-R (+1d)	D3-R (+/-1d)	D7-R (+/-2d)	D10-R (+/-2d)	D14-R (+/-2d)	D21-R (+/-3d)	D28-R (+/-3d)	M2-R, M3-R, M4-R, M5-R, M6-R (+/-14d)	M9-R, M12-R (+/-14d)	Pre-Reinfusion	Reinfusion Day 0	Safety Follow-up
DNA (qPCR CART19 persistence)[5]		X				X	X	X	X	X	X	X			X[26]
Exploratory analyses (i.e. flow cytometry		X				X	X	X	X	X					
Bone Marrow/LN aspirate[23] (~5 cc lavender top, EDTA)		X[14]								X[1]	X[1]	X[1]			
Exploratory analyses (i.e. qPCR huCART19 homing)		X								X[1]	X[1]	X[1]			
Marrow Serum (~2 cc red top)		X								X[1]	X[1]	X[1]			
Exploratory analyses (i.e. cytokines)		X								X[1]					
Disease Monitoring[20]															
Tumor response assessments		X								X[1]	X[1]	X[1]	X[27]		
Physical exam (extramedullary disease)[16]		X													
Bone marrow aspirate/biopsy (cytogenetics/FISH if appropriate)		X[14]								X[1]	X[1]	X[1]			

	Lymphodepleting Chemotherapy	Pre-Infusion	huCART19 Infusion	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up[17]	Follow-Up	Follow-Up	Monthly Follow-Up	Quarterly Follow-Up	REINFUSIONS[21]		
													Prior to Reinfusion	huCART19 Reinfusion Day 0	+3 (+/- 1d), +7 (+/- 1d), +10 (+/-1d), +14 (+/- 1d) Post-Reinfusion[28]
	~ -1W-R	~ -1D-R	D0-R	D1-R (+1d)	D3-R (+/-1d)	D7-R (+/-2d)	D10-R (+/-2d)	D14-R (+/-2d)	D21-R (+/-3d)	D28-R (+/-3d)	M2-R, M3-R, M4-R, M5-R, M6-R (+/-14d)	M9-R, M12-R (+/-14d)	Pre-Reinfusion	Reinfusion Day 0	Safety Follow-up
CNS evaluation[9]		X								X	As clinically indicated				
Mediastinal disease assessment (Chest X-ray)[16]															
MRD by flow cytometry		X								X	X[1]	X[1]			
BCR-ABL (Ph+ patients only)[27]										X	X[1]	X[1]			
Safety															
Adverse events[6]	X----------											----------X	X----------		----------X
Total clinical blood draw (mL)	0	19	12.5	12.5	12.5	12.5	12.5	12.5	12.5	16.5	16.5	16.5	0	12.5	12.5
Total research blood draw (mL)	0	30	5	0	0	30	30	30	30	30	30	30	0	30	30
Total blood draw (mL)	0	49	17.5	12.5	12.5	42.5	42.5	42.5	42.5	46.5	46.5	46.5	0	42.5	42.5
Total blood draw (Tbsp.; approximately)	0	3	1	1	1	3	3	3	3	3	3	3	0	3	3

Note on outpatient visits: the Oncology clinic is not open on holidays or weekends. Visits that fall in these days will be rescheduled as soon as is practical.

[1] Tumor response assessments will be performed at Day 28, Months 3, 6, 9 and 12 after huCART19 cell infusion (Refer to **Section 6.14** for further details and frequency).

[2] TCSL has requested lab samples for research be sent to TCSL as soon as collected. If required to keep research labs after hours, please keep red tops upright, lavender tubes should be room temperature on rotating platforms. In the event that something unexpected occurs, additional research sample collection may be done as necessary. Blood collections are not to exceed 3 tablespoons of blood twice in one week time window. Marrow/LN collections would not exceed more than one procedure per month. This would be at the PI's discretion.

[3] RCL VSV-G testing will not be routinely performed as of Protocol V2. Blood samples will be collected and banked at pre-infusion and post-infusion at Months 3, 6, and 12. These samples will be used for future RCL VSV-G testing if indicated.

[4] Lymphodepleting chemotherapy prior to huCART19 cell infusion is NOT required if WBC ≤ 1,000/uL. The chemotherapy will be planned so that the last dose is completed approximately 2-5 days BEFORE the planned infusion of huCART19 cells. Please refer to **Section 6.5** for complete details.

[5] Testing for huCART19 persistence by qPCR will continue until any 2 sequential tests are negative documenting loss of CAR T cells.

[6] Collection of adverse events during retreatment will be continuous from the primary Schedule of Evaluations and will continue until the subject is off-study.

[7] Vital sign assessments include blood pressure, body temperature, respiration rate, and heart rate, and oxygen saturation via pulse oximetry as clinically indicated.

[8] Repeated as clinically indicated or if HLH/MAS or CRS is suspected. If fevers are observed following huCART19 infusion, every attempt will be made to monitor additional Ferritin and CRP levels **daily** at fever onset and until resolution of the fever.

[9] CNS evaluations will then be performed at Day -1 and Day 28. If CNS symptoms are identified at these timepoints, additional testing may be performed as per clinical discretion.

[10] Months 3, 6, 9 and 12

[11] Pregnancy test for females of childbearing potential only. Pre-infusion pregnancy test to be performed within 48 hours prior to the huCART19 retreatment infusion (Day 0-R).

[12] Vital signs will be measured within 10 minutes prior to the infusion and within 15 minutes after the infusion. Vital signs will also be measured at 30 (+/- 5) minutes, 45 (+/- 5) minutes, and 60 (+/- 5) minutes after the infusion. If the subject's vital signs are not satisfactory and stable one hour post-huCART19 infusion, vital signs will continue to be monitored as clinically indicated until stable.

[13] D-dimer required on Days 3 and 10.

[14] Bone marrow biopsy/aspirate to be performed between initiation of LD chemo and huCART19 T-cell infusion.

[15] See **Section 5.3** regarding criteria to proceed with huCART19 infusion.

[16] If extramedullary disease is present prior to treatment, this will be followed as clinically appropriate (including chest x-ray).

[17] Visit windows have been incorporated to allow for flexibility in scheduling however these visits cannot overlap and all individual follow-up visits must be performed.

[18] All subjects must undergo influenza testing within 10 days prior to the huCART19 retreatment infusion during the October-May flu season. If the subject is positive for influenza, oseltamivir phosphate (Tamiflu®) or equivalent should be administered per package insert. The subject must complete this course treatment prior to receiving huCART19. The test does not need to be repeated prior to the huCART19 infusion, however if influenza symptoms are present, the huCART19 infusion should be delayed until the subject is asymptomatic.

[19] Research blood (5 cc red top) to be taken between 20-120 minutes post-huCART19 infusion.

[20] Please refer to **Table 6-3** for details regarding Disease Monitoring requirements.

[21] Subjects in continued remission post-retreatment may also receive additional huCART19 infusions at the physician-investigator's discretion. Subjects must be evaluated by a physician-investigator for eligibility to receive additional CART19 cell infusions according to criteria in **Section 5.3**. If performed, these additional infusions post-retreatment will be defined as "reinfusions". For the purposes of study timepoint identification and reporting, each reinfusion post-retreatment will be considered a new treatment number under the Retreatment Schedule of Evaluations (i.e. the first huCART19 retreatment infusion on Day 0-R will be Treatment #1, the first reinfusion post Day 0-R will be Treatment #2, the second reinfusion post Day 0-R will be Treatment #3, etc). If reinfusions are administered, additional safety follow-up visits are required. While all study visits will continue to be calculated based on the date of the retreatment CART19 cell infusion (Day 0-R), subjects will also enter into a Reinfusion Section of the Retreatment Schedule of Evaluations at the time reinfusion of CART19 cells is initiated. Where Reinfusion Study Visits may overlap with existing study timepoints (i.e. Reinfusion Day 0 may overlap with the Month 3-R study timepoint), all study tests/procedures required for both visits should be completed per protocol requirements. When post-reinfusion follow-up is completed per protocol, the subject will resume the study follow-up timepoints per the Retreatment Schedule of Evaluations. Additional follow-up post reinfusion may also be performed per physician-investigator discretion. Please refer to **Section 6.13** for additional information.

[22] Testing for huCART19 persistence by qPCR will continue until any 2 sequential tests are negative documenting loss of CAR T cells.

[23] If LN aspirate performed as per clinical discretion.

[24] Subjects who relapse post huCART19 infusion may also receive additional huCART19 infusions post-relapse at the physician-investigator's discretion. Subjects must be evaluated by a physician-investigator for eligibility to receive additional huCART19 infusions according to criteria in **Section 5.3**. If performed, additional infusions administered post-relapse will be defined as "retreatment". If a decision is made to retreat a subject post-relapse, the subject will officially discontinue the Primary Schedule of Evaluations (**Appendix 1**) at the time of their first retreatment study visit/procedure (i.e. lymphodepleting chemotherapy) and enter the Retreatment Schedule of Evaluations (**Appendix 2**). Retreatment study visit timepoints will be distinguished from the primary Schedule of Evaluations by a "-R" identifier (i.e. Day 0-R, Day 1-R, etc). Follow-up will continue under the Retreatment Schedule of Evaluations for up to 12 months post retreatment. Please refer to **Section 6.13** for additional information.

[25] Lymphodepleting chemotherapy may be repeated prior to huCART19 reinfusions per clinical discretion. Please refer to **Section 6.5** for additional information.

[26] qPCR persistence testing performed post reinfusion on Days +7, +10, and +14 only.

[27] Disease assessments may be performed prior to reinfusion of huCART19 cells per clinical discretion. If a bone marrow aspirate is performed, a portion of this sample may also be used for research purposes.

[28] Additional follow-up post-reinfusion may also be performed per physician-investigator discretion.

[29] Research samples to be collected pre-infusion.