University of Pennsylvania

Phase 2 Study of CD19-directed Chimeric Antigen Receptor-modified T cells (CART19) for Adult Patients with Minimal Residual Disease During Upfront Treatment for Acute Lymphoblastic Leukemia

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Protocol Number:	UPCC#39416; IRB# 825668
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LIST OF ABBREVIATIONS

ACC	Abramson Cancer Center
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
CHOP	Children's Hospital of Philadelphia
CFR	code of federal regulations
CLL	chronic lymphoblastic leukemia
CMV	Cytomegalovirus
CNS	central nervous system
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 toxicity 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 safety and monitoring board
DSMC	data safety and monitoring committee
ECOG	Eastern Cooperative Oncology Group
EFS	event free survival
FAS	full analysis set
FDA	food and drug administration
FISH	fluorescent in situ hybridization

GCP	good clinical practices
GMP	good manufacturing practices
HAMA	human anti-murine antibody
HSCT	hematopoietic stem cell transplantation
IBC	Institutional Biosafety Committee
IRB	Institutional Review Board
MAS	macrophage activation syndrome
MRD	minimal residual disease
MRI	magnetic resonance imaging
NCCN	National Comprehensive Cancer Network
ORR	overall remission rate
OS	overall survival
PFS	Progression free survival
PBMC	peripheral blood mononuclear cells
PDCS	Product Development and Correlative Studies Laboratory
PD	progressive disease
PK	pharmacokinetics
PR	partial remission
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 variable fragment
SCT	stem cell transplant
TCR	T cell receptor
TCSL	Translational and Correlative Studies Laboratory
TLS	tumor lysis syndrome
TRM	treatment related mortality
UPenn	University of Pennsylvania
Vβ	a rearranged T cell specific gene that can be used to determine clonality of a T cell population
VSV-G	Vesicular Stomatitis Virus, Glycoprotein
WBC	white blood cell

STUDY SUMMARY AND STUDY SCHEMA

Title	Phase 2 Study of CD19-directed Chimeric Antigen Receptor-modified T cells (CART19) for Adult Patients with Minimal Residual Disease during upfront treatment for acute lymphoblastic leukemia (ALL)
Short Title	CD19 redirected autologous T cells for MRD positive patients during upfront treatment for ALL
Protocol Numbers	UPCC # 39416; IRB # 825668; [redacted]
Phase	2
Methodology	This is a single center, single arm, open-label phase 2 study to determine the efficacy of autologous T cells expressing CD19 chimeric antigen receptors expressing tandem TCRζ and 4-1BB (TCRζ/4-1BB) co-stimulatory domains (referred to as "CART19" cells) in adults with minimal residual disease (MRD) during upfront treatment for CD19+ acute lymphoblastic leukemia.
Study Duration	The duration of active protocol intervention is approximately 12-15 months from screening visit. The protocol will require approximately 12-18 months to complete enrollment.
Study Center(s)	Single-center
Objectives	**Primary Objective:** Describe the incidence of conversion of minimal residual disease (MRD) to <0.01% after CART19 therapy in patients with MRD+ ALL during upfront treatment **Secondary Objectives:** • Evaluate best overall response rate • Evaluate overall survival, duration of remission, relapse free survival, and event free survival • Evaluate manufacturing feasibility of CART19 • Assess safety and tolerability of CART19 **Exploratory Objectives**: • Describe anti-tumor response to CART19 by deep sequencing • Characterize the CART19 pharmacokinetic (PK) profile • Evaluate bioactivity of CART19 cells
Number of Patients	24 evaluable subjects
Diagnosis a*nd Main* Inclusion Criteria	Adult patients with MRD+, CD19+ ALL during upfront treatment within 18 months of initial diagnosis

Study Product, Dose, Route, Regimen	CART19 cells transduced with a lentiviral vector to express anti-CD19 scFv TCRz:41BB administered by IV infusion. Subjects will receive 1-5 x 10^8 transduced CAR T cells as a split dose over three days as follows: • Day 1, 10% fraction: 1-5x10^7 CART19 cells • Day 2, 30% fraction: 3x10^7-1.5x10^8 CART19 cells • Day 3, 60% fraction: 6x10^7-3x10^8 CART19 cells
Duration of administration	Based on the total volume to be infused and the recommended infusion rate of 10-20mL per minute.
Reference therapy	None.
Statistical Methodology	For the primary objective, the proportion of subjects that are MRD negative (MRD<0.01%) by 28 days along with 90% Clopper-Pearson confidence intervals will be estimated. With 24 patients, there will be at least 80% power to detect an ORR of 40% or greater against a null rate of 15% using a two-sided exact binomial test at 10% type I error. At least 8 out of 24 patients would need to achieve MRD^- by day 28 (ORR=33%; exact 90% CI [17.8%, 52.1%]) to indicate meaningful efficacy of 15% or greater. If the true MRD^- rate were 84%, we have more than 80% power to reject a rate of 60% or smaller with 90% confidence. The confidence intervals below also provide the precision for 90% confidence intervals around adverse events. Adverse events will be collected and evaluated for all patients during the protocol specified AE reporting periods. AEs will be graded for severity using the National Cancer Institute (NCI) – Common Toxicity Criteria (v4.03). All adverse events will be described and exact 90% confidence intervals will be produced for adverse event rates, both overall and within major categories. Results will be tabulated and summarized. Descriptive statistics will be applied to determine the persistence, trafficking of CART-19cells, and the change of blast counts in blood and marrow. Data regarding the number of CART-19 cells in blood, marrow and the immune cell components in the marrow will be presented graphically to explore the pattern over time. We will compute 90% confidence intervals for proportions and means. For the secondary clinical objectives, overall complete response rate (ORR) by Day 28 will be computed and summarized by exact 90% confidence intervals. Distributions of overall, progression-free, and duration of response will be presented graphically using Kaplan-Meier curves. The six-month survival rates will be presented.

1. INTRODUCTION

1.1. Background

B cell malignancies comprise a heterogeneous group of neoplasms including a vast majority of non-Hodgkin's lymphomas, as well as acute lymphoblastic leukemias (ALL) and chronic lymphocytic leukemias (CLL). An estimated 87,000 new cases of leukemia and non-Hodgkin's lymphomas are diagnosed in the US annually[1], and most of these are of B cell origin. Current treatments for B cell malignancies include chemotherapy, radiation therapy, bone marrow transplantation, monoclonal antibodies and peripheral blood stem cell transplantation. Despite these treatment modalities, most patients will remain incurable.

B cell lineage acute lymphoblastic leukemia (B-ALL) is responsive to chemotherapy, however the ability to uniformly eradicate the disease has not been achieved as about 65% of adults and 20% of children have disease recurrence[2,3]. The standard upfront treatment approach for patients with newly diagnosed B-ALL is 2-3 years of chemotherapy. Cycles are often described as Induction, Consolidation, Intensification then Maintenance and consist of multiple chemotherapy agents. Minimal residual disease (MRD) as assessed by flow cytometry powerfully predicts outcome in both adults and children with ALL. The presence and persistence of MRD during upfront treatment (from Induction through Maintenance) is the most important adverse prognostic factor for patients with B-ALL and identifies patients who have chemotherapy refractory disease[4-6]. The clinical significance and medical decision-making based upon MRD results is less well-established as it relates to surveillance and decisions related to proceeding with allogeneic stem cell transplantation, particularly in adults. The median time to overt morphologic relapse after testing MRD+ is 4-5 months[7]. The only standard option for cure in this situation is allogeneic stem cell transplantation but outcome is suboptimal due to high risk of treatment related morbidity and mortality and risks of relapse. Clinical decision-making on the basis of MRD status is at the discretion of the investigator. Given the overall uncertainty and rapidly evolving evidence in the field, non-chemotherapy based treatment options for patients with MRD+ disease is needed. The benefit of this approach has been shown with the T cell engaging, bispecific single-chain (BiTE) antibody blinatumomab. In a Phase 2 study, 20 patients with MRD+ ALL during upfront treatment were given blinatumomab with 60% relapse free survival at 33 months follow up [8].

In most cancers, tumor-specific antigens for targeting are not well defined, but in B-cell neoplasms such as B-ALL, CD19 is an attractive target. CD19 is a 95kDa glycoprotein present on B cells from early development until differentiation into plasma cells[9-11]. 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[10-12]. 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[11,13].

CD19 is not present on most normal tissues, other than normal B cells, including pluripotent blood stem cells[14], 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[15-19] and human and non-human primates [17,20-29]. Outcomes using anti-CD19 CAR T cells, including CTL019, for patients with relapsed or refractory ALL have been outstanding [30-32]. The most significant treatment related adverse event with this line of therapy is cytokine release syndrome (CRS). It is known that a significant risk factor for severe CRS from anti-CD19 CAR T cells is baseline disease burden[31,32]. We

hypothesize that patients with MRD+ B-ALL treated on this study will therefore have minimal toxicity from CRS.

Adoptive immunotherapy

Adoptive transfer is a term coined by Medawar[33] to study allograft rejection, and the term adoptive immunotherapy denotes the transfer of immunocompetent cells for the treatment of cancer or infectious disease[34]. Adoptive immunotherapy appears to be the most robust form of immunotherapy for treatment of established tumors[35], as powerful effects have been noted in patients with metastatic melanoma after the adoptive transfer of tumor infiltrating lymphocytes and gene modified peripheral blood T cells[36]. However, several problems remain to be solved before this therapy becomes routine[37].

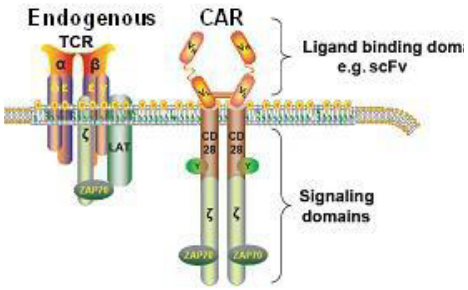

Fig. 1-1. CAR design. Bispecific T cells are created by the introduction of genes encoding CAR proteins that recognize target surface antigens in an MHC-independent fashion.

Engineered T cells with redirected specificity: chimeric antigen receptors (CARs)

As shown in **Figure 1-1**, the CAR approach uses genetically programmed, patient-derived lymphocytes transfected with chimeric receptor genes to combine the effector functions of T lymphocytes with the high specificity antibody recognition of predefined surface antigens in a non-MHC restricted manner[38,39]. In principle, universal targeting vectors can be constructed because the CAR's scFv region binds to native cell surface epitopes and bypasses the need for specific antigen processing. The scFv region is engineered for tumor binding function and contains the V_H and V_L chains joined by a peptide linker of about 15 residues in length[40]. First generation CARs contain a minimal T cell receptor (TCR) signaling domain consisting of TCRζ. Second generation CARs contain double costimulatory signaling domains such as CD28 and TCRζ or 4-1BB and TCRζ. Third generation CARs contain triple costimulatory modules comprised of CD28, 4-1BB, and TCRζ. See reviews of CARs for details[41-46].

1.2. Investigational Agent

The investigational agent in this protocol is CART19 cells, also referred to as CTL019. CART19 is the most recent adaptation of adoptive cellular immunotherapy that uses the patient's own peripheral blood T cells that have been genetically re-directed to kill CD19+ cells. As shown in **Figure 1-1**, the CAR approach uses genetically programmed, patient-derived lymphocytes transduced with chimeric receptor genes to combine the effector functions of T lymphocytes with the ability of antibodies to recognize predefined surface antigens with high specificity in a non-MHC restricted manner[38,39]. These receptors have the ability to recognize intact membrane proteins independent of antigen processing. CARs or T-bodies typically encode an extracellular domain to bind tumor or virus linked to an intracellular signaling domain that mediates T cell activation (reviewed in[42,45]). In principle, universal targeting vectors can be constructed because the scFv bind to native cell surface epitopes and bypass the requirement for MHC restriction. The tumor binding function of CAR is usually accomplished by the inclusion of a single chain variable fragment (scFv) antibody, containing the V_H and V_L chains joined by a peptide linker of about 15 residues in length[40]. First generation CARs contain a minimal TCR signaling domain consisting of TCRζ. Second generation CARs contain double stimulatory signaling domains either CD28 and TCRζ or 4-1BB and TCRζ. The 3rd generation CARs contains further advancements such as triple stimulatory modules comprised of CD28, 4-1BB, and TCRζ. See reviews of CARs for details[43,44,47].

Autologous T cells will be engineered using a clinical-grade lentiviral vector to express an extracellular single chain antibody (scFv) with specificity for CD19. This will be expected to redirect specificity of the transduced T cells for cells that express CD19, a molecule that is restricted in expression on the surface of the malignant cells and on normal B cells. In series with the CD19 scFv, the transduced cells will express an intracellular tandem signaling domain comprised of 4-1BB and TCRζ signaling modules. The extracellular single chain antibody (scFv) with specificity for CD19 was previously reported[23]. The scFv is derived from a mouse monoclonal antibody using hybridoma cell line FMC63 described in Nicholson et al.[23]. The signaling domains are entirely of the native human sequences[48,49].

The CART19 cells will be manufactured in the Clinical Cell and Vaccine Production Facility (CVPF) at the [redacted] At the end of cell cultures, the cells are cryopreserved in infusible cryomedia for administration as split dose fractions. The split dose infusion will be administered as a 10%, 30% and 60% fraction of 1-5 x 10^8 CART19 transduced cells. The target dose of CART19 cells is calculated based on the scFv percent transduction efficiency. The infusion bag(s) will contain an aliquot (volume dependent upon dose) of cryomedia containing the following infusible grade reagents (% v/v):31.25% plasmalyte-A, 31.25% dextrose (5%), 0.45% NaCl, 7.5% DMSO, 1% dextran 40, 5% human serum albumin.

Mechanism of action

Redirected T cells have been shown in experimental models to bind to cells that express the target antigen. Over the past decade, CARs directed against a wide variety of tumor antigens have been developed[45,50]. There are several potential limitations to the CAR T cells: 1) the target antigen must be expressed on the cell surface; 2) large amounts of shed or soluble antigen may inhibit the CAR T cells; 3) the chimeric receptor may be immunogenic, resulting in the elimination of the redirected T cells by the host immune system.

Absorption, distribution and metabolism

Lymphocytes have complex trafficking and survival kinetics, and after adoptive transfer several fates have been demonstrated: 1) margination; 2) exit from the peripheral blood trafficking to lymphoid tissues; and 3) death by apoptosis. Following an intravenous dose, retrovirally modified and adoptively transferred T cells have been shown to persist in the circulation for at least 10 years in immunodeficient SCID patients due to the replicative competence of T cells[51]. Human CD8 CTLs have an elimination half-life from the peripheral blood of about 8 days, and this increases to about 16 days when low doses of IL-2 are given[52]. In patients with HIV infection, it was determined that the mean half-life of lentivirally modified CD4 T cells in the circulation of 5 patients following a single infusion was 23.5 (± 7.7) days in patients. Adoptively transferred human T cells have been shown to traffic to tumor and secondary lymphoid tissues[52-55].

Drug interactions

CART19 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.

Immune elimination

An important consideration is that the CAR can be immunogenic either because foreign sequences are expressed, or because of novel epitopes that are created at the fusion joint of human signaling domains

that are not normally juxtaposed. Immunogenicity of the CAR can lead to the rejection of the adoptively transferred T cells. The basis for this supposition is that human retrovirally-modified CTLs expressing a fusion protein consisting of hygromycin:HSV thymidine kinase were eliminated by host CTLs in patients with advanced HIV infection[56]; importantly, this immune mediated elimination was not accompanied by adverse effects and required 6 to 8 weeks to occur. There has been one case of immune-based CART19 rejection at the National Cancer Institute[31]. Other CART studies at UPENN using a CAR construct in which the scFv is of murine origin suggest that a humoral anti-mouse immune response develops following CART infusion which may contribute to the limited persistence of the CART cells.

Rationale for lymphodepletion

Adoptive immunotherapy strategies may be able to capitalize on homeostatic T cell proliferation[57], a recent finding that naive T cells begin to proliferate and differentiate into memory-like T cells when total numbers of naive T cells are reduced below a certain threshold[58,59]. Lymphodepletion 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[60]. This hypothesis has been tested clinically in patients with metastatic melanoma refractory of conventional treatments[53]. The patients received a lymphodepleting conditioning regimen consisting of cyclophosphamide (60mg/kg x 2 days) and fludarabine (25 mg/m^2 x 5 days) prior to adoptive transfer of T cells. Patients with myeloma, NHL, CLL and ALL have been treated with infusions of ex-vivo co-stimulated and expanded autologous T cells after lymphodepleting chemotherapy, and observed improved engraftment [61-66]. In this protocol, we will transfer CART19 cells into subjects that are given lymphodepleting chemotherapy. This approach has been taken with previous recipients of CART19 cells (**Section 1.4**). Recent data indicates that the increased antitumor efficacy of adoptive transfer following host conditioning is more than simply "making room" because the quantitative recovery of adoptively transferred T cells in mice reveals that in vivo proliferation following adoptive transfer is identical in mice with or without previous irradiation.

1.3. Preclinical Data

Extensive literature supports the use of engineered T cells for tumor immunotherapy in rodent tumor models[45,67-70]. Others have used electroporation or retroviral vectors to create CART19 T cells, and have shown in vivo safety and efficacy of adoptively transferred T cells in immunodeficient mouse models[27,28,71-73]. The incorporation of signaling modules such as CD28 and 4-1BB in 2nd generation CARs increases potency of the engineered T cells in pre-clinical studies[48,74-79]. The pre-clinical data supporting CART19 has been published[80-82].

1.4. Previous Clinical Data with CART-19 cells

[redacted]

[redacted]

[redacted]						
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted] for children. ≤ 10cc/kg of total volume Dose 1 (10%), Dose 2 (30%)
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
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[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

[redacted]

Historical Clinical Data with CART19

CART19 appears to have greater success than previous CAR constructs, since *in vivo* these cells proliferate more, persist longer, and retain effector functions, therefore amplifying and sustaining effective anti-tumor responses. Please see recent reviews of CAR T cell trials[44,46,83-90].

UPCC04409 was the first-in-human adult CLL and ALL clinical protocol where $1.5x10^7$-$5x10^8$ CART19 cells are infused in a split dosing regimen (10%, 30% and 60%) on Day 0, 1 and 2. Only one patient 04409-03 received the optional Day 11 dose ($1.5x10^7$-$5x10^8$ CART19). Response rate in the ALL cohort was 80% (5/6). The main toxicity that was observed was CRS, but was managed clinically in all cases. Results from five of the six subjects have been published [32]. Based on encouraging response rates and manageable toxicity profile, a phase 2 study in ALL, UPCC21413, was opened. The dosing regimen in UPCC21413 has been adjusted (as described in **Section 1.5.1**) to maximize safety after five patient deaths early in the study and improve efficacy [redacted]

CART19 has also been used in other disease indications at UPenn and CHOP, including CLL [63 66], non-hodgkin's lymphoma, multiple myeloma [89] and pediatric ALL [32 91]. CART19 is well tolerated in these disease indications, with the main toxicity being clinically manageable CRS. In the setting of pediatric ALL, a very high response rate of 92% (55/60) has been observed. In addition, the first pediatric patient treated, CHP959-100, remains in complete remission as of her 48 month follow-up visit.

CRS has been the most significant SAE seen in adult and pediatric patients treated with CART19. CRS is described in detail below (**section 1.5.2**) but typically begins within 2 weeks of CART-19 infusion. The CRS typically starts with several days of fevers. In all cases, evaluation for infections is done. Fevers tend to be spiking and can be associated with rigors, anorexia, nausea, diarrhea, diaphoresis, capillary leak, hypoxia and hypotension. In several cases ICU level care, ventilator support and pressors have been needed. Observations have noted experimentally very high levels of IL6 during the CRS. In addition, the reaction typically appears to be associated with MAS. This can be manifest by evidence of hemolysis, cytopenias, elevated ferritin, altered mental status, and other complications.

Teachey and colleagues (2016) reported that, while there have been three cases of refractory CRS which resulted in death in the adult ALL population at the University of Pennsylvania, nine out of 12 CRS cases observed in this population were severe but reversible. It is hypothesized that CRS severity may be related to tumor burden, so that patients with high tumor burden may develop more severe CRS compared to patients with low tumor burden. In ALL patients, high grade CRS is mechanistically linked to the antitumor effect of CART19 in patients with high baseline tumor burden[92]. However, additional patient and CART19-related factors may also contribute to such an anti-tumor effect.

CRS/MAS was initially managed in one patient with corticosteroids. Since then, as more data have become available, a CRS management algorithm has been developed and is described in detail in **Section 8.5.2** and outlined in **Figure 8-1**. Briefly, CRS is managed with supportive care and with anti-cytokine therapy, when appropriate. Specifically, tocilizumab is an anti-IL6 receptor antibody that has been administered for CRS with worsening respiratory distress, including pulmonary infiltrates, increasing oxygen requirement including high-flow oxygen and/or need for mechanical ventilation or hemodynamic instability despite intravenous fluids and moderate vasopressor support or rapid clinical deterioration. Systemic immunosuppression with corticosteroids following CART19 infusion is avoided and corticosteroids are given only under life threatening situations due to their known lympholytic effects.

1.5. Dose Rationale and Risk/Benefits

1.5.1. Dose Rationale

Based on the clinical experience with adult ALL patients treated with CART19 as part of the UPenn protocol UPCC21413 and UPCC04409, subjects will receive 1-5x10^8 CART19 cells as a 10%, 30% and 60% fraction administered over 3 days.

The first 6 ALL patients treated on UPCC21413, received a dose of 1-5 x 10^8 CART19 cells administered as a single infusion. Based on three patient deaths at this dose level, the dose was reduced to 1-5 x 10^7 CART19 cells administered as a single infusion. This change was implemented in response to three patient deaths that were possibly related, in part, to refractory cytokine release syndrome, an expected toxicity of CART19. At this reduced, single CART19 dose, an additional two deaths occurred. As a measure to enhance subject safety, the CART19 dose schedule was changed from a single infusion back to the well-tolerated split dose administration (10% on Day 1, 30% on Day 2 and 60% on Day 3) used in the first CART19 adult ALL study, UPCC04409. Three adult subjects were then treated on with 1-5 x 10^7 CART19 cells as a split dose. Two of the three subjects developed and recovered from CRS after treatment with anti-cytokine therapy, demonstrating safety of the split dose approach. However, efficacy was diminished compared to the original split dose of 1-5x10^8 CART19 cells seen for the adult ALL subjects on UPCC04409; only one of the two evaluable subjects had a response (CR). Therefore, the dose was increased back to the well-tolerated split dose administration of 1-5x10^8 CART19 cells that had a 100% Day 28 CR rate on the original CART19 adult ALL study, UPCC04409. As of December 2015, six subjects have been treated with 1-5 x 10^8 CART19 cells as fractionated doses, which has been well tolerated and has resulted in CR at Day 28 in four of the six subjects.

Additionally, the spilt dose administration schedule has been well tolerated in multiple adult patients with CLL (UPCC04409, UPCC03712 and UPCC18415) as well as pediatric ALL subjects (CHP959). Clinical responses at doses ranging from 1.4x10^7 to 1.1x10^9 CART-19 cells have been observed and CRS events have been clinically manageable under the split dosing strategy. Unlike standard drugs that are metabolized, CART cells are able to proliferate extensively in the patients. Thus, the administered dose may underestimate the CART19 T cells *in vivo* following engraftment and expansion and will vary from patient to patient.

1.5.2. Risks/Benefits

Potential Risks

Participation in this study will expose the patient to genetically engineered autologous T cells. The risk of the cells alone is low based on clinical experience. The unknown risk is that of the signaling domains in the CAR. T cell proliferation could be uncontrolled; however, we have not observed this in our pre-clinical models in treating nearly 200 adults and children with CART19 cells in our clinical trials as of December 2015. In this case, corticosteroids and chemotherapy would be given to eradicate the CART19 cells; this has worked in previous cases[93].

Immunogenicity

Another risk is that the cells may be immunogenic, and that the patients will have an immune response directed against the scFv or novel sequences generated by the fusion protein; this has not had clinical consequences in previous trials studying lentivirus transduced T cells. If an immune response to the cells occurs, it is possible that the cells will be rejected. Three of 3 patients in an anti-carbonic anhydrase IX (CAIX) CAR trial developed Human Anti-Murine Antibody (HAMA) and loss of T cell engraftment[93]. This

has generally not been an issue in patients treated with CARs directed towards B cells as temporary or persistent B cell aplasia results in reduced antibody formation. Persistent B cell aplasia leads to hypogammaglobulinemia and may increase the risk of infection. This can be managed with IVIG repletion. In the UPenn and CHOP CART19 trials, hypogammaglobulinemic CLL and ALL patients have received IVIG. No significant unusual infection patterns have been identified in patients receiving IVIG repletion at regular intervals.

Transformation

There is a risk that people who receive gene-modified cells may develop new tumors derived from their genetically modified cells. This risk is primarily associated with viral gene transfer vectors that integrate into the cellular DNA where they may dysregulate genes controlling proliferation and/or survival. Transformation has not been observed following adoptive T cell transfer in hundreds of cancer and HIV patients receiving gammaretroviral modified T cells treated on multiple protocols at many academic centers[43], and in the 21 HIV patients treated with lentiviral modified T cells treated at Penn[94] or any of the nearly 200 patients treated to date on CTL019/CART19.

Risk of tumor lysis syndrome related to cytoreductive chemotherapy or CAR T cells

The risk of tumor lysis syndrome (TLS) is dependent on the disease and burden of disease. Several of the patients treated with CART19 have developed delayed TLS presumably due to T cell proliferation and tumor cell killing at that time. Therefore, all patients will be closely monitored both before and after chemotherapy and CART19 infusions including blood tests for potassium and uric acid. All patients will receive allopurinol prophylactically for 30 days after infusion and appropriate clinical therapy will be administered should any significant tumor lysis occur.

Infusion reactions

Immediately following T cell infusions, reactions could occur and may include transient fever, chills, and/or nausea. Patients must be pre-medicated with acetaminophen and an anti-histamine prior to the infusion of CART19. A review of infusion-related adverse events of 381 T cell products administered to 180 recipients, enrolled on 18 studies, over a 10 year period was conducted by Cruz et al.[95] and found no grade 3-4 infusion reactions during initial monitoring or 24-hour follow-up. Grade 1-2 adverse events were observed in 21 patients during or shortly after infusion and included nausea, vomiting, fever, and/or chills. A mild infusion reaction was recorded in one of more than 20 CLL patients with CART-19 infusion.

Intracranial Hemorrhage

As of February 2017, there have been three events of intracranial hemorrhage on CART19 trials under [redacted]. One event occurred on UPCC#21413 (adult ALL) in the setting of thrombocytopenia (related to prior lymphodepleting chemotherapy and underlying leukemia), was determined to be possibly related to the CART-19 T-cell infusion, occurred with concurrent Grade 4 CRS, and resulted in death. Another event occurred on the CNS3 cohort on CHP959 (pediatric ALL) in the setting of active circulating leukemia, CRS, sepsis/bacteremia, and acute renal failure requiring dialysis. The subject died, and intracranial hemorrhage (suspected clinically, unconfirmed radiologically) was felt to be the immediate cause of death in this critically ill patient. A third event occurred on 16CT022 (pediatric ALL study). In February 2017, a 3-year-old subject with refractory ALL and CNS2 disease experienced severe CRS complicated by DIC and multi-organ failure. While on extracorporeal membrane oxygenation, the subject experienced an intracranial hemorrhage, with associated cerebral edema as a terminal event.

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

Overview and Clinical Manifestations

Patients treated with CART19 may experience a cytokine release syndrome (CRS), which has correlated with disease response. Clinical manifestations have included high fevers, fatigue, anorexia, nausea, vomiting, diarrhea, myalgias, arthralgias, headache, rash, hypotension (occasionally requiring pressor support), tachypnea, hypoxia (occasionally requiring ventilator support), altered mental status including delirium and confusion (in several patients), word finding difficulties, evidence of disseminated intravascular coagulation, as well as macrophage activation syndrome (MAS). Additional symptoms of CRS may also include rigors, sweating, dyspnea, and seizures. In some cases CRS, TLS and hypotension have led to acute kidney injury and several patients have required at least transient dialysis. The CRS has been effectively abrogated with cytokine-directed therapy, typically tocilizumab, in most patients requiring therapy. As of December 2015, five adult ALL patients treated with CART19 have died of complications related to refractory CRS and intercurrent infections. In addition, it is unclear if treating the CRS with cytokine-directed therapy adversely impacts the anti-tumor response.

Features consistent with MAS or HLH have been observed in patients treated with CART19, coincident with clinical manifestations of the CRS. MAS appears to be a reaction to immune activation that occurs from the CRS. Macrophage activation syndrome can also be referred to as hemophagocytic lymphohistiocytosis (HLH); it is a reaction to immune stimulation by infection, autoimmune diseases or other precipitants, but is distinguished from familial or genetically mediated HLH. Some but not all features of MAS are typically observed. The clinical syndrome of MAS is characterized by high grade non-remitting fever, cytopenias affecting at least two of three lineages, and hepatosplenomegaly. It is associated with biochemical abnormalities, such as high circulating levels of serum ferritin (prominent feature of CART19-associated MAS), and triglycerides, together with a decrease of circulating NK activity. Other findings include variable levels of transaminases up to signs of acute liver failure and coagulopathy with findings consistent with DIC. D-dimer is nearly universally elevated in CART19 patients, with no evidence of bleeding, but very low fibrinogen has been associated with bleeding.

Supportive clinical criteria include neurologic symptoms and cerebrospinal fluid pleocytosis, conjugated hyperbilirubinemia, and transaminitis, hypoalbuminemia and hyponatremia. Typically high fevers, cytopenias, and when performed hemophagocytosis in the bone marrow is observed (though marrow specimens at the time of the reaction are not often taken).

At this time it is still unknown whether CRS/MAS are required for the anti-tumor response. Research monitoring data showed that IL6 levels were extraordinarily high during the CRS, prompting the use of an anti-IL6 receptor antibody tocilizumab to treat the CRS/MAS. The majority of patients treated with tocilizumab for CRS and MAS had rapid (within hours) resolution of dramatic fevers, and continuous improvement in hypotension and hypoxia over hours to several days, and showed improvement in biochemical evidence of CRS and MAS within 48 hours. Treatment and timing of treatment of this toxicity will be at the discretion of the patient's physician and the study investigator, and occur in the setting of hemodynamic instability.

Pediatric ALL patients treated with CART19 on CHP959 have experienced a similar CRS and MAS. CHP959-100 experienced a severe CRS and had high fevers, hypotension, acute vascular leak syndrome and acute respiratory distress. The patient was treated with tocilizumab, as described in *Grupp et al., NEJM, 2013*[91], and all associated adverse events resolved.

Replication-competent lentivirus

Replication-competent lentivirus (RCL) may be generated during the CART19 manufacturing phase or subsequently after introduction of vector transduced cells into the patient. 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 for detection of RCL before it can be released to a patient. Nevertheless, generation of an RCL following infusion of the vector product remains a theoretical possibility. The consequences of such recombination events in patients without a known lentiviral infection are unknown, and therefore patients with coexistent HIV infection are excluded from participation in this study in order to minimize this possibility.

Clonality and insertional oncogenesis

The occurrence of adverse events caused by insertional mutagenesis in five patients in a gene therapy trial for X-linked SCID following stem cell therapy emphasizes the potential for problems in translating this approach to the clinic[96-99]. The T cell leukemias were attributable to clonal expansion conferred by gammaretroviral vector integration sites in the CD34+ bone marrow stem cell modification[97]. This represents the most severe adverse event caused by vector integration. However, there is also evidence for retroviral vector integration site dominance in a gene therapy trial of β-thalassaemia without malignancy[100]. The lentiviral vector used for CART19 manufacturing is part of a vector class that may have a lower risk for integration in or near oncogenic regions than oncoretroviral vectors[101].

Uncontrolled T cell proliferation

CART19 cells could proliferate without control of normal homeostatic mechanisms. In pre-clinical studies, CART19 cells have only proliferated in response to physiologic signals or upon exposure to CD19. In the context of this protocol it is possible that the T cells will proliferate in response to signals from the malignant tumor or normal B cells. This could be beneficial or harmful depending on the extent of proliferation. Clonal dominance of adoptively transferred T cells has been associated with tumor reduction in adoptive transfer trials[53,102].

B cell aplasia

Transient or permanent host B cell depletion is also a potential risk with CART19 cells, since B cells express CD19. This is expected to resolve when the CART19 cells are cleared. B cell aplasia has been seen in CART19-treated subjects as well as B cell recovery in some subjects who had lost CART19 [32]. Persistent B cell aplasia leads to hypogammaglobulinemia and may increase the risk of infection. This is common with anti-CD20 directed therapies[104]. This can be managed with IVIG repletion by established clinical dosing guidelines to support IgG levels. In previous CART19 trials, responding CLL and ALL patients who are hypogammaglobulinemic have received IVIG. No significant unusual infection patterns have been identified.

Fatal SAEs with CARs

There have been instances of fatal SAEs following CD19-directed CAR T cell infusion in patients with B cell malignancies. In one study, Brentjens et al utilized a second generation, retrovirally transduced anti-CD19 CAR, containing CD28 and CD3ζ, in patients with CLL. Of the 7 subjects treated on this protocol at the time of the study report, 6 infusions proceeded without SAE while one subject experienced complications leading to death approximately four days after anti-CD19 CAR infusion. This subject received lymphodepleting chemotherapy with 1.5g/m^2 of cyclophosphamide two days prior to infusion with genetically modified anti-CD19 CAR T cells at 1.2-3x10^7 cells/kg. Twenty hours following T cell infusion, the patient developed persistent fever (transient fever was observed in the first 3 subjects on the study

too) and hypotension that was rapidly followed by respiratory distress (despite negative chest x-ray), hypoxemic respiratory failure, and acute renal failure. The family decided to remove further life sustaining therapies and the patient expired 44 hours post-T cell infusion. The post-mortem pathology report failed to support a diagnosis of tumor lysis syndrome as the primary source of renal failure. Analysis of serum cytokines revealed elevated levels of IL-2, IL-7, IL-15, and IL-12 following cyclophosphamide therapy which may have been secondary to a prior subacute infection exacerbated by the immune suppression associated with cyclophosphamide-mediated lymphodepletion. The authors concluded that concomitant sepsis was the most likely cause of death and attributed the etiology of the death as "possibly related" to CAR T cell infusion[105].

Other fatal SAEs using CD19-targeting T cells have been reported, including five fatal events at the University of Pennsylvania and two events in April 2014 at the Memorial Sloan Kettering Cancer Center. At the University of Pennsylvania, three of the first six adult ALL subjects infused on UPCC21413 died as a result of refractory Cytokine Release Syndrome (CRS) in the setting of intercurrent infections. Thereafter, the single dose administered in UPCC21413 was reduced to 1-5x10^7 CART19 cells. In the next six adult ALL subjects treated at the de-escalated dose, there were two deaths: one subject died from an intracranial bleed and one subject death was in the setting of neutropenic sepsis/bacteremia and non-responsive acidosis.

Potential benefits

The presence and persistence of minimal residual disease (MRD) during upfront treatment (from Induction through Maintenance) is the most important adverse prognostic factor for patients with B-ALL and identifies patients who have chemotherapy refractory disease[4-6]. The median time to overt morphologic relapse after testing MRD+ is 4-5 months[7]. The only standard option for cure in this situation is allogeneic stem cell transplantation but outcome is suboptimal due to high risk of treatment related morbidity and mortality and risks of relapse. Non-chemotherapy based treatment options for patients with MRD+ disease is needed. The benefit of this approach has been shown with the T cell engaging, bispecific single-chain (BiTE) antibody blinatumomab. In a Phase 2 study, 20 patients with MRD+ ALL during upfront treatment were given blinatumomab with 60% relapse free survival at 33 months follow up [8].

Outcomes using anti-CD19 CAR T cells, including CART19/CTL019, for patients with relapsed or refractory ALL have been outstanding[30-32]. The most significant treatment related adverse event with this line of therapy is cytokine release syndrome. It is known that a significant risk factor for severe CRS from anti-CD19 CAR T cells is baseline disease burden[31,32]. Therefore, we hypothesize that the risk of toxicity from CRS in patients with MRD+ disease treated on this study will be minimal and that the overall risk:benefit ratio is favorable.

2. STUDY OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
Describe the response to CART19 in adult patients with ALL who fail to achieve MRD negativity during upfront treatment	• Incidence of achievement of MRD negative state at Day 28 post-CART19

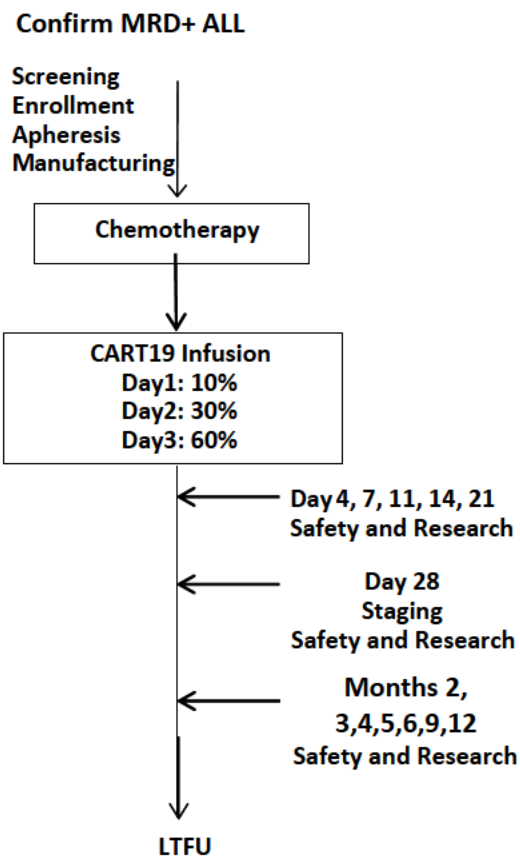
Objectives	Endpoints
Secondary	
Evaluate overall survival (OS), duration of remission (DOR) , relapse free survival (RFS), and event free survival (EFS)	• Overall survival (OS), duration or remission (DOR), relapse free survival (RFS), and event free survival (EFS).
Evaluate manufacturing feasibility of CART19	• Percentage of manufacturing products that do not meet release criteria for vector transduction efficiency, T cell product purity, viability, sterility or due to tumor contamination.
Assess safety and tolerability of CART19	• Frequency and severity of adverse events, including, but not limited to, cytokine release syndrome (CRS) and macrophage activation syndrome (MAS).
Exploratory	
Describe anti-tumor response to CART19 by deep sequencing	• Evaluate MRD by deep sequencing of patient bone marrow samples pre- and post-CART19 infusion (Day 28, for all, and Month 3 and 12 if subject has morphologic response).
Characterize the CART19 pharmacokinetic (PK) profile	• Engraftment and persistence of CART19 in blood as determined by Q-PCR (and flow cytometry) performed at a minimum of weekly for the first month, monthly until Month 6 and every three months until Month 12 or until any 2 sequential negative tests documenting loss of CART19 provided that a specific blood or tissue sample is available at that time point. • Trafficking to target tissue (bone marrow) or other tissues (cerebral spinal fluid and other tissues if available) as determined by Q-PCR (or flow cytometry).
Evaluate bioactivity of CART-19 cells	• Systemic soluble immune and inflammatory factors pre- and post-CART-19 infusion as determined by Luminex-based analyses. • CD19 antigen and peripheral B cell levels in marrow and other biopsied tissues pre- and post-CART19 infusion as determined by multi-parametric flow cytometry.

3. STUDY DESIGN

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 CART19 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**.

Figure 3-1 Study Schema



After signing informed consent, patients will undergo screening tests and procedures to determine eligibility. Adult patients with MRD ≥ 0.01% during upfront treatment for CD19+B cell ALL (during induction, consolidation, intensification or maintenance) will be eligible. Once patient eligibility is confirmed by an investigator, patients will undergo leukapheresis to obtain peripheral blood mononuclear cells (PBMC) for CART19 manufacturing, unless adequate numbers of cells are available from a prior apheresis. 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 CART19 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. Enrolled patients may continue to receive chemotherapy while cell manufacturing is ongoing.

Unless contraindicated and medically not advisable based on previous chemotherapy, patients will be given conditioning chemotherapy prior to CART19 cell infusion with the intent of lymphodepletion. Additionally, if the patient's white blood cell (WBC) count is ≤ 1,000 /uL, conditioning/lymphodepleting chemotherapy is NOT required. The chemotherapy will be planned so that the last dose is completed ~1-4 days BEFORE the planned infusion of CART19 cells. The chemotherapy start date will vary based on the duration of the selected chemotherapy regimen. If the delayed period from chemotherapy to CART19 infusion is 4 or more weeks, the patient will need to be re-treated with lymphodepleting chemotherapy prior to CART19 infusion. Please refer to **Section 6.4.15** for selection guidance of the preferred conditioning chemotherapy regimens.

We will enroll 24 evaluable patients for the primary endpoint analysis. Primary evaluable patients are those who have received at least 1-5x10^7CART19 cells and completed the response assessments for the primary efficacy endpoint as planned by the protocol. Primary evaluable patients also include those with disease progression or death prior to the primary endpoint response assessment.

Subjects with a manufactured cell dose that is less than the protocol-specified dose will be scored as a manufacturing failure. These subjects will receive their cell infusion, provided that all other manufacturing release criteria are met, however these subjects will be considered non-evaluable for the primary endpoint.

All patients will have blood tests to assess CART19 safety, engraftment and persistence at regular intervals throughout the study (**Visit Evaluation Schedule in Appendix 1**). Circulating CART19 T cells subsets will be assessed at various times after infusion. CART19 trafficking will be assessed in bone marrow aspirates, and other tissues, if available. Follow up is planned at a minimum of weekly for 4 weeks, monthly for 6 months, then quarterly for the remainder of the year to obtain a medical history, undergo a physical examination, and blood tests. Additional sample collections and assessments between schedule visits after CART19 cell infusion will be performed as clinically indicated.

Following these evaluations, patients will enter a long-term follow-up study for long term follow-up for up to an additional fourteen years to assess for safety assessments per the FDA guidelines.

4. PATIENT SELECTION AND WITHDRAWAL

Exceptions to eligibility will not be granted for this study.

4.1. Inclusion Criteria

1. Patients with CD19+, B cell Acute Lymphoblastic Leukemia (B-ALL) who have 0.01%≤MRD<10% during upfront treatment
2. Patients must be within 18 months of initial ALL diagnosis
3. Age ≥18 years
4. Adequate organ function defined as:
    a. Creatinine ≤ grade 2
    b. ALT/AST ≤3x upper limit of normal range for age
    c. Direct bilirubin ≤2.0 mg/dl
    d. Adequate pulmonary function defined as ≤ grade 2 dyspnea and ≤ grade 2 hypoxia
    e. Cardiac Left Ventricle Ejection Fraction (LVEF) ≥ 40% confirmed by ECHO/MUGA
5. Patients with CNS3 disease will be eligible if CNS disease is responsive to therapy. [At infusion-must meet criteria in Section 5.2].
6. Expression of CD19 on leukemic blasts demonstrated by flow cytometry or immunohistochemistry of bone marrow or peripheral blood
7. Adequate performance status defined as ECOG Performance Status 0 or 1
8. Provides written informed consent
9. 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, uncontrolled infection
2. Active hepatitis B or hepatitis C
3. HIV Infection

4. Class III/IV cardiovascular disability according to the New York Heart Association Classification (see Appendix 2)
5. Subjects with clinically apparent arrhythmia or arrhythmias who are not stable on medical management within two weeks of enrollment.
6. Pregnant or nursing (lactating) women
7. Patients with a known history or prior diagnosis of optic neuritis or other immunologic or inflammatory disease affecting the central nervous system, and unrelated to leukemia or previous leukemia treatment.

Note: For the purposes of this study, enrollment is defined as the date the Investigator confirms subject eligibility.

Please refer to the Concomitant Therapy Section 5.6 for windows for related to apheresis and CART19 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, and publicized via University of Pennsylvania or Abramson Cancer Center press releases. No direct-to-patient advertising will be performed.

Female patients of reproductive potential (women who have reached menarche or women who have not been post-menopausal for at least 24 consecutive months, i.e., who have had menses within the preceding 24 months, or have not undergone a sterilization procedure [hysterectomy or bilateral oophorectomy]) must have negative serum pregnancy test performed at the time of enrollment and a negative urine pregnancy test within 72 hours of T cell infusion.

Patients must agree not to participate in a conception process (e.g., active attempt to become pregnant or to impregnate, sperm donation, in vitro fertilization). Additionally, if participating in sexual activity that could lead to pregnancy, the study patient must agree to use at least one reliable method of contraception during their participation in the study.

Acceptable birth control includes one of the following methods:

- Total abstinence (no sexual relations)
- Female sterilization- surgical removal of both ovaries (woman's reproductive system that stores and releases eggs for fertilization and produces female sex hormones), or tubal ligation (having your “tubes tied”) at least six weeks prior to signing this consent.
- Male sterilization (i.e. vasectomy)
- 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 (women who have been post-menopausal for at least 24 consecutive months or have undergone hysterectomy, salpingectomy, and/or bilateral oophorectomy or men who have documented azoospermia) do not require the use of contraception. Acceptable

documentation of sterilization, azoospermia, and menopause is specified below:

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

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

4.4. Early Withdrawal of Patients

4.4.1. When and How to Withdraw Patients

Subjects who enroll but do not receive CART19 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 principal investigator judges that the subject, prior to CART19 infusion, is too ill to continue.
3. Pregnancy is documented prior to CART19 infusion. If pregnancy occurs after the subject has received CART19 cells, she will be kept active in the study for safety follow-up and pregnancy outcome.
4. 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.
5. Significant and rapid progression of malignancy, requiring alternative medical, radiation or surgical intervention prior to CART19 infusion.
6. A serious adverse event that requires the subject withdrawn from the trial prior to the CART19 T cell infusion.
7. Technical difficulties are encountered in the T cell genetic modification and expansion procedure that precludes the generation of clinical cells doses that meet all Quality Control release criteria as specified by FDA.
8. Termination of the study by the Principal Investigator, the Sponsor, the Funding Sponsor, the IRB, ACC CTSRMC, ACC DSMC, DSMB, or the Food and Drug Administration.

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

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. Termination of the study by the Principal Investigator, the Sponsor, the Funding Sponsor, the IRB, ACC CTSRMC, ACC DSMC, DSMB, or the Food and Drug Administration.

The reasons for discontinuation (for example, voluntary withdrawal, toxicity, death) must be recorded on the case report form (CRF). 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 FDA. As long as subjects have detectable cells transduced with the lentiviral vector, they should be followed for toxicity, immune reactions, and any long-term adverse events. Therefore, subjects will continue to be followed for: 1) engraftment as long as patients are at risk (until evidence of loss of detectable transduced T cells), 2) Disease Free Survival (DFS) until there is disease progression, and 3) survival until the time of death or until the patient withdraws consent for clinical data collection, enrolls into a 15 year long-term follow-up protocol, or the end of the study (Last Subject/Last Visit). Subjects who complete follow-up as part of this protocol or discontinue participation early for any reason, will be encouraged to enroll in a 15 year long term follow-up protocol to further evaluate long term adverse events related to the study product. Once subjects are enrolled on the long-term follow-up protocol, all follow-up data collection under this protocol will be discontinued.

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 to the University of Pennsylvania for protocol required analysis. The subject and local provider will also be contacted via telephone by a member of the study team to assess any potential toxicity.

Every effort will be made to contact patients who appear to be lost to follow-up in order to at least obtain survival data. 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.

5. STUDY DRUG

5.1. Description

CART19 cells are autologous T cells that have been engineered to express an extracellular single chain antibody (scFv) with specificity for CD19 linked to an intracellular signaling molecule consisting of a tandem signaling domains comprised of the TCRζ signaling module linked to the 4-1BB costimulatory domain. The CART19 cells are cryopreserved in infusible cryomedia (volume dependent upon dose).

5.2. Subject Eligibility to Receive CART19 Transduced Cells

For Day 1 infusion:

1. All subjects must undergo a Respiratory Virus Panel (RVP) within 10 days prior to the first planned CART19 infusion. If the subject is positive for influenza, Tamiflu® or equivalent, should administered per package insert. The subject must complete treatment prior to receiving CART19. The test does not need to be repeated prior to the first CART19 infusion, however if influenza sign and symptoms are present, the CART19 infusions should be delayed until the subject is asymptomatic. If the subject is positive for another virus on the RVP, the CART19

infusion will be delayed for at least 7 days to be sure clinical symptoms of a viral infection do not develop. If clinical symptoms develop, the infusion will be delayed until resolution of these symptoms.

2. Subject should not experience a significant change in performance or clinical status compared to initial eligibility criteria that would, in the opinion of the treating physician, increase the risk of experimental cell infusion.
3. Subjects should not be experiencing signs/symptoms of an active infection.
4. Subject experiencing laboratory abnormalities after enrollment that, in the opinion of the treating investigator or PI, may impact subject safety or the subjects' ability to receive CART19 T cells, may have their infusion delayed until both the treating investigator and PI determine it is clinically appropriate to proceed with the CART19 infusion.
5. Subjects experiencing toxicities from their preceding cytoreductive chemotherapy can have their infusion schedule delayed until these toxicities have resolved. **Note:** If subjects' CART19 infusion is delayed > 4 weeks from cytoreductive chemotherapy, the cytoreductive chemotherapy should be repeated. The specific toxicities warranting delay of T cell infusions include:
    a. Pulmonary: Requirement for supplemental oxygen to keep saturation greater than 92% or presence of radiographic abnormalities on chest x-ray that are progressive
    b. Cardiac: New cardiac arrhythmia not controlled with medical management
    c. Hypotension requiring pressor support
    d. Active Infection(s) as evidenced by positive blood cultures for bacteria or fungus within 48 hours of CART19 cell infusion
6. For subjects with CNS3 disease, it must be > 8 weeks from radiation therapy (if used) and stable or improving CNS disease by the following criteria as applicable:
    a. If CNS3 by spinal fluid involvement, CSF WBC count stable or decreasing and <100 by lumbar puncture within 72 hours of infusion
    b. If CNS3 by MRI findings, improvement in MRI findings within 2 weeks of infusion
    c. If CNS3 by cranial nerve findings, stable or improving cranial nerve exam
7. Subjects must not have received any investigational therapy within 4 weeks of CART19 cell infusion. Please refer to **Section 5.6** for other concomitant therapy washout requirements.

For Subsequent CART19 infusions:

1. Subject 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.
2. Subjects should not be experiencing signs/symptoms of an active infection.
3. Subject experiencing new laboratory abnormalities that, in the opinion of the treating investigator or PI, may impact subject safety or the subjects' ability to receive CART19 cells, may have their infusion delayed until both the treating investigator and PI determine it is clinically appropriate to proceed with the CART19 infusion.

5.3. Treatment Regimen

The infusion will be scheduled to occur approximately 1 to 4 days following lymphodepleting chemotherapy but may be delayed as outlined above (**Section 5.2**). CART19 cells will be given on Days 1, 2 and 3.

Subjects will receive 1 to 5 x 10^8 transduced CAR T cells as a split dose over three days as follows:

- Day 1, 10% fraction: 1-5x10^7 CART19cells
- Day 2, 30% fraction: 3x10^7-1.5x10^8 CART19 cells

- Day 3, 60% fraction: $6x10^7$-$3x10^8$ CART19 cells

Note: Doses less than the goal range will be released and infused if all release criteria are met.

5.4. Preparation and Administration of Study Drug

Cell manufacturing is performed at the University of Pennsylvania Clinical Cell and Vaccine Production Facility (CVPF). The CART19 cells are prepared in the CVPF and are not released from the CVPF until FDA approved release criteria for the infused cells (e.g., cell dose, cell purity, sterility, average copy number of vectors/cell, etc.) are met. The CART19 dose is formulated according to transduced cell dose (flow cytometry testing) in cryobags.

The total cell number and volume depends on the transduction efficiency. Each bag will contain an aliquot of cryomedia (volume dependent upon dose).

Package and Labeling

Each infusion bag will be affixed with a label containing information regarding the dose, the method of manipulation, the vector and the following statements "FOR AUTOLOGOUS USE ONLY" And "Caution: New Drug- Limited by Federal Law to Investigational Use". In addition the label will have at least two unique identifiers. Prior to each infusion, two individuals will independently verify all unique identifier information in the presence of the patient and to confirm that the information is correctly matched to the patient.

Cell Thawing

The cells will be transported to the subject's bedside on the day of infusion. The cells will be thawed by trained personnel using a water bath maintained between 36°C to 38°C. The bag will be gently massaged until the cells have just thawed. There should be no frozen clumps left in the container at the time of infusion. If the CART19 cell product appears to have a damaged or leaking bag, or otherwise appears to be compromised, it should not be infused, and should be returned to the CVPF.

Premedication

Side effects following T cell infusions include transient fever, chills, and/or nausea. It is recommended that the patient be pre-medicated with 650mg acetaminophen and an antihistamine prior to each CART19 cell infusion. These medications may be repeated every six hours as needed. A course of non-steroidal anti-inflammatory medication may be prescribed if the patient continues to have fever not relieved by acetaminophen. Patients should not receive systemic corticosteroids such as hydrocortisone, prednisone, prednisolone (Solu-Medrol) or dexamethasone (Decadron) at any time, except in the case of a life-threatening emergency, since this may have an adverse effect on CART19 cell expansion and function.

Neutropenic subjects will be administered preventive antibiotics treatment starting on the day of infusion. Broad-spectrum antibiotics will be administered orally until recovery of neutrophil counts, or until judged by the investigator to no longer be at increased risk of infection.

Return or Destruction of Study Drug

CART19 cells may need to be returned to the CVPF for a variety of reasons, including but not limited to: 1) Mislabeled product; 2) Condition of patient prohibits infusion/injection, and 3) Subject refuses infusion. Any unused product will be returned to CVPF by CVPF personnel as per CVPF SOP.

There is an ongoing reconciliation of drug shipped, drug consumed, and drug remaining performed by the CVPF. Final disposition of the investigational product will also be documented in the site Investigational Product Accountability logs appropriately.

5.5. CART19 Product Infusions

CART19 will be administered via i.v. infusion at the [redacted] by a licensed Registered Nurse. The investigational CART cell products should be infused into the subject immediately after they are thawed. There should be no frozen clumps left in the bag prior to infusion. The CART19 cells will be infused into an intravenous catheter, either through a peripheral vein (preferred) or central vein. A macrodrip intravenous tubing will be used to infuse the CART cells by gravity (i.e., no infusion pump). The macrodrip intravenous tubing will be connected to a "Y" adapter with one end of the adapter spiked to the CART cell product bag(s) and the other to a normal saline solution bag. A **leukoreduction filter must not be used for the infusion of the CART cell product**. The duration of the infusion will be based on the total volume to be infused (on the order of 1-10 minutes).

Vital signs (temperature, respiration rate, pulse, blood pressure, and oxygen saturation by pulse oximetry) will be measured within 10 minutes prior to the infusion and within 15 minutes after the infusion. Thereafter, vital signs will be measured at 30 (+/- 5) minutes, 45 (+/- 5) minutes, and 60 (+/- 5) minutes after the infusion, and then every hour (+/- 10 minutes) for the next 2 hours until these signs are satisfactory and stable. If the subject's vital signs are not satisfactory and stable three hours post-CART19 infusion, vital signs will continued to be monitored at a minimum of every hour or as clinically indicated until stable. If the infusion is administered in the outpatient setting, the subject will be discharged after the physician managing their care on the day of each infusion has determined that they are in satisfactory condition.

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 CART19 T cell product is suspected, the product can be retested for sterility using archived samples that are stored in the CVPF. Consideration of a CRS should be given.

Additional Safety Procedures Prior to Administration

The on-site pharmacy must confirm that a dose of tocilizumab is on site prior to infusion and available for administration in order to manage suspected toxicities.

Emergency medical equipment (i.e., emergency trolley) must be available during the infusion in case the patient has an allergic response, or severe hypotensive crisis, or any other reaction to the infusion.

5.6. Concomitant Therapy

All prescription and nonprescription medication, vitamins, herbal and nutritional supplements, taken by the patient during the 30 days prior to screening will be recorded. At every visit following the CART19 infusions and until the patient 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:

- 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.
- Chemotherapy, including corticosteroids and tyrosine kinase inhibitors, or immunosuppressive medications should NOT be used within one week prior to the apheresis procedure.
- Steroids or other immunosuppressant drugs should NOT be used within 24 hours prior to (refer to **Section 5.4**) or 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. However, the following physiological replacement doses of steroids are allowed: 6-12 mg/m^2/day hydrocortisone or equivalent
- Patients with severe signs and symptoms attributable to cytokine release syndrome (i.e. CRS) should be managed with administration of tocilizumab or other anti-cytokine directed therapies (Refer to **Section 8.5.2** for administration details).
- Neutropenic subjects will be administered broad-spectrum antibiotics on the day of infusion.

6. STUDY PROCEDURES

Overview

The schedule of evaluations and study procedures are described in Visit Evaluation Schedule 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/Enrollment Assessments (~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 Visit Evaluation Schedule **(Appendix 1)**. For the purposes of this study, enrollment is defined as the date the Investigator confirms subject eligibility.

- 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 child bearing 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, BSA, blood pressure, body temperature, heart rate and oxygen saturation via pulse oximetry)
- ECOG performance status
- ECHO/MUGA-performed within 12 weeks of enrollment and after last treatment with induction chemotherapy (excluding maintenance chemotherapy)
- Electrocardiogram (EKG)
- Complete blood count and differential, comprehensive metabolic panel, and coagulation panel. See Table 6-2 for complete details.
- Viral serologies - including HIV, Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody, Hepatitis B core antibody, and Hepatitis C Antibody. 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 enrollment is provided.

- Serum pregnancy test for females of child bearing potential
- Serum immunoglobulin levels
- Bone marrow aspirate for disease and MRD assessment as described in **Appendix 1** within 4 weeks of enrollment
- Chest X-ray for mediastinal disease assessment- performed only if clinically indicated.
- 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.

In the event that the time between the screening visit and the infusion of CART19 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, and HIV and Hepatitis B/C tests. An ECHO/MUGA scan must be performed within 6 months prior to the CART19 infusion.

6.2. Subject Enrollment

At the time a subject consents to participate in this study, a Consent Notification Form should be completed. When eligibility of the subject is confirmed by a Study Investigator, and follow-up on this trial commences, an Enrollment Notification should be completed. Both completed forms should be emailed in real-time to:

Protocol Monitor and Sponsor Project Manager
[redacted]

Once subject eligibility has been confirmed by an investigator, the subject may undergo the apheresis procedure and CART19 manufacturing may commence.

Each Subject is identified in the study by a Subject Number that is assigned when the subject is first consented and is retained as the primary identifier for the subject throughout his/her entire participation in the trial. The Subject Number consists of the Protocol Number with a sequential subject number suffixed to it, so that each subject is numbered uniquely across the entire database. 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. If the subject fails screening for any reason, the reason will be entered into the End of Study eCRF.

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

After the patient has been enrolled, patients will be scheduled for apheresis (leukapheresed) to obtain a target of $5x10^9$ PBMCs for CART19 manufacturing. Apheresis can occur any time after the subject is enrolled and up to 3 weeks prior to CART19 infusion. Baseline blood leukocytes for FDA look-back requirements and for research are also obtained and cryopreserved (1×10^8 cells from apheresis to TCSL and $1\text{-}2 \times 10^9$ to PDL, if available after cells required for manufacturing are obtained). Apheresis should be scheduled prior to any planned chemotherapy administration. The cell product is expected to be released approximately 3-4 weeks after manufacturing has commenced.

Historical Apheresis Sample
Cryopreserved historical apheresis products collected from the patient prior to study entry are usable for CART19 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/uL. If the ALC <500/uL 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. The Investigator or designated staff 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 pre-entry) concomitant medications.

6.4.2. Physical Exam

A complete physical examination will be performed by the investigator 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.

Significant findings that are present prior to the administration of the investigational product must be recorded as part of the subjects' medical history. All findings made after the start of investigational product which meet the definition of an Adverse Event must be recorded and reported appropriately. Please see Section 8 for additional details.

6.4.3. Vital Signs

Blood pressure, body temperature, oxygen saturation by pulse oximetry, and heart rate will be measured as indicated in **Appendix 1.**

On infusion days, vital signs (temperature, respiration rate, pulse, blood pressure, and oxygen saturation by pulse oximetry) will be measured within 10 minutes prior to the infusion and within 15 minutes after the infusion. Thereafter, vital signs will be measured at 30 (+/- 5) minutes, 45 (+/- 5) minutes, and 60 (+/- 5) minutes after the infusion, and then every hour (+/- 10 minutes) for the next 2 hours until these signs are satisfactory and stable. If the subject's vital signs are not satisfactory and stable three hours post-CART19 infusion, vital signs will continued to be monitored at a minimum of every hour or as clinically indicated until stable.

If high fevers (≥ 101.5° F / 38.6° C) occur in the days to weeks following CART19 infusion, additional assessments are required to more closely monitor the patient until resolution of the fever (below 101.5°

F / 38.6^{o} C).

6.4.4. ECOG Performance status

At Visits according to **Appendix 1**, the ECOG performance scale index will be used to evaluate the performance status of the patients.

Table 6-1: ECOG Performance status grade	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

6.4.5. Cardiac Assessment: ECHO/MUGA

An ECHO/MUGA scan is required to confirm protocol eligibility. This needs to be completed within 12 weeks of enrollment and after last treatment with induction chemotherapy (excluding maintenance chemotherapy). Patients must have a LVEF ≥40% to be included into the study. An ECHO/MUGA scan will need to be repeated prior to CART19 infusion if has been greater than 6 months since this scan was performed.

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 AEs or CART19 expected events. For all laboratory assessments that occur on Infusion Days (Day 1, 2 or 3) these should be performed **prior** to CART19 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 study related to CART19 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 (SGPT), AST (SGOT), Magnesium, Phosphate, LDH, Uric Acid; Direct bilirubin (to be performed at screening only)
HLH/MAS and CRS screen – repeated if clinically indicated	Ferritin, CRP, Haptoglobin, triglycerides, LDH
Coagulation	Prothrombin time (PT), International normalized ratio (INR), Partial thromboplastin time (PTT), fibrinogen, D-dimer
Viral Serologies	HIV, Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody, Hepatitis B core antibody, and Hepatitis C Antibody. 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 enrollment is provided.
Respiratory Virus Panel (RVP)	Influenza A, Influenza B, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Parainfluenza Virus Type 1, Parainfluenza Virus Type 2, Parainfluenza Virus Type 3, Adenovirus
T-Cell Subsets	CD4, CD3, CD8
Donor chimerism	Whole blood (minimum required) and CD3+ lineage-specific chimerism preferred
Additional Assessments	Serum immunoglobulin levels, Serum and Urine Pregnancy Test

6.4.7. Hematology, Coagulation and T cell Subsets

Hematology & Coagulation safety assessments will be performed at screening, pre-infusion (Day-1), prior to CART19 infusions on Days 1, 2 and 3, and at each study visit according to **Appendix 1**. Assessments will include WBC (total) with differential count including % lymphoblasts, hematocrit, hemoglobin, platelets, prothrombin time, INR, PTT, Fibrinogen and d-dimer.

T-cell subsets analysis (CD3, CD4 and CD8) will be performed prior to the first CART19 infusion on Day 1 and again on Day 28, and Months 2, 3, 4, 5 and 6.

6.4.8. Chemistry

Biochemical safety assessments will be performed at screening, pre-infusion (Day-1), prior to CART19 infusions on Days 1, 2 and 3, and at each study visit according to **Appendix 1**. Assessments will include Glucose, BUN, Creatinine, Sodium, Potassium, Calcium, Total Protein, Albumin, Total Bilirubin, Alkaline phosphatase, ALT (SGPT), AST (SGOT), Magnesium, Phosphate, LDH, Uric Acid. Direct bilirubin will be performed at Screening only.

Additional assessment of Ferritin, LDH and CRP levels for CRS:
As noted, side effects following CART19 cell infusions can induce high fevers and should be expected. If high fevers (≥ 101.5° F / 38.6° C) occur following CART19 infusion, every attempt will be made to monitor additional Ferritin, LDH and CRP levels **daily** at fever onset and until resolution of the fever (below 101.5° F / 38.6° C). Other chemistries should be monitored per **Appendix 1** or as clinically indicated if CRS is suspected.

Additional assessments of Haptoglobin and Triglycerides for HLH/MAS screen
Haptoglobin and triglycerides will be assessed at pre-infusion[106] and should be monitored per **Appendix 1** or as clinically indicated if HLH/MAS is suspected.

6.4.9. Viral Serology

Blood will be taken for HIV, Hepatitis B, and Hepatitis C at Screening per Table 6-2 above. 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 enrollment is provided.

6.4.10. Serum Immunoglobulin Levels

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

6.4.11. Pregnancy Testing

Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, must use effective contraception (see Section 4.3 for details). For women of childbearing potential, a serum pregnancy test (β-HCG) will be performed according to **Appendix 1** at screening. During treatment, an additional pregnancy test will be performed prior to (within 72 hours) the first CART19 infusion and again at the end of study (Month 12). Patients should be instructed to inform site 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 CART19 T cell infusion, patients must be withdrawn from the study.

6.4.12. Research Assessments to Assess Engraftment, Persistence and Bioactivity

The following assessments will be collected according to **Appendix 1** and will be analyzed as described below at the TCSL. These tests may include:

- Serum Cytokines
- DNA Q-PCR CART19 persistence
- DNA RCL (VSV-G Q-PCR)
- CART19 Immune phenotyping
- MRD by deep sequencing

For molecular studies (Q-PCR and deep sequencing), immune phenotyping and functional assays, peripheral blood and marrow samples will be collected in Lavender top (K2EDTA) tubes. For cytokine analyses peripheral blood and marrow samples will be collected in red top (no additive) tubes. For all subjects, deep sequencing will be performed on marrow samples collected pre-infusion (~Day -1) and at Day 28; for subjects experiencing a morphological response, deep sequencing will also be performed on marrow samples collected at months 3 and 12. Samples will be delivered, processed, and frozen as

per SOP to the Translational and Correlative Studies Laboratory (TCSL) at the [redacted]. Samples will be stored in the TCSL at the [redacted] for storage and bulk analyses. Documentation for sample receipt, processing, and storage and primary data from the research analyses will be collected and stored in the TCSL.

Translational and Correlative Studies Laboratory (TCSL),

[redacted]

In the event that something unexpected occurs, the research team may request an additional sample collection be performed to collection additional blood or marrow/LN samples for research analysis. This is being done with the intention of evaluating the likely effects from the investigational product received. Additional research sample collection may be performed. The total amount of extra blood that may be collected will be 3 tablespoons of blood twice in one week. The total amount of extra bone marrow or lymph node biopsies collected will be up to 1 extra procedure per month.

An additional larger blood draw (~30cc) will be drawn on Days 14 and 28 and archived for future research purposes. This blood sample will also be sent to TCSL as indicated above.

6.4.13. Cytogenetics/FISH

Cytogenetics/FISH assessments will be performed as clinically indicated at each time point a bone marrow aspirate/biopsy is sampled (**per Appendix 1**).

6.4.14. Cytoreductive chemotherapy

Prior to CART19 cell infusion, an additional chemotherapy cycle is planned. A selection is provided below for guidance; however, the regimen of chemotherapy will be at the discretion of the investigator and dependent on the patient's prior history, disease burden and other patient specific factors.

Note, the lymphodepleting chemotherapy prior to CART19 cell infusion is **NOT required if the patients WBC ≤ 1,000 /uL. Additionally, if the delayed period from chemotherapy to CART19 infusion is 4 or more weeks, the patient will need to be re-treated with lymphodepleting chemotherapy prior to CART19 infusion.

For B-cell ALL patients, Fludarabine and Cyclophosphamide [(Fludarabine (30mg/m^2/d x 4 days) and Cyclophosphamide (500mg/m^2/d x 2 days)] will be the preferred lymphodepletion regimen. An alternative regimen may be used at the discretion of the PI based on the subject's prior treatment history. The suggested alternative regimens include the following:

1. Fludarabine (30mg/m^2/d x 4 days) and Cyclophosphamide (500mg/m^2/d x 2 days)
2. Clofarabine 40 mg/m^2/d x 5 d
3. High dose methotrexate 3 gm/m^2
4. AraC 1.5-3 gm/m^2 q12 hr x 6-12 doses
5. Methotrexate 1mg/m^2 with AraC 1-3gm/m^2 x 4 doses
6. CVAD (cytoxan, vincristine, adriamycin, decadron)
7. Cyclophosphamide 1.5-3gm/m^2 over 1-3 days

8. ICE (ifosphamide, carboplatin, etoposide)
9. Daunorubicin /AraC

The chemotherapy will be planned so that the last dose is completed ~1-4 days BEFORE the planned infusion of CART19 cells for ALL. Each regimen is of different duration so the start day of chemotherapy will vary. The purpose of the chemotherapy is to induce lymphopenia in order to facilitate engraftment and homeostatic expansion of CART19 cells. In addition, chemotherapy can potentiate the ability of T cells to kill tumor cells. The chemotherapy is not investigational and may be given by a patient's local oncologist within the specified time frame.

All subjects must undergo a Respiratory Virus Panel (RVP) within 10 days prior to the first planned CART19 infusion. If the subject is positive for influenza, Tamiflu® or equivalent, should be administered per package insert. The subject must complete treatment prior to receiving CART19. The test does not need to be repeated prior to the first CART19 infusion, however if influenza sign and symptoms are present, the CART19 infusions should be delayed until the subject is asymptomatic. If the subject is positive for another virus on the RVP, the CART19 infusion will be delayed for at least 7 days to be sure clinical symptoms of a viral infection do not develop. If clinical symptoms develop, the infusion will be delayed until resolution of these symptoms.

6.4.15. CART19 Infusions

Subject infusions are to begin 1 to 4 days after completion of chemotherapy (if administered) as indicated in **Section 6.4.15**. Subjects will undergo tests and procedures in accordance with the Visit Evaluation Schedule 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.

Patients will be infused and premedicated as described in **Section 5.4**.

6.4.16. Day 28: Follow Up

At the Day 28 visit, subjects will undergo tests and procedures in accordance with the Visit Evaluation Schedule in Appendix 1. Tumor response assessments will be done according to National Comprehensive Cancer Network (NCCN) v1 2013 guidelines (**See Sections 6.5 and 6.6**)

6.4.17. Monthly Evaluations 2 to 6 Months Post Infusion

Subjects will return to the clinic on a monthly basis during months 2 to 6 post CART19 cell infusion. At these study visits, subjects will undergo tests and procedures in accordance with the Visit Evaluation Schedule in Appendix 1. Tumor response will be measured accordingly to **Sections 6.5** and **6.6** at Months 3 and Month 6.

6.4.18. Quarterly Evaluations for up to 1 Year Post Infusion

Subjects will be evaluated on a quarterly basis until 1 year post infusion. At these study visits, subjects will undergo tests and procedures in accordance with the Visit Evaluation Schedule in Appendix 1. Tumor response will be measured accordingly to **Sections 6.5** and **6.6** at Months 9 and 12.

6.4.19. Long-term Follow-up

Subjects who complete follow-up as part of this protocol or discontinue participation early for any reason will be encouraged to enroll in a 15 year long term follow-up protocol to further evaluate long term adverse events related to the study product. Once subjects are enrolled on the long-term follow-up protocol, all follow-up data collection under this protocol will be discontinued.

6.5. Efficacy Assessments

Tumor response assessments will be done at Screening/Enrollment, Pre-infusion (prior to CART19 infusions), and then at Day 28 and Months 3, 6, 9 and 12 after CART19 cell infusions or until the patient requires alternative therapy for their disease. Assessments will be made as clinically indicated by physical exam, chest x-ray, 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

Procedure	Pre-Infusion Assessments	Post Infusion Assessments
Bone marrow aspirate and biopsy for blast cell counts	Mandated	Mandated: Day 28, Months 3, 6, 9 and 12
Peripheral Blood for blast, neutrophil and platelet cell counts	Mandated	Mandated: Day 28, Months 3, 6, 9 and 12
Lymph node biopsy	Optional; Performed if accessible and/or as clinically indicated	Optional; performed if accessible and/or as clinically indicated at Day 28 and Months 3, 6, 9, and 12
CSF Assessment for CNS disease	Mandated- within 4 weeks of the first CART19 infusion.	Mandated Day 28 and then as clinically indicated by the presence of neurologic symptoms
CNS Brain Imaging (MRI/CT)	As clinically indicated	As clinically indicated
Chest x-ray for mediastinal disease	If clinically indicated	If clinically indicated
Chest CT/MRI scan for mediastinal disease	Required if the screening chest x-ray suggests mediastinal enlargement	Mandated only if mediastinal enlargement confirmed at or before screening: Day 28 and Month 3
Physical exam for extramedullary disease	Mandated	Mandated Day 28 and Months 3, 6, 9, and 12
BCR-ABL PCR of blood and bone marrow aspirate for patients with Ph+ ALL	Mandated	Mandated Day 28 and Months 3, 6, 9, and 12
MRD assessment of bone marrow by flow cytometry (every patient)	Mandated	Mandated Day 28, Months 3, 6, 9 and 12

Table 6-3: Disease Assessment Collection Plan		
Procedure	**Pre-Infusion Assessments**	**Post Infusion Assessments**
Transfusion dates	Assess dependency	Record as needed during the course of the trial

6.5.1. Physical Exam

A physical examination will be used to assess evidence extramedullary disease as clinically appropriate in the liver, spleen, lymph node, skin, gum infiltration, testicular involvement and other sites as applicable. Extramedullary involvement is to be assessed at screening and at each response assessment visit.

6.5.2. Bone Marrow Aspirate/Biopsy and Peripheral Blood

Bone marrow biopsies and aspirate will be measured for tumor evaluations and efficacy analysis per Appendix 1. When aspirate smear and differential is not interpretable by the pathologist (e.g., hemodilute or dry tap) response assessment as evaluated by bone marrow biopsy may substitute for bone marrow response.

6.5.3. Cerebrospinal Fluid (CSF) Assessment

If CNS symptoms are present at Screening/Enrollment, a lumbar puncture and brain imaging by MRI/CT will be performed to assess CNS leukemic involvement. All subjects will have cerebrospinal fluid (CSF) evaluations performed at baseline (within 4 weeks of the first CART19 infusion) and at Day 28. Subsequent CSF assessments will be performed as clinically indicated (i.e. when new neurological symptoms are present). Subjects without baseline CNS involvement who do not have a day 28 evaluation will still be considered evaluable for response. CSF will be analyzed for cell count and differential, cytology, and for the presence of CART19 cells. Additionally, CSF may be assessed during the height of cytokine release syndrome (CRS).

6.5.4. Mediastinal Disease Assessment

A chest x-ray will be performed at screening. If the chest x-ray reveals mediastinal widening/enlargement, then a CT/MRI scan is required at baseline to confirm the presence of mediastinal disease and subsequent visits are required at Day 28 and at Month 3. If the screening chest x-ray suggests no mediastinal disease then no further chest imaging is required.

If at any time point, mediastinal disease is present by CT/MRI assessment, then follow-up CT/MRI is required to document the absence of mediastinal involvement whenever the patient meets all other criteria for complete response. Two evaluations at least 28 days apart are needed to confirm a response in mediastinal disease. The timing of the CT/MRI must be within the required time window of the other disease response components (**Table 6-3**).

For optimal evaluation of patients, the same imaging method of assessment (i.e. CT or MRI) and technique (i.e. with or without contrast) should be used to characterize each identified and reported lesion at baseline and during follow-up. A change in methodology can be defined as either a change in contrast use (e.g. keeping the same technique, like CT, but switching from intravenous contrast use to non-contrast

enhanced CT, regardless of the justification for the change) or a change in technique (e.g. from CT to MRI, or vice-versa).

6.5.5. Minimal Residual Disease (MRD)

All patients will have multi-parameter flow cytometry on bone marrow aspirate for MRD status at each time point a bone marrow aspirate is collected (**Appendix 1**).

MRD testing will be performed at the University of Washington using a multicolor flow measurement that has been validated and reproducible (see http://www.fda.gov/downloads/drugs/newsevents/ucm300749.pdf). The sensitivity of the test is 0.01%.

6.5.6. BCR-ABL: Ph+ ALL Patients

Bone marrow aspirates sampled at the time points for tumor assessments will additionally be analyzed for BCR-ABL for Ph positive ALL patients only. Where applicable, peripheral blood testing for quantitative BCR-ABL levels should be sent concurrently.

6.5.7. Evaluation of Transfusion Dependency

Information on transfusion dependency will be assessed at screening as well as during the course of the trial for all patients. Transfusion of blood products will be recorded in a separate module of the eCRF. The type of transfusion, start and end date as well as the number of units will be captured at each visit with hematologic assessment.

A period of at least one week without any transfusion has been taken as a convention to define the status of transfusion independence to assess a CR vs. CRi response[107]. Any sample of peripheral blood which was taken less than seven days after a transfusion will be considered as taken while the patient is transfusion dependent.

6.6. ALL Response Criteria

The response criteria will be evaluated according 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)[108] and the International Working Group (IWG) guideline for acute myeloid leukemia (AML)[39,109]. 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 6.5**) will be performed based on bone marrow and blood morphologic criteria, physical examination findings, 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% blasts

Table 6-4: Overall disease response classification at a given evaluation time	
Response category	**Definition**
	Peripheral blood Neutrophils > 1.0 x 10^9/L, and Platelets > 100 x 10^9/L, and Circulating blasts < 1% **Extramedullary disease** No evidence of extramedullary disease (no CNS disease, mediastinal disease CR, 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 x 10^9/L, or Platelets ≤ 100 x 10^9/L, or Platelet and/or neutrophil transfusions within week before peripheral blood sample for disease assessment
Complete remission (CR) with residual mediastinal disease	All criteria for CR or CRi as defined above are met, except that mediastinal disease as defined by CRu or PR is observed:
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 blasts in the blood (≥ 1%), or Reappearance of blasts in bone marrow (≥ 5%), or (Re-)appearance of any extramedullary disease after CR
Unknown	In case the response assessment was not done, the baseline assessment was not done, the assessment was incomplete or was not done within the respective time frame. If there is evidence of relapse, the overall response will assessed as relapse with the relapsed component alone.

Note:
The NCCN guideline has defined mediastinal response criteria including CRu and PR. In the case a patient achieves CR or CRi at all other non-mediastinal disease sites, and has residual mediastinal disease [110], a category for of overall disease response of CR or CRi with residual mediastinal disease has been included in this document, which is not part of the NCCN guidance.

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) [109] guideline. The difference between PD and “No response” in ALL is not believed to be clinical meaningful.

7. STATISTICAL PLAN

7.1. Design Overview

This is a single center, single arm, open-label phase 2 study to determine the efficacy of autologous T cells expressing CD19 chimeric antigen receptors expressing tandem TCRζ and 4-1BB (TCRζ/4-1BB) co-stimulatory domains (referred to as "CART19" cells) in adults with minimal residual disease (MRD) during upfront treatment for CD19+ acute lymphoblastic leukemia.

7.2. Sample Size Justification

The study will enroll 24 evaluable subjects with MRD positive ALL. The primary endpoint is conversion to MRD^- (MRD< 0.01%) by day 28. The table below provides 90% exact Clopper-Pearson confidence intervals for different possible event (conversion) rates. For example, if 21 out of 24 evaluable subjects (87.5%) are observed to convert to MRD^- (<0.01%), then the observed rate 90% confidence interval is (70.8%, 96.5%). With 24 patients, there will be at least 80% power to detect an ORR of 40% or greater against a null rate of 15% using a two-sided exact binomial test at 10% type I error. At least 8 out of 24 patients would need to achieve MRD^- by day 28 (ORR=33%; exact 90% CI [17.8%, 52.1%]) to indicate meaningful efficacy of 15% or greater. If the true MRD^- rate were 84%, we have more than 80% power to reject a rate of 60% or smaller with 90% confidence. The confidence intervals below also provide the precision for 90% confidence intervals around adverse events.

90% Clopper-Pearson confidence intervals for different possible observed event rates seen in 24 evaluable subjects.

Observed rate (%)	90% Confidence Interval (%)
0/24 (0)	(0.0, 11.7)
1/24 (4.2)	(0.2, 18.3)
2/24 (8.3)	(1.5, 24.0)
3/24 (12.5)	(3.5, 29.2)
6/24 (25.0)	(11.5, 43.5)
9/24 (37.5)	(21.2, 56.3)
12/24 (50.0)	(31.9, 68.1)
15/24 (62.5)	(43.7, 78.8)
18/24 (75.0)	(56.5, 88.5)
21/24 (87.5)	(70.8, 96.5)
24/24 (100)	(88.3, 98.0)

7.3. Analysis Sets

- The **Enrolled Set** comprises all patients who sign an informed consent form and are enrolled in the study, excluding screen failure patients.
- The **Primary Evaluable Set** comprises all patients who receive the CART19 cells at the intended dose range and completed the response assessments for the primary endpoint as planned by the protocol. Primary evaluable patients also include those with disease progression or death prior to the primary endpoint response assessment. These are the primary evaluable patients as defined below. The Primary Evaluable Set will be used for the primary endpoint analysis.
- The **Full Analysis Set (FAS)** comprises all patients who received the CART19 cells. This set includes both primary efficacy evaluable and non-evaluable patients as defined below. The Full Analysis

Set will be used for the secondary efficacy, safety and correlative endpoints or other exploratory analyses.

Definitions relevant to the Analysis Sets:

1) **Screening failure -** Any patient who fails to meet the inclusion/exclusion criteria specified by the protocol.
2) **Manufacturing failure** – Any patient who has manufactured CART19 cells that do not meet the manufacturing release criteria.
3) **Primary evaluable patient** – Any patient who is infused with at least 1-5x10^7 CART19 cells and completed the response assessments for the primary efficacy endpoint as planned by the protocol. Primary evaluable patients also include those with disease progression or death prior to the primary endpoint response assessment.
4) **Primary non-evaluable patient** – Any patient who is infused with the CART19 cells at less than the protocol-specified dose. These patients are also counted as manufacturing failures. Patients who are infused and drop out before the Day 28 assessment due to reasons other than disease progression or death are also considered non-evaluable.

7.4. Analysis of Primary Objective

The primary endpoint is efficacy as measured by the incidence of MRD conversion to < 0.01% (MRD^-) at Day 28 post-CART19 in patients with MRD+ ALL during upfront treatment.

7.5. Analysis of Secondary Objectives

Efficacy will also be determined as the overall complete remission rate (ORR) at day 28, computed as the proportion of subjects with CR or CRi according to the response criterion described in Section 6.12. The two-sided exact Clopper-Pearson 90% confidence intervals for Day 28 ORR will be computed. For the secondary efficacy objectives for this study, the proportion of subjects will be computed with a best overall disease response of CR or CRi, where the best overall disease response is defined as the best disease response recorded from the start of the treatment until death, last follow up, relapse or start of new anticancer therapy, whichever comes first. Proportion of subjects achieving CR or CRi before or at Month 6 (prior to receiving other anticancer therapy if any), and the proportion of subjects with a minimal residual disease (MRD) negative bone marrow as determined according to Section 6.13 will also be computed. Two-sided exact Clopper-Pearson 90% confidence intervals for the proportions will be provided.

Secondary efficacy objectives for this study also include the evaluation of the following time to event endpoints: overall survival (OS), duration of remission (DOR), relapse free survival (RFS), and event free survival (EFS). Definitions for each of the endpoints are described below. The survival function of those endpoints using the Kaplan-Meier method will be estimated. 90% confidence interval for the survival probability at a specific time point (e.g., 3-month overall survival) will be computed based on the log-log transformation. Median survival time along with the associated 90% confidence intervals will be presented if appropriated. **Overall survival (OS)** is defined as the time from the date of the first CART19 infusion to the date of death due to any reason. In case a subject is alive at the date of last contact on or before the date of data cutoff, OS is censored at the date of last contact. Cause of death will be described when applicable. **Duration of remission (DOR)** is defined as the duration from the date when the response criteria of CR or CRi is first met to the date of relapse or death due to ALL. DOR will be assessed only in subjects with the best overall response of CR or CRi.

In case a subject does not relapse or die due to ALL prior to the date of data cutoff, DOR will be censored at the date of the last adequate assessment on or prior to the earliest censoring event. The censoring event could be:

- Lost to follow-up
- Withdrew consent
- Death due to reason other than ALL
- New anticancer therapy (including HSCT when performed in CR or CRi)
- Event after at least two missing scheduled disease assessment

Although subjects in remission might choose to receive HSCT, data on relapse, survival, and response status after HSCT will not be used for the calculation of DOR. This is because HSCT procedure could affect remission duration independent of CART19 therapy; in addition it is likely to remove any remaining CART19 cells in the subject thus outcomes after HSCT cannot be solely attributed to the CART19 treatment. If a substantial number of subject choose to receive HSCT while in CR or CRi, sensitivity analysis will be performed in which subjects who receive HSCT while in CR or CRi are not censored at time of HSCT; an analysis with HSCT regarded as a competing risk to the event of interest (e.g., relapse after CART19 treatment) may also be considered. In a competing risk analysis, the cumulative incidence function (CIF) would be computed to estimate the probability of relapse in the presence of the competing risk due to HSCT. Analyses that treat death for reasons unrelated to ALL as a competing risk will also be considered.

Relapse free survival (RFS) is defined as the duration between the date when the response criteria of CR or CRi is first met to the date of relapse or death due to any cause. RFS will be assessed only in subjects with the best overall response of CR or CRi.

In case a subject does not relapse or die due to any cause prior to the date of data cutoff, RFS will be censored at the date of the last adequate assessment on or prior to the earliest censoring event. The censoring event could be:

- Lost to follow-up
- Withdrew consent
- New anticancer therapy (including HSCT when performed in CR or CRi)
- Event after at least two missing scheduled disease assessment

Sensitivity analyses similar to those described for the analysis DOR will be performed.

Event free survival (EFS) is defined as the time from start of the first CART19 infusion to the earliest of the following:

- Death from any cause
- Relapse
- Treatment failure: Defined as no response in the study and discontinuation from the study due to any of the following reasons:
    - Adverse event(s)
    - Abnormal laboratory value(s)
    - Abnormal test procedure results
    - New cancer therapy (excluding HSCT when performed in CR or CRi)

In case a subject does not experience an event of interest prior to the date of data cutoff, EFS is censored at the last adequate response assessment date on or prior to the date of data cutoff.

All secondary efficacy endpoints will be assessed using the FAS.

7.6. Analysis of other secondary objectives

Descriptive statistics will be calculated for correlative endpoints and patient reported outcomes. For continuous variables, mean, median, standard deviation, inter-quartile range will be provided. For discrete variables, frequency and proportions will be used. The correlation between measures of immunogenicity and the loss of CART19 engraftment will be evaluated using Pearson's correlation coefficient or the nonparametric equivalent of Spearman rank correlation. When endpoints of interest are obtained from the same patient at multiple time points (e.g., CART19 in vivo survival is measured by Q-PCR weekly for the first month, monthly until Month 6 and every three months until Month 12), statistical methods appropriate for longitudinal data will be implemented. To evaluate manufacturing feasibility, the proportion of manufacturing products that do not meet release criteria for vector transduction efficiency, T cell product purity, viability, sterility or due to tumor contamination will be computed using patients in the enrolled set. 90% confidence interval appropriate for each statistic will be used.

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 that 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 severity and/or frequency is not described in the investigator brochure or protocol. Please refer to the investigator brochure for additional detail related to severity and/or frequency of a particular event.

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.

Adverse Event Reporting Period
For this study, adverse events are reported starting on Day 1 (from the start of the first CART19 infusion) until the subject is off study or until the 1 year end of study visit. Events not related to apheresis procedure which occurs after enrollment but before CART19 infusion will be excluded from the adverse event reporting period.

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. After 30 days, any SAE that the investigators become aware of should be reported to the sponsor if the investigator suspects the event may reasonably be related to the study treatment.

Preexisting Condition/General Physical Examination Findings
A preexisting condition is one that is present at the start of the study. At screening, any clinically significant abnormality 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 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 Toxicity Criteria version 4.03 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 if a diagnosis can be assigned.

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/ not suspected) 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 (if applicable) or protocol].
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.

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.

CTCAE Grading System of Cytokine Release Syndrome

A protocol specific grading system (**Table 8-1**) has been developed to capture cytokine release syndrome (CRS) in CAR T-cell protocols. Please refer to Section 1.5.2 and the CART19 investigator brochure for additional detail on CRS in CART19.

For the purposes of reporting and grading on clinical trials using CAR T-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 100.4°F/38°C.

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	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	Life-threatening complications such as hypotension requiring high dose pressors (see Table 8-2), or hypoxia requiring mechanical ventilation	Death

Table 8-1: CRS grading criteria				
CRS Toxicity Grade (Modified)				
1	**2**	**3**	**4**	**5**
	with associated neutropenia.	(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.		

*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 or ≥ 20 mcg/min (if institutional practice is to use flat dosing)
Dopamine monotherapy	≥ 10 mcg/kg/min or ≥ 1000 mcg/min (if institutional practice is to use flat dosing)
Phenylephrine monotherapy	≥ 2 mcg/kg/min or ≥ 200 mcg/min (if institutional practice is to use flat dosing)
Epinephrine monotherapy	≥ 0.1 mcg/kg/min or ≥ 10 mcg/min (if institutional practice is to use flat dosing)
If on vasopressin	High-dose if vaso + Norepinephrine Equivalent (NE) of >0.1 mcg/kg/min (or 10mcg/min) (using Vasopressin and Septic Shock Trial (VASST) formula)
If on combination vasopressors (not vasopressin)	Norepinephrine equivalent of ≥ 0.2 mcg/kg/min (or ≥ 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[111].

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 [redacted] of learning of its occurrence. The original SAE notification may take place by email to meet the [redacted] reporting window. However within [redacted] days of 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.

Follow-up information on SAEs should be reported when updates are available, as a follow-up to the initial SAE form, and should include both the follow-up number and report date. New information on ongoing serious adverse events should be provided promptly to the sponsor. 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

[redacted]

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

1. Study identifier
2. Subject number
3. A description of the event
4. Date of onset
5. Current status
6. Whether study treatment was discontinued
7. The reason the event is classified as serious
8. Investigator assessment of the association between the event and study treatment
9. Expectedness relative to investigational product

8.3.1. Investigator Reporting: Notifying the Penn IRBs

This section describes the requirements for safety reporting by investigators who are Penn faculty, affiliated with a Penn research site, or otherwise responsible for safety reporting to the IRB. The IRB requires expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below. The IRB requires researchers to submit reports of the following problems within [redacted] days from the time the investigator becomes aware of the event:

Any adverse event (regardless of whether the event is serious or non-serious, on-site or off- site) that occurs any time during or after the research study, which in the opinion of the principal investigator is:

Unexpected (An event is "unexpected" when its specificity and severity are not accurately reflected in the

protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.)

AND
Related to the research procedures (An event is "related to the research procedures" if, in the opinion of the principal investigator or sponsor, the cause of the event was deemed probably or definitely related to the investigational product or procedure that was performed for the purposes of the research.)

Reporting Process
Unanticipated problems posing risks to patients or others as noted above will be reported to the IRB. This will include a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

Reporting Deaths: more rapid reporting requirements
Concerning deaths that occur during the course of a research study, the following describes the more rapid reporting requirement of the Penn IRB for specific situations:

- Report the event within [redacted] hours, when the death is unforeseen (unexpected) and indicates participants or others are at increased risk of harm.

For reportable deaths, the initial submission to the IRB may be made by contacting the IRB Director or Associate Director.

Other Reportable events:
For clinical drug trials, the following events are also reportable to the IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as a granulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human patients.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality.
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard

to a research participant.

- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the patient to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of patients.

8.3.2. Investigator Reporting: Notifying the DSMC of the Abramson Cancer Center (ACC)

All events that meet the ACC DSMC definition of reportable AE's must be promptly entered into Velos. The DSMC requires AE/SAE submission as follows:

- Unless covered by exclusions below, grade 3 or higher events must be reported within 10 days of knowledge of the adverse event.

  EXCEPTIONS:
  - Grade 3 and 4 events that are typical in the disease population - with the exception of those that could be symptoms/early indicators of any of the toxicities defined in the Toxicity Management section of the protocol, signs/symptoms of an allergic response, severe hypotensive crisis or any other reaction to the infusion.
  - All grade 3 or 4 events that are judged by a study investigator to be clearly unrelated to protocol therapy.
  - Grade 3 or 4 events that are probably or definitely related to progression of disease as judged by a study investigator.
  - Grade 3 or 4 events that are probably or definitely related to an FDA-approved agent.
- All unexpected deaths within one business day of knowledge
- All others deaths within 30 days of knowledge. Deaths of subjects off-study for greater than 30 days from the last study treatment/ intervention are not reportable unless a longer time frame is specified in the protocol.

In the event of a grade 4 or 5 unexpected event regardless of attribution, the study team must meet or have a teleconference within [redacted] hours of knowledge of the event to have a thorough discussion of the event. These types of events will not be vetted via e-mail. The sponsor should not be involved in discussions about attribution. The PI and Research Coordinator will schedule a meeting with the study team to discuss the grade 4 or 5 unexpected event. Meeting minutes capturing the review of any ongoing investigations of the grade 4 or 5 unexpected event, including next steps in the management of the subject and any proposed changes to the protocol will be documented appropriately.

8.3.3. IBC Notification by Investigator

Notify the Institutional Biosafety Committee of serious adverse events according to institutional requirements.

8.3.4. FDA Notification by Sponsor

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The sponsor must report an IND safety reports as described in:

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM227351.pdf

The following describes the safety reporting requirements by timeline for reporting an associated type of event:

- ***Within 7 Calendar Days***

Any study event that is:

- *Unexpected* fatal or life-threatening *suspected adverse reaction*
- Expected and unexpected Grade 3 or higher events of cytokine release syndrome (CRS) per the modified CRS grading scale in Table 8-1
- All fatal events occurring within 30 days of T-cell infusion, regardless of attribution and expectedness

- ***Within 15 Calendar Days***

Any study event that is:

- unexpected
- Suspected adverse reaction that is serious, but not fatal or life-threatening

  -or-
- a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

Any finding from tests in laboratory animals that:

- suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity or reports of significant organ toxicity at or near the expected human exposure.

Increase in rate of occurrence of serious suspected adverse reactions:

- any clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol.

Additional Reporting Requirements

Sponsors are also required to review all adverse events to make a causality determination on the basis of information from investigators and report these findings to the FDA in accordance with 21 CFR 312.32.

If the adverse event does not meet expedited reporting requirements, the Sponsor will report the SAE in the IND Annual Report.

8.4. Pregnancies

To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to protocol sponsor within [redacted] 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.

Pregnancy should be recorded on a Clinical Trial Pregnancy Form and reported by the investigator to the protocol sponsor Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the study drug for any pregnancy outcome. Any SAE experienced during pregnancy must be reported on 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 CART19 cells would define a stopping rule.

Premature termination of the clinical trial may occur because of a regulatory authority decision, change in opinion of the IRB, ACC DSMC, the DSMB, 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.
- Premature study termination may occur if the Investigator, Study Funder, Sponsor, DSMB, 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 or Study Funder decides to discontinue the development of the intervention to be used in this study.

The study will be paused if:

- The protocol will be paused pending submission to the FDA and review by IRB, DSMC, CTSRMC and the DSMB if any patient experiences any of the following events within two weeks of the first CART19 infusion:
    - Life-threatening (grade 4) occurring within two weeks of the first CART19 infusion that is unmanageable, unexpected and unrelated to chemotherapy and attributable to protocol therapy. High fevers, hypotension, possible ICU admission and even mechanical ventilation are expected. These side effects can result in grade IV liver toxicity, nephrotoxicity and other organ involvement.
    - Death within 100 days of the first CART19 infusion which is not attributable to the subject's leukemia.

If all parties are in agreement as to the event resolution and any proposed modifications (if applicable),

then the pause will be lifted.

The protocol manufacturing will be paused to review the manufacturing process should there be ≥33% primary efficacy non-evaluable patients (i.e. the manufacturing process fails to meet the protocol-specified dose range of 1-5x10^7 CART19 cells).

If the study is paused for manufacturing reasons, required team members will meet to identify the manufacturing failure. The team will make recommendations for process improvements to be implemented. Pending successful completion of a process validation run, the manufacturing pause will be lifted.

8.5.2. General Toxicity Management Considerations

RCL will be monitored by a suitable Q-PCR assay for the detection of the lentivirus (VSV-g DNA)
If a positive VSV-g DNA assay or suitable alternative result is obtained, the Investigator will be informed and the patient rescheduled for a retest for the DNA test. If the second DNA test is positive, then infusions will be temporarily halted. The patient will undergo a blood draw for isolation of HIV from his/her cells. The virus will be sequenced and compared to sequences of the transfer vector and packaging constructs, as well as to available HIV sequences to determine the origin of the virus. Determination of the origin of the virus can be easily performed by evaluation for HIV accessory genes such as vif, vpr and vpu which are not present in the packaging constructs. If the sequence is derived from wt-HIV then infusions for all patients can resume, and the patient will be referred to treatment for HIV. If an RCL is confirmed, or the virus cannot be isolated from the blood draw, the patient will be scheduled for apheresis and will undergo a full biological RCL testing for detection and/or characterization of the RCL.

Clonality and insertional oncogenesis
Monitoring for T cell clonal outgrowth will be performed by qPCR for CART19 and by CBC count. CART19 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.

A summary of integration site analyses will be presented in the annual report to the FDA. If oligo- or monoclonality of a vector integration site is observed, this data will be reported as an information amendment to the IND with best efforts made to submit this amendment within 30 days of data confirmation.

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[93]. If uncontrolled T cell proliferation occurs (grade 3 or 4 toxicity related to CART19 cells), patients may be treated with corticosteroids. Patients will be treated with pulse methylprednisolone (2 mg/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, as has been done with Rituximab.

Infusion reaction
Acetaminophen and an antihistamine may be repeated every 6 hours as needed. It is recommended that patients not receive corticosteroids at any time, except in the case of a life-threatening emergency, since this may have an adverse effect on CART19 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 CART19 T cell product is suspected, the product can be retested for sterility using archived samples that are stored in the CVPF. Consideration of a cytokine release syndrome (see below) should be given.

Cytokine Release Syndrome (CRS) / Macrophage Activation Syndrome (MAS)
Given the dramatic clinical improvement of most patients treated with anti-cytokine therapy, patients with moderate to severe cytokine toxicities should be first managed with administration of tocilizumab.

Tocilizumab should be used as a single, weight-based dose of 8 mg/kg at the time of hemodynamic instability (initial dose of tocilizumab within one hour of ordering drug is highly recommended). This management approach is designed to avoid life-threatening toxicities, while attempting to allow the CART19 cells to establish a proliferative phase that appears to correlate with anti-tumor efficacy. Thus, 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 CART19 function and efficacy, if used, they should be rapidly tapered.

Upon developing the prodrome of high-persistent fevers following CART19 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

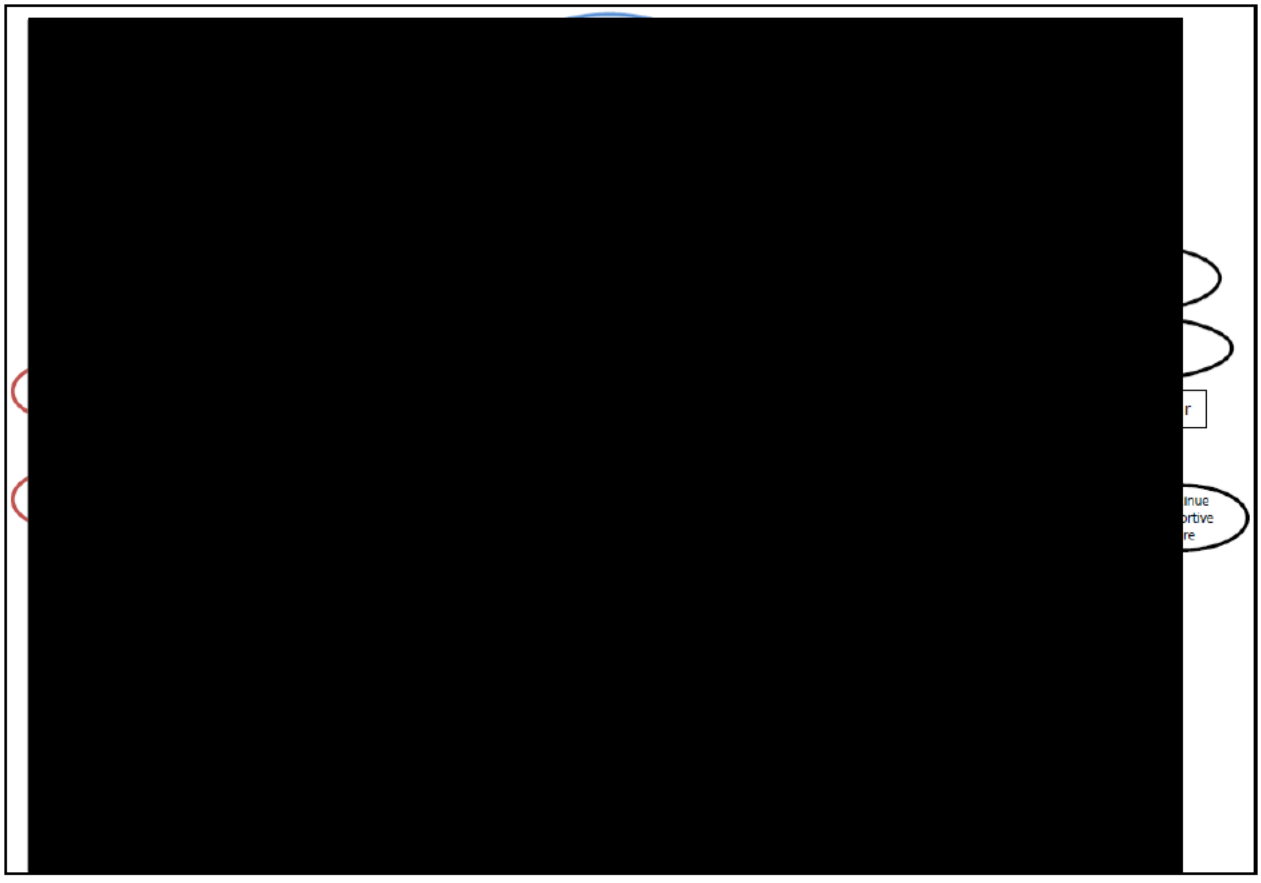
The recommended dosing for tocilizumab is 8 mg/kg i.v. single dose. Not all Grade 4 CRS reactions following CART19 have been immediately treated with tocilizumab and decisions are, in part, based upon the rapidity of the syndrome onset and underlying patient reserve.

Siltuximab, an anti-IL6 therapy, may be administered beginning 2-24 hours after the first dose of tocilizumab, at the physician-investigator's discretion. Other anti-cytokine therapies, such as repeat administration of tocilizumab or siltuximab or etanercept, may also be considered if the patient does not respond to initial dose therapy. If the patient experiences ongoing CRS despite administration of anti-cytokine directed therapies, anti T-cell therapies such as cyclophosphamide, ATG, or alemtuzumab (Campath) may also be considered.

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*. **Refer to Figure 8-1 below for a CRS Management Algorithm.**

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 CAR T-cell delayed CRS events.

Figure 8-1 [redacted]



Tumor lysis syndrome

Patients will receive allopurinol prophylactically for 30 days after infusion to prevent complications from TLS. TLS resulting in renal insufficiency, or rapidly rising uric acid, or evidence of organ dysfunction will be managed with fluids and rasburicase as clinically indicated and determined by the treating physicians.

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, local IRB, ACC DSMC, and other local regulatory review committees per institutional guidelines is required. Approval from the Regulatory Sponsor must be received prior to submission to the IRB, ACC DSMC and local regulatory review committees for approval.

No exceptions to eligibility will be granted for this study.

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 and ACC DSMC within 5 business days and the IRB within 10 business days of PI knowledge.

Any departure from the protocol that meets the following criteria should be submitted:

- Impacts subject safety
- Impacts the integrity of the study design or outcome
- Based on the PI's judgment is reportable

Include the following information on the Sponsor supplied exception/deviation form: Study identifier, subject study number, description of the exception/deviation and rationale. Ensure all completed exception/deviation forms are signed by the Principal Investigator (or sub-investigator) and submitted to the Sponsor Project Manager for review.

Attention: Sponsor Project Manager
[redacted]

The Sponsor Project Manager will submit the exception/deviation form to the Regulatory Sponsor for review and approval. 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 the IRB, ACC DSMC and all other applicable committees for review and approval.

Other deviations should be explained in a protocol deviation form (such as a patient missing a visit) is not an issue unless a critical/important treatment or procedure was missed and must have been done at that specific time.

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

An Independent Data and Safety Monitoring Board (DSMB) comprised of a minimum of four individuals including physicians with experience in oncology and/or gene transfer therapy and a statistician will be assembled, and will work under a charter specifically developed for safety oversight of this study. The DSMB will provide guidance/advice to the Sponsor. The DSMB will evaluate patient-subject safety as specified in the DSMB Charter.

The DSMB will meet approximately every 4 months. If necessary, additional meeting 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 the IRB and other local regulatory review committees per institutional requirements.

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 subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject 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 subjects 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 subject 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 subject in the study, consisting of case and visit notes (hospital or clinical medical records) containing demographic and medical information, laboratory data, 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.

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 or quality assurance 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, government regulatory bodies, and University compliance and quality assurance groups. 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 and quality assurance offices.

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.

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. A HIPAA authorization has been integrated into this consent and therefore a combined consent-authorization document will be used. 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 and the investigator-designated research professional obtaining the consent.

The protocol is listed on clinicaltrials.gov.

12. STUDY FINANCES

12.1. Funding Source

This study is funded by Novartis Pharmaceuticals Corp and the Leukemia and Lymphoma Society (LLS).

12.2. Conflict of Interest

All University of Pennsylvania Investigators will follow the University of Pennsylvania 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. However subjects will be reimbursed for some of the cost for travel and lodging to the research study site during their participation in this research study.

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: VISIT EVALUATION SCHEDULE

	Screening and Enrollment	Apheresis	Chemo-Therapy	Pre-Infusion	Infusion #1	Infusion #2	Infusion #3	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Monthly Follow-Up	Quarterly Follow-Up
	~-12W to -4W	~-4W to -3W	~ -1W	~ -1D	D1	D2 (+) 1d	D3 (+) 1d	D4 (+) 1d	D7 (+/-) 2d	D11 (+/-) 1d	D14 (+/-) 2d	D21 (+/-) 3d	D28 (+/-) 3d	M2 M3 M4 M5 M6 (+/-) 7d	M9 M12 (+/-)14d
Informed Consent	X														
Interventions															
Apheresis[6]		x													
Lymphodepleting Chemotherapy[4]			x												
CART19 Infusion[24]					x	x	x								
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]														
Prior/concomitant medications	X		x	x	x	x	x	x	x	x	x	x	x	x	x
Physical examination	X			x	x	x	x	x	x		x	x	x	x	x
Performance status (ECOG)	X			x	x	x	x	x	x		x	x	x	x	x

	Screening and Enrollment	Apheresis	Chemo-Therapy	Pre-Infusion	Infusion #1	Infusion #2	Infusion #3	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Monthly Follow-Up	Quarterly Follow-Up
	~-12W to -4W	~-4W to -3W	~ -1W	~ -1D	D1	D2 (+) 1d	D3 (+) 1d	D4 (+) 1d	D7 (+/-) 2d	D11 (+/-) 1d	D14 (+/-) 2d	D21 (+/-) 3d	D28 (+/-) 3d	M2 M3 M4 M5 M6 (+/-) 7d	M9 M12 (+/-)14d
Height	X														
Weight	x				x										
Vital signs[25]	x			x	x[12]	x	x	x	x	x	x	x	x	x	x
Laboratory assessments[27]															
Hematology (5 ml lavender top, EDTA)	x			x	x	x	x	x	x	x	x	x	x	x	x
Chemistry (3 ml SST)	x[17]			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	x[13]	x	x[13]	x	x	x	x	x
Serum Pregnancy Test[11] (1 ml SST)	X														
Urine Pregnancy Test[11]				x											x
HIV Test (1ml SST)	x														
Viral Serology (Hepatitis B and C) (5ml red top, serum)	x														
Serum Immunoglobulin levels (1ml SST) IgG only	x												x	x	x
HLH/MAS (triglycerides, haptoglobin)(4mL SST; 2.5mL lavender top, EDTA)				X[8]											
Ferritin, LDH and CRP for Cytokine Release Syndrome				X[8]											

	Screening and Enrollment	Apheresis	Chemo-Therapy	Pre-Infusion	Infusion #1	Infusion #2	Infusion #3	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Monthly Follow-Up	Quarterly Follow-Up
	~-12W to -4W	**~-4W to -3W**	**~ -1W**	**~ -1D**	**D1**	**D2 (+) 1d**	**D3 (+) 1d**	**D4 (+) 1d**	**D7 (+/-) 2d**	**D11 (+/-) 1d**	**D14 (+/-) 2d**	**D21 (+/-) 3d**	**D28 (+/-) 3d**	**M2 M3 M4 M5 M6 (+/-) 7d**	**M9 M12 (+/-)14d**
Respiratory Virus Panel (RVP)			x[18]												
T cell Subsets-CD3, CD4, CD8 (4mL lavender top, EDTA)					X								X	X	
Research Analyses[2]															
Serum ~6cc (Red top)				x	x[19]	x[19]	x[19]	x	x	x	x	x	x	x[5]	
Cytokines				x	x	x	x	x	x	x	x	x	x	x[5]	
PBMC ~25cc (purple, EDTA)				x					x	x	x	X	x	x	x
Additional 30cc for research blood sample (Lavender, EDTA)											x		x		
DNA (Q-PCR CART19 persistence)				x					x	x	x	x	x	x	x
DNA RCL (VSV-G Q-PCR)				x										x[3]	x[3]
CART19 Immune-phenotyping, CART19) and B cell enumeration, functional assays				x					x	x	x	x	x	x	x
Bone Marrow/LN aspirate[22] (5 cc lavender top, EDTA)	x[26]		x[23]	x[14]									x[1]	x[1]	x[1]
DNA (Q-PCR CART19 homing)	x		x	x									x[1]	x[1]	x[1]

	Screening and Enrollment	Apheresis	Chemo-Therapy	Pre-Infusion	Infusion #1	Infusion #2	Infusion #3	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Monthly Follow-Up	Quarterly Follow-Up
	~-12W to -4W	**~-4W to -3W**	**~ -1W**	**~ -1D**	**D1**	**D2 (+) 1d**	**D3 (+) 1d**	**D4 (+) 1d**	**D7 (+/-) 2d**	**D11 (+/-) 1d**	**D14 (+/-) 2d**	**D21 (+/-) 3d**	**D28 (+/-) 3d**	**M2 M3 M4 M5 M6 (+/-) 7d**	**M9 M12 (+/-)14d**
CART19 and B cell enumeration, immunophenotyping	x		x	x									x[1]	x[1]	x[1]
MRD by deep sequencing				x[15]									x[15]	x[15]	x[15]
Marrow Serum (2 cc red top)	x		x	x									x[1]	x[1]	x[1]
Cytokines	X		x	x									x[1]	x[1]	x[1]
Disease Monitoring[20]															
Tumor response assessments				x									x[1]	x[1]	x[1]
Physical exam (extramedullary disease)[16]	X			x									x[1]	x[1]	x[1]
Bone marrow aspirate/biopsy (cytogenetics/FISH if appropriate)	x[26]		x[23]	x[14]									x[1]	x[1]	x[1]
Lymph node biopsy[22]	x			X									x[1]	x[1]	x[1]
CSF evaluation[9]	x	x											x	As clinically indicated	
Mediastinal disease assessment (Chest X-ray → CT/MRI scan)[16]	x												x[16]	x[16]	
MRD by flow cytometry	x			x									x	x[1]	x[1]
BCR-ABL (Ph+ patients only)[28]	x												x	x[1]	x[1]
Safety															
Adverse events					x	x	x	x	x	x	x	x	x	x	x

	Screening and Enrollment	Apheresis	Chemo-Therapy	Pre-Infusion	Infusion #1	Infusion #2	Infusion #3	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Monthly Follow-Up	Quarterly Follow-Up
	~-12W to -4W	~-4W to -3W	~ -1W	~ -1D	D1	D2 (+) 1d	D3 (+) 1d	D4 (+) 1d	D7 (+/-) 2d	D11 (+/-) 1d	D14 (+/-) 2d	D21 (+/-) 3d	D28 (+/-) 3d	M2 M3 M4 M5 M6 (+/-) 7d	M9 M12 (+/-)14d
ECHO/MUGA	x[21]														
Electrocardiogram (EKG)	x														
Total clinical blood draw (mL)	27	0	0	19	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	16.5	16.5	16.5
Total research blood draw (mL)	0	0	0	30	5	0	0	0	30	30	60	30	60	30	30
Total blood draw (mL)	27.0	0	0	49	17.5	12.5	12.5	12.5	42.5	42.5	72.5	42.5	76.5	46.5	46.5
Total blood draw (Tbsp.; approximately)	2	0	0	3	1	1	1	1	3	3	5	3	5	3	3

[1] Tumor response assessments will be performed at Day 28, Months 3, 6, 9 and 12 after CART19 cell infusions (Refer to Sections 6.5 and 6.6 for further details and frequency)

[2] Translational and Correlative Studies Laboratory (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 collects 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] Months 3, 6 and 12 only

[4] Lymphodepleting chemotherapy prior to CART19 cell infusion is NOT required if WBC ≤ 1,000 /uL. The infusion will be scheduled to occur approximately 1 to 4 days following lymphodepleting chemotherapy. Please refer to Section 6.4.11 for complete details.

[5] Month 2 only.

[6] Apheresis will be performed to obtain a target of $5x10^9$ PBMCs for CART19 manufacturing. Apheresis can occur any time after the subject is enrolled and up to 3 weeks prior to CART19 infusion. Cryopreserved historical apheresis products collected from the patient prior to study entry are usable for

CART19 manufacturing if collected at an appropriately certified apheresis center and the product meets adequate mononuclear cell yields. Please see Section 6.3 for additional details.

[7] Prior antineoplastic therapy to be collected up through the lymphodepleting chemotherapy prior to CART19 cell infusion.

[8] Repeated as clinically indicated or if HLH/MAS or CRS is suspected. If high fevers (≥ 101.5° F / 38.6° C) occur following CART19 infusion, every attempt will be made to monitor additional Ferritin, LDH and CRP levels **daily** at fever onset and until resolution of the fever (below 101.5° F / 38.6° C).

[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. CNS evaluations will be performed in all patients within 4 weeks of the first CART19 infusion and at Day 28, and will be repeated thereafter as clinically indicated by the presence of neurologic symptoms.

[10] Months 3, 6, 9 and 12

[11] Pregnancy test (quantitative) for females of childbearing potential only. Pre-infusion pregnancy test to be performed within 48 hours prior to the first CART19 infusion. End of Study pregnancy test to be performed at the Month 12 visit or at the time of study discontinuation.

[12] Vital signs (temperature, respiration rate, pulse, blood pressure, and oxygen saturation by pulse oximetry) will be measured within 10 minutes prior to the infusion and within 15 minutes after the infusion. Thereafter, vital signs will be measured at 30 (+/- 5) minutes, 45 (+/- 5) minutes, and 60 (+/- 5) minutes after the infusion, and then every hour (+/- 10 minutes) for the next 2 hours until these signs are satisfactory and stable.

[13] D-dimer required on Days 4 and 11.

[14] Bone marrow biopsy/aspirate to be performed within 48 hours prior to the CART19 infusion. The results of this baseline bone marrow are not required prior to infusion.

[15] For all subjects, deep sequencing for MRD detection will be performed on marrow samples collected pre-infusion (~Day -1) and at Day 28; for subjects experiencing a morphological response, deep sequencing will also be performed on marrow samples collected at **months 3** and **12.**

[16] A chest x-ray for mediastinal disease will be performed at Screening/Enrollment if clinically indicated. Chest CT/MRI required pre-infusion and post-infusion on Day 28 and Month 3 if chest x-ray suggests mediastinal enlargement.

- If extramedullary disease is present prior to treatment, this will be followed at each response assessment visit.

[17] Direct bilirubin will be performed at screening only

[18] All subjects must undergo a Respiratory Virus Panel within 10 days prior to the planned CART19 infusion. If the subject is positive for influenza, oseltamivir phosphate (Tamiflu®) or equivalent should be administered per package insert (see Tamiflu® package insert for dosing information). The patient must complete treatment prior to receiving the CART19 infusion. If the patient is positive for influenza and is also experiencing flu-like symptoms, all clinical symptoms must also be resolved prior to the CART19 infusion. If the subject is positive for another virus on the RVP, the CART19 infusion will be delayed for at least 7 days to be sure clinical symptoms of a viral infection do not develop. If clinical symptoms develop, the infusion will be delayed until resolution of these symptoms.

[19] Research blood (~6 cc red top) to be taken between 20-120 minutes post-CART19 infusion.

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

[21] ECHO/MUGA must be performed within 12 weeks prior to enrollment (after last treatment with induction chemotherapy, excluding maintenance chemotherapy) and within 6 months of CART19 infusion.

[22] Lymph node biopsy is optional and performed if accessible and/or as clinically indicated

[23] If subjects receive treatment for their ALL after study eligibility is confirmed, a repeat bone marrow should be performed prior to lymphodepleting chemotherapy (if administered) and within 4 weeks prior to the CART19 infusion. If lymphodepleting chemotherapy is not administered, the pre-infusion bone marrow (to be performed within 48 hours prior to the CART19 infusion) is sufficient.

[24] Neutropenic subjects will be administered preventive antibiotics treatment starting on the day of infusion. Broad-spectrum antibiotics will be administered orally until recovery of neutrophil counts, or until judged by the investigator to no longer be at increased risk of infection. All patients will receive allopurinol prophylactically for 30 days after infusion and appropriate clinical therapy will be administered should any significant tumor lysis occur. The Investigator will review all labs to determine that it is appropriate to proceed with the infusion. See Section 5.2 regarding criteria to proceed with CART19 infusion each day.

[25] Vital sign assessments include blood pressure, body temperature, heart rate and oxygen saturation via pulse oximetry.

[26] Bone marrow performed for disease and MRD assessment. Must be performed within 4 weeks of enrollment.

[27] Please refer to Table 6-2 for additional details.

[28] Bone marrow aspirates will be analyzed for BCR-ABL for Ph+ ALL patients only. Where applicable, peripheral blood testing for quantitative BCR-ABL levels should be sent concurrently.

Appendix 2: NEW YORK HEART ASSOCIATION (NYHA) FUNCTIONAL CLASSIFICATION

Class	Functional Capacity: How a patient with cardiac disease feels during physical activity
I	Patients with cardiac disease but resulting in no limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea or anginal pain.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea or anginal pain.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea or anginal pain.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort increases.