University of Pennsylvania

PHASE II STUDY OF REDIRECTED AUTOLOGOUS T CELLS ENGINEERED TO CONTAIN ANTI-CD19 ATTACHED TO TCRζ AND 4-1BB SIGNALING DOMAINS IN PATIENTS WITH CHEMOTHERAPY RESISTANT OR REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA

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Study Product:	*CD19 redirected autologous T cells (CART-19 T Cells)*
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Medical Monitor	[redacted]

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LIST OF ABBREVIATIONS

ALL	acute lymphoblastic leukemia
APC	antigen presenting cell
aAPC	artificial APC
AE	adverse event
B-ALL	B lineage acute leukemia
B-cell ALL	B cell acute lymphoblastic leukemia
CAR	chimeric antigen receptor
CART-19 cells	CD19 redirected autologous T cells
CCI	Center for Cellular Immunotherapies
CHOP	Children's Hospital of Philadelphia
CIR	chimeric immune receptor, interchangeable with CAR
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
GVHD	graft versus host disease
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
PBMC	peripheral blood mononuclear cells
PD	progressive disease
PK	pharmacokinetics
PR	partial remission
QoL	quality of life
RAC	NIH Office of Biotechnology Recombinant DNA Advisory Committee
RCR/L	replication competent lentivirus
RFS	relapse free survival
SAE	serious adverse event
scFv	single chain Fv fragment
SCT	stem cell transplant
TCR	T cell receptor
TCR-ζ	signaling domain found in the intracellular region of the TCR zeta, gamma and epsilon chains
TCSL	Translational and Correlative Studies Laboratory
TLS	tumor lysis syndrome
UPenn	University of Pennsylvania
Vβ	a rearranged T cell specific gene that can be used to determine clonality of a T cell population
VSV-G	Vesicular Stomatitis Virus, Glycoprotein
WBC	white blood cell

STUDY SUMMARY AND STUDY SCHEMA

Title	**Phase II Study Of Redirected Autologous T Cells Engineered To Contain Anti-CD19 Attached To TCRζ And 4-1BB Signaling Domains In Patients With Chemotherapy Resistant Or Refractory Acute Lymphoblastic Leukemia**
Short Title	CD19 redirected autologous T cells for ALL
Protocol Numbers	[redacted]
Phase	Phase 2
Methodology	This is a single center, single arm, open-label phase II study to determine the efficacy and safety of autologous T cells expressing CD19 chimeric antigen receptors expressing tandem TCRζ and 4-1BB (TCRζ/4-1BB) co-stimulatory domains (referred to as "CART-19" cells) in adult patients with relapsed or refractory B-cell acute lymphoblastic leukemia. This protocol also allows for retreatment of any subject who received CART-19 T-cells and has subsequently relapsed with CD19+ disease.
Study Duration	The duration of active protocol intervention is approximately 12-15 months from screening visit. The protocol will require approximately 24-30 months to complete enrollment.
Study Center(s)	Single-center
Objectives	**Primary Objective** Evaluate the efficacy of CART-19 therapy as measured by complete remission rates which includes complete remission (CR) and CR with incomplete blood count recovery (CRi) at Day 28 (see Section 6.15 for response definitions). **Secondary Objectives:** 1. Evaluate best overall response rate. 2. Evaluate overall survival (OS), duration of remission (DOR), relapse free survival (RFS), and event free survival (EFS). 3. Describe cause of death (COD) when appropriate. 4. Describe response in terms of minimal residual disease (MRD) negative and positive testing using flow cytometry (standard) and quantitative molecular technologies (deep sequencing). 5. Evaluate manufacturing feasibility of CART-19. 6. Assess safety and tolerability of CART-19. 7. Characterize the *in vivo* cellular pharmacokinetic (PK) profile (levels, persistence, trafficking) of CART-19 cells in target tissues (blood, bone marrow, cerebral spinal fluid and other tissues if available). 8. Describe the incidence of immunogenicity to CART-19, and assess correlation for immunogenicity with loss of detectable CART-19 (loss of engraftment). 9. For patients treated for relapse after allogeneic SCT, describe the risks of GVHD.

	10. Evaluate bioactivity of CART-19 cells. 11. Describe Patient Reported Outcomes 12. Assess safety and efficacy of re-infusion of CART-19 cells in previously treated patients
Number of Patients	30 evaluable subjects
Diagnosis a*nd Main* Inclusion Criteria	Inclusion criteria are designed to include adult patients aged ≥18 with B cell ALL, relapsed or refractory, with no available curative treatment options (such as autologous or allogeneic stem cell transplantation) who have limited prognosis with currently available therapies.
Study Product, Dose, Route, Regimen	CART-19 cells transduced with a lentiviral vector to express anti-CD19 scFv TCRζ:41BB administered by i.v. infusion. • The first 6 subjects received 1 to 5 x 10^8 transduced CAR T cells via a single i.v. infusion. • The next 6 subjects received 1 to 5 x 10^7 transduced CAR T cells via a single i.v. infusion. • The next 3 subjects received 1 to 5 x 10^7 transduced CAR T cells via split dosing: 10% on Day 1, 30% on Day 2, 60% on Day 3. • The remaining subjects will receive 1 to 5 x 10^8 transduced CAR T cells via split dosing: 10% on Day 1, 30% on Day 2, 60% on Day 3. <u>Retreatment Cohort</u>: The target dose for this retreatment cohort will be 1-5 x 10^8 transduced CAR T cells via split dosing: 10% on Day 1, 30% on Day 2, 60% on Day 3. Subjects may receive either murine or humanized CART-19 as part of this retreatment cohort. For retreatment, preference will be for subjects to receive humanized CART-19. If not determined to be feasible and/or a subject has additional cryopreserved murine CART-19 doses available, they will receive retreatment with murine CART-19.
Duration of administration	Based on the total volume to be infused and the recommended infusion rate of 10-20mL per minute
Reference therapy	None. This protocol will be offered to patients with unmet medical needs for which there are no effective therapies known at this time.
Statistical Methodology	A total of 30 evaluable patients will be treated. The primary objective in this study is to determine overall complete remission rate (ORR) at Day 28. The two-sided exact Clopper-Pearson 95% confidence intervals (CI) for 28-day ORR will be computed. The half-width of the 95% exact confidence interval for ORR will be no larger than 19% for a cohort of size 30 and no larger than 26% for a cohort of size 15. For the secondary efficacy objectives, proportion of patients with a best overall disease response of CR or CRi, proportion of patients achieving CR or CRi before or at Month 6, and the proportion of patients with a minimal residual disease (MRD) negative bone marrow as determined

	according to Section 6.14 will be computed along with a two-sided 95% exact CI. Secondary time to event endpoints include overall survival (OS), duration of remission (DOR), relapse free survival (RFS), and event free survival (EFS). Kaplan-Meier method will be used. Median survival time and the associated 95% confidence intervals will be presented if appropriated. Incidence and severity of AEs, and changes in laboratory values and vital signs from baseline will be summarized and tabulated. Descriptive statistics will be calculated for correlative endpoints, manufacturing feasibility endpoint, and patient reported outcomes. Exploratory subset analyses of safety and efficacy for specific dosing-groups [e.g. fractionated 1-5 $x10^8$ (N=15)] will be considered. Safety and efficacy analyses for subjects in the retreatment cohort will be exploratory and used to generate hypotheses for future studies. Feasibility endpoints will be analyzed using all patients who are evaluated for cell expansion.

1. INTRODUCTION

1.1. Background

CD19 positive hematologic malignancies. B cell malignancies comprise a heterogeneous group of neoplasms including a vast majority of non-Hodgkin's lymphomas (NHL), 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, and peripheral blood stem cell transplantation. Despite these treatment modalities, most patients will remain incurable.

B lineage acute 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]. Improved response rates have thus far only been achieved with intensified cytotoxic chemotherapy, resulting in substantial morbidity. Adoptive immunotherapy with allogeneic donor leukocytes has potent anti-leukemic effects, however the benefit is confined largely to patients with myeloid leukemias, as B-ALL has a durable remission rate of less than 10%[4], and often at the cost of substantial morbidity due to graft versus host disease (GVHD)[5, 6].

Adoptive immunotherapy. Adoptive transfer is a term coined by Medawar[7] to study allograft rejection, and the term adoptive immunotherapy denotes the transfer of immunocompetent cells for the treatment of cancer or infectious disease[8]. Adoptive immunotherapy appears to be the most robust form of immunotherapy for treatment of established tumors[9], 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[10]. However, several problems remain to be solved before this therapy becomes routine; see reviews for details[11-13].

CD19 as a therapeutic target for leukemia and lymphoma. CD19 is a 95kDa glycoprotein present on B cells from early development until differentiation into plasma cells[14-16]. 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[14, 17, 18]. 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[14, 19]. Expression of CD19 is restricted to B lineage cells and is not expressed by pluripotent blood stem cells[20]. CD19 is also expressed by most B cell lymphomas, mantle cell lymphoma, ALLs, CLLs, hairy cell leukemias, and a subset of acute myelogenous leukemias[15, 21, 22]. CD19 thus represents a highly attractive target for immunotherapy[20]. Furthermore, CD19 is not present on most normal tissues, other than normal B cells, including pluripotent blood stem cells[20], 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[23-27] and human and non-human primates[28-38].

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[39, 40]. 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[41]. 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[11, 42-45].

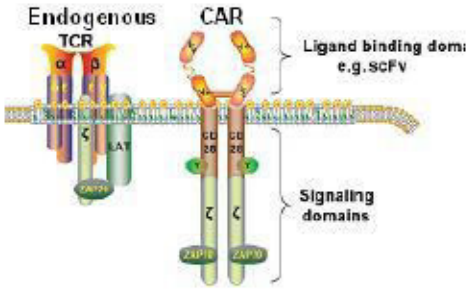


Fig. 1-1. CAR design. Bispecific T cells are created by the introduction of genes encoding CAR proteins that recognize target surface antigens in an MHC-independent fashion.

Rationale to evaluate CART-19 in patients with chemotherapy resistant or refractory adult ALL. Outcome remains poor for adult patients with relapsed or refractory (r/r), B cell acute lymphoblastic leukemia (B cell ALL). Treatment options for r/r B-cell ALL include further treatment with salvage chemotherapy, a 2nd allogeneic hematopoietic stem cell transplantation (HSCT) or supportive care. For this population, 5 yr overall survival (OS) after relapse is estimated at 7%[46]. For the small minority of patients with relapsed ALL who have a donor identified and go on to transplant, OS is estimated at 23% and those patients who receive chemotherapy only is 4%. Therefore, the risk:benefit ratio for a novel therapy with the potential to induce remission in ALL is quite favorable.

Moreover, in ongoing clinical trials with CART-19, T cells expressing a second generation CAR with an anti-CD19 scFv and 4-1BB and TCR signaling domains, in patients with B-cell ALL and CLL (both CD19 expressing B cell malignancies) show that CART-19 therapy has potent anti-tumor activity in pediatric and adult patients[47, 48] (described in detail in Section 1.4). Eighteen adult CLL subjects are evaluable as of April 2013 and include: 5 CR, 6 PR, and 7 NR to give an overall response rate of 11/18 (61%). Of the nine evaluable pediatric B cell ALL patients, 7 CR, 1 PR and 1 NR have been reported for an overall response rate of 8/9 (89%). The one adult ALL patient treated remains in CR after 2 months of follow up and has gone on to have an allogeneic SCT; of note, he was considered previously ineligible for allogeneic SCT before CART19 cell infusion because of his refractory disease.

For ALL patients, there is little benefit from allogeneic SCT with relapsed and active disease and many centers, do not offer patients in this situation transplantation because of futility. Therefore, any benefit that may be seen with CART-19 cells will have a major impact for patients.

1.2. Investigational Agent

The investigational agent in this protocol is CART-19 cells. Autologous T cells will be engineered using a 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 addition to the CD19 scFv, the cells will be transduced to express an intracellular tandem signaling domain comprised of 4-1BB and TCRζ signaling modules. Clinical grade CD19 TCRζ/4-1BB lentiviral vector will be manufactured at the Children's Hospital of Philadelphia (CHOP) and City of Hope Center for Applied Technology Development. The extracellular single change antibody (scFv) with specificity for CD19 is derived from a mouse monoclonal antibody using hybridoma cell line FMC63 described in Nicholson et al.[49] or will be a humanized version of that sequence developed by Novartis Biomedical Institutes of Research. The humanized version will only be used in the retreatment cohort. The signaling domains are entirely of the native human sequences[50, 51].

The CART-19 cells will be manufactured in the Clinical Cell and Vaccine Production Facility at the University of Pennsylvania. At the end of cell cultures, the cells are cryopreserved in infusible cryomedia. The infusion bag/syringe 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[52, 53]. There are several potential limitations to the CAR T cells: 1) the tumor must express the target antigen on the cell surface; 2) large amounts of shed or soluble antigen could 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[54]. 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[55]. 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[55-58].

Drug interactions. CART-19 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 such as antibiotic selection genes or mouse antibody 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[59]; importantly, this immune mediated elimination was not accompanied by adverse effects and required 6 to 8 weeks to occur. It is important to note that it is possible the CART-19 T cells may be rejected in patients.

Rationale for lymphodepletion. Adoptive immunotherapy strategies may be able to capitalize on homeostatic T cell proliferation[60], 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[61, 62]. 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[63]. This hypothesis has been tested clinically in patients with metastatic melanoma refractory of conventional treatments[56]. 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, and CLL have been treated with infusions of ex-vivo co-stimulated and expanded autologous T cells after lymphodepleting chemotherapy, and observed improved engraftment[64-67]. In this protocol transfer CART-19 cells into subjects that are given lymphodepleting chemotherapy. This approach has been taken with previous recipients of CART19 cells (section 1.4 below). 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, reviewed[68-72]. Others have used electroporation or retroviral vectors to create CART-19 T cells, and have shown in vivo safety and efficacy of adoptively transferred T cells in immunodeficient mouse models[73-77]. 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[78-84]. The pre-clinical data supporting CART-19 has been published[51, 85].

1.4. Previous Clinical Data with CART-19 cells

[redacted]

[redacted]

[redacted]

CRS has been the most significant SAE seen in adult and pediatric patients treated with CART-19 has been on target CRS. CRS is described in details below (section 1.5.2) but typically begins up to 2 weeks after CART-19 infusion. The CRS typically starts with several days of fevers. In all cases, evaluation for infections are 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.

CRS/MAS was managed in 1 patient initially with corticosteroids. Subsequently, as more data became available, it has been successfully managed with supportive care and when needed, tocilizumab therapy. Tocilizumab is an anti-IL6 receptor antibody and has been administered at a dose of 4 to 8 mg/kg. This may be preferable to systemic immunosuppression with corticosteroids. In many cases, CRS has been severe but reversible. However there have been several cases of refractory CRS that resulted in death. It is hypothesized this may be related to tumor burden, so that treating patients with less tumor burden may result in less severe cytokine release syndrome. However, additional contributory patient and CART-19 related factors cannot be ruled out.
Since CRS mechanistically is a required part of the antitumor mechanism of *in vivo* CART-19 cell expansion and tumor killing, tocilizumab was 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. Steroids following CART-19 infusion were avoided and given only under life threatening situations due to the known lympholytic effects.

1.5. Dose Rationale and Risk/Benefits

We have chosen to use split dosing via an intravenous route of administration for this protocol. The dose that was initially selected for administration was a single dose of 1-5x10^8 CART-19 cells. As part of protocol version 5 dated June 19, 2014, the dose was reduced to a single dose of 1-5 x 10^7 CART-19 cells. In the protocol amendment dated November 21, 2014, the dose remained 1-5 x 10^7 CART-19 cells, but was revised to be administered via split dosing: 10% on Day 1, 30% on Day 2, 60% on Day 3. In the protocol amendment dated May 5, 2015, the dose was changed to 1-5 x 10^8 CART-19 cells administered via split dosing: 10% on Day 1 (1-5x10^7), 30% on Day 2 (3x10^7-1.5x10^8), 60% on Day 3 (6x10^7-3x10^8). The current dose is the same dose and split fraction administration schedule that the first six adult subjects were treated with on the first CART19 protocol, UPCC0[redacted] All six of those subjects achieved complete responses and received either the 10% fraction only or all three dose fractions.

1.5.1. Dose Rationale

Animal studies support a threshold dose of CART-19 cells and therefore the initial clinical dose selection was within the range of 1 x 10^7 to 1 x10^9 CART-19 transduced cells. For safety reasons, initial phase I dosing was divided among three split infusions (10%, 30% and 60% of the total dose). Many patients failed to receive more than the initial dose due to the onset of fevers, yet efficacy responses were observed. Phase II adult patient dosing has utilized either a single infusion or split dosing administration of 1-5 x 10^7 or 1-5 x 10^8 CART-19 transduced cells to study dose optimization with good clinical tolerance. No significant infusional toxicities have been observed.

In multiple adult patients with CLL (UPCC[redacted] and UPCC[redacted]2) and in the six adult ALL patient treated as of December 2014 (UPCC[redacted]), as well as many pediatric ALL patients (CHP959), clinical responses at doses ranging from $1.4x10^7$ to $1.1x10^9$ CART-19 cells have been observed even after a single infusion. In an initial pilot trial, the median CART19 cell dose infused was $1.6x10^8$ cells. No obvious dose:response relationship can be identified. Unlike standard drugs that are metabolized, CAR T cells are able to proliferate extensively in the patients. Thus, the administered dose may underestimate the number of CART-19 T cells *in vivo* following engraftment and expansion and will vary from patient to patient.

The first 6 ALL patients treated on this protocol received a single dose of 1-5 x 10^8 CART-19 T-cells. The second 6 ALL patients treated on this protocol received a single reduced dose of 1-5 x 10^7 CART-19 T-cells. This change was implemented in response to three patient deaths at the 1-5 x 10^8 dose level, which were felt to be possibly related to refractory cytokine release syndrome, an expected toxicity of CART-19 T-cells. We hypothesized that this reduced cell dose would elicit the same clinical responses seen at the higher dose level and in pilot trials of CART-19, but result in less rapid and severe cytokine release syndrome that could be better controlled. Two subjects treated at the reduced dose died from an intracranial bleed and sepsis, respectively. While the relationship of these deaths to the infused dose of CART-19 cells is uncertain, a relationship cannot be excluded, prompting an additional modification of the dosing scheme. In order to limit dose related toxicity, the dosing regimen was modified in a protocol amendment dated November 21, 2014, to be administered at a reduced dose of 1-5 x 10^7 CART-19 cells via split dosing: 10% on Day 1, 30% on Day 2, 60% on Day 3. As this dosing regimen has proven to be safe, the dose was re-escalated to 1-5 x 10^8 [as of the protocol amendment dated 5/05/2015] administered via split dosing: 10% on Day 1 (1-5x10^7), 30% on Day 2 (3x10^7-1.5x10^8), 60% on Day 3 (6x10^7-3x10^8).

1.5.2. Risks/Benefits

Safety information outlined in the risk section is largely representative of the murine CART-19 experience. Based on our limited experience thus far with humanized CART-19, no unexpected events have been observed. Periodic safety review will be performed and the risk language will be updated accordingly.

Potential Risks. Participation in this study will expose the patient to genetically engineered autologous T cells. The risk of administering unmodified cells alone is low based on extensive past clinical experience. One unknown risk is that T cell proliferation could be uncontrolled, however it has not been observed in pre-clinical models. In this case, corticosteroids, anti-cytokine therapy, and chemotherapy would be given to eradicate the CAR cells; this has worked in previous cases[47]. It is possible the cells may be immunogenic, and that the patients will have an immune response directed against the scFv; this has not had clinical consequences in previous trials. If an immune response to the cells occurs, it is possible that the cells will be rejected. Three of 3 patients developed Human Anti-Murine Antibody (HAMA) and loss of T cell engraftment in the Lamers study, but this has generally not been an issue in patients with B cell malignancies. Transient or permanent host B cell depletion is also a potential risk with CART-19 cells, since normal B cells express CD19. This is expected to resolve when the CART-19 cells are cleared. Persistent B cell aplasia leads to hypogammaglobulinemia and may increase the risk of infection. This can be managed with IVIG repletion. In previous trials, responding CLL patients who are hypogammaglobulinemic have received IVIG. No significant unusual infection patterns have been identified.

Transformation. There is a risk that people who receive gene transfer 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. 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[86], and in the 21 HIV patients treated with lentiviral modified T cells treated at Penn[87].

General Safety. At the University of Pennsylvania >20 patients with HIV infection have been treated with autologous T cells modified with lentiviral vector. In the first protocol, each subject received a single i.v. infusion of $1x10^{10}$ lentiviral modified T cells; in the second protocol, each subject received up to 6 doses of 0.5-1 x 10^{10} cells. The lentiviral engineered T cells were well tolerated in all patients, with follow up of up to 5 years. Doses of up to $5x10^{10}$ autologous *ex vivo* non-gene modified and expanded T cells have been administered in five protocols to 128 patients with hematologic malignancies and HIV, and have found this to be well tolerated[64, 88-91]. More than 20 patients with CLL and ALL have been treated with CART19 cells. Again, in all cases the infusions were well tolerated. Follow-up has been 3 years in 2 cases.

Risk of tumor lysis syndrome (TLS) related to cytoreductive chemotherapy or CAR T cells. The risk of TLS is dependent on the disease and burden of disease. Several of the patients treated with CART-19 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 CART-19 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 could occur and may include transient fever, chills, and/or nausea. Patients must be pre-medicated with acetaminophen and diphenhydramine hydrochloride prior to the infusion of CART-19. 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.[92]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 CART-19 trials under [redacted]. One event occurred on UPCC[redacted] (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 [redacted] (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 experience 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 CART-19 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), delirium and confusion (in several patients), evidence of disseminated intravascular coagulation as well as MAS. 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 anti-cytokine directed therapy, including tocilizumab, in most patients. As of June 2014, three patients have died of complications related to refractory CRS and intercurrent infections. In addition, it is unclear if treating the CRS with anti-cytokine directed therapy adversely impacts the anti-tumor response.

Features consistent with MAS or HLH have been observed in patients treated with CART 19, coincident with clinical manifestations of the CRS. MAS appears to be a reaction to immune activation that occurs from the CRS, and therefore should be considered a manifestation of CRS.

Macrophage activation syndrome is similar to 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. There are no definitive diagnostic criteria for MAS, but it is typically diagnosed by meeting HLH criteria.

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, soluble interleukin-2 receptor (sCD25), 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. A pathologic feature of MAS is the presence of hemophagocytic CD163+ macrophages (HPC) in bone marrow or lymph-node aspirates.

Diagnosis is based on the fulfillment of criteria established in 2004[93] for HLH associated with autosomal recessive disorders (familial HLH, fHLH).

A diagnosis of non-familial HLH/MAS is made by having 5/8 criteria:

- Fever
- Splenomegaly
- Cytopenias (affecting 2 or more lineages in the peripheral blood; hemoglobin <9 g/dL, platelets <100,000/μL, Absolute neutrophil count <1000/μL)
- Fasting triglycerides >265 mg/dL, Fibrinogen < 1.5 g/L

- Hemophagocytosis in bone marrow or spleen or lymph nodes
- Low or absent NK-cell activity
- Ferritin > 500 g/L
- Soluble CD25R > 2400 U/L

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). Soluble CD25R and NK cell activity are not standard tests, though samples are taken for retrospective CD25R analysis. Therefore, patients may not meet strict definition of HLH/MAS, but given the constellation of findings, and the consistent dramatic elevation in Ferritin, this is indeed the reaction associated with the CRS.

At this time it is still unknown whether CRS/MAS is beneficial or harmful to the anti-tumor response. Research monitoring data showed that IL6 levels were extraordinarily high during the CRS, prompting to 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. Adult patients were treated with tocilizumab 4mg/kg or 8 mg/kg. It is unclear if early treatment will negate the antitumor response. 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 CART-19 on CHP9595 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 etanercept and tocilizumab, as described in *Grupp et al., NEJM, 2013*, and all associated adverse events resolved. CHP959-104 and CHP959-105 received the 10% dose only and experienced CRS. CHP959-103 received the 10% and 30% doses, respectively, and experienced a mild CRS after the 10% dose, with no CRS experienced after the 30% dose. None of these patients experienced were severe enough CRS (i.e. there were no instances of more than transient oxygen requirements, or hypotension requiring pressor support) requiring treatment with steroids or cytokine blockade.

Fatal SAEs with CARs: Two studies have reported fatal SAEs following CAR infusion in patients with malignancy. Brentjens et al designed a retrovirally-transduced CAR against the CD19 molecule for patients with B cell lymphoma. The **CD19** CAR was the second generation design containing CD28 and CD3ζ signaling domains. A total of 7 subjects have been treated on this protocol, 6 without SAE. However, subject four in this study was a 69 year old man with refractory CLL and who had a significant past medical history of myocardial infarction, coronary artery disease, hypertension, and chronic renal failure. This was the 4th patient in the study and the first one on the cohort undergoing lymphodepletion. This subject received pre-T cell conditioning with 1.5g/m^2 of cyclophosphamide followed 2 days later by infusion with genetically modified 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 44h 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[94].

The second case of a fatal SAE related to CAR T cells was reported by the NCI group (Morgan et al. 2010). This study attempted to treat cancer patients with overexpressing ERBB2 tumors with an anti-**ERBB2 CAR** of 3rd generation (containing CD28, 41BB and CD3ζ signaling domains). The first subject in the study was a 39-year-old female with colon cancer metastatic to lungs and liver. The patient received lymphodepleting regimen (60mg/kg cyclophosphamide daily for 2 days followed by fludarabine 25mg/m^2 for the next 5 days) followed the next day by retrovirally-transduced 10^{10} ERBB2 CAR T cell (transduction efficiency 79%). At 15min post-infusion, the patient began to develop dyspnea and hypoxia with pulmonary infiltrates on chest x-ray. The patient progressed into hypoxemic respiratory failure requiring mechanical ventilatory support, vasopressor-dependent hypotension, and cardiopulmonary arrest. The patient was initially resuscitated and started on high dose steroids, but despite aggressive supportive care, the patient expired 5 days after infusion. Serum cytokine measurements demonstrated a dramatic rise in pro-inflammatory cytokines (IFN-γ, TNF-α, IL-6, GM-CSF) within 4 hours of infusion consistent with a cytokine storm initiating multi-system organ failure. Dr. Morgan postulates that upon first pulmonary circulation passage[95], the CAR ERBB2 T cells bound to native low level expression pulmonary epithelial cell ERBB2 proteins[96], leading to CAR activation and pulmonary microvascular injury.

Other fatal SAEs have been reported. Two events occurred in April 2014 at the Memorial Sloan Kettering Cancer Center using CD19 specific CAR redirected T cells. Five fatal events have occurred at the University of Pennsylvania using CART-19 T-cells (a CD19-specific CAR using a lentiviral vector) in patients with acute lymphoblastic leukemia. Three of the first six adult ALL subjects infused on UPCC[redacted] died as a result of refractory Cytokine Release Syndrome (CRS) in the setting of intercurrent infections. Thereafter, the single dose administered in UPCC[redacted] was reduced to 1-5x10^7 CART19 cells. In the next six adult ALL subjects treated at the de-escalated dose, two died from an intracranial bleed and sepsis, respectively.

Grading of CRS: The CTC grading system was originally developed to capture a cytokine syndrome occurring during infusional therapy; therefore, it is inadequate to capture the delayed CRS that occurs after CART-19 infusions. University of Pennsylvania has proposed to modify the CTC grading specifically to capture toxicity for protocols using CART-19 cells. MAS/HLH observed signs and symptoms are a manifestation of CRS and will therefore not be graded separately (See Table **8-1** in Section 8.2).

Graft versus host disease (GVHD): The chance of GVHD occurring is low, but it is a potential risk with CART-19 therapy. A prior UPenn/CHOP study of activated DLI (*ex vivo* activated cells collected from the donor and grown in the same fashion as CART-19 but without CAR introduction) did not show high rates of GVHD (2/18 patients with grade 3 GVHD and none with Grade 4)[97].

Patients with active, acute or chronic GVHD at screening are excluded from enrolling in this study. However, due to the possibility of some degree of residual donor engraftment, which will include T cells of donor origin, patients that received a previous HSCT at the Hospital of the University of Pennsylvania will be assessed for donor chimerism at screening and will be monitored closely throughout the study for signs of GVHD. To date, no patient that had a prior allogeneic HSCT developed GVHD after autologous CART-19 infusion, even when "autologous" cell included cells of donor origin.

Potential benefits.
Outcome remains poor for adult patients with relapsed or refractory (r/r), B cell acute lymphoblastic leukemia (B cell ALL). Treatment options include further treatment with salvage chemotherapy, allogeneic hematopoietic stem cell transplantation (HSCT) or supportive care. For this population, 5 year overall survival (OS) after relapse is estimated at 7%46. For the small minority of patients with relapsed ALL who have a donor identified and go on to transplant, OS is estimated at 23% and those patients who receive chemotherapy only is 4%.

Based on inclusion criteria, patients will be incurable with standard available therapies. Furthermore, most patients will not be eligible for allogeneic transplant. For ALL patients, there is little benefit from allogeneic SCT with relapsed and active disease and many centers do not offer patients in this situation transplantation because of futility. Therefore, any benefit that may be seen with CART-19 cells will have a major impact for patients.

For patients who relapse after allogeneic transplant, treatment options are even more limited and outcomes dismal. Conventional chemotherapy is not curative and often highly toxic and ineffective. 2nd allogeneic transplant is associated with extensive morbidity, mortality, high relapse rate, and is ineffective for the majority of patients. Donor lymphocyte infusions result in response rates between 0-13% and there are very few long-term survivors.

Ongoing clinical trials with CART-19, T cells expressing a second generation CAR with an anti-CD19 scFv and 4-1BB and TCR signaling domains, in patients with B-cell ALL and CLL (both CD19 expressing B cell malignancies) described above show that CART-19 therapy has potent anti-tumor activity in pediatric and adult ALL patients[47, 48] (described in detail in Section 1.4). Of the nine evaluable pediatric B cell ALL patients, 7 CR, 1 PR and 1 NR have been reported for an overall response rate of 8/9 (89%). The one adult ALL patient treated remains in CR after 2 months of follow up and has gone on to have an allogeneic SCT; of note, he was considered previously ineligible for allogeneic SCT before CART19 cell infusion because of his refractory disease.

Therefore, the risk:benefit ratio for a novel therapy with the potential to induce remission in ALL is quite favorable.

2. STUDY OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
Evaluate the complete remission rate at day 28 after CART-19 therapy.	• Overall Complete Remission Rate (ORR) at Day 28 which includes CR and CR with incomplete blood count recovery (CRi) (see section 6.15)
Secondary	
Evaluate best overall response rates	• Percentage of patients 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. • Percentage of patients achieving CR/CRi before or at Month 6
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).
Describe cause of death (COD) when appropriate	• Cause of death (COD) when appropriate.
Describe response in terms of minimal residual disease (MRD) negative and positive testing using: - flow cytometry (standard) - quantitative molecular technologies (deep sequencing) (exploratory)	• Percentage of patients who achieve a CR associated with minimal residual disease (MRD) negative bone marrow as determined by high sensitivity flow cytometry. • Percentage of patients who achieve a CR associated with minimal residual disease (MRD) negative bone marrow as determined by quantitative molecular technologies (deep sequencing). These analyses will be performed on nucleic acid isolated from pre- and post-treatment.
Evaluate manufacturing feasibility of CART-19	• 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 CART-19	• Frequency and severity of adverse events, including, but not limited to, cytokine release syndrome (CRS) and macrophage activation syndrome (MAS).
Characterize the *in vivo* cellular pharmacokinetic (PK) profile (levels, persistence, trafficking) of CART-19 cells in target tissues (blood, bone	• Duration of CART-19 *in vivo* survival as determined by Q-PCR performed at a minimum of weekly for the first month, monthly until Month 6 and every three months until Month 12

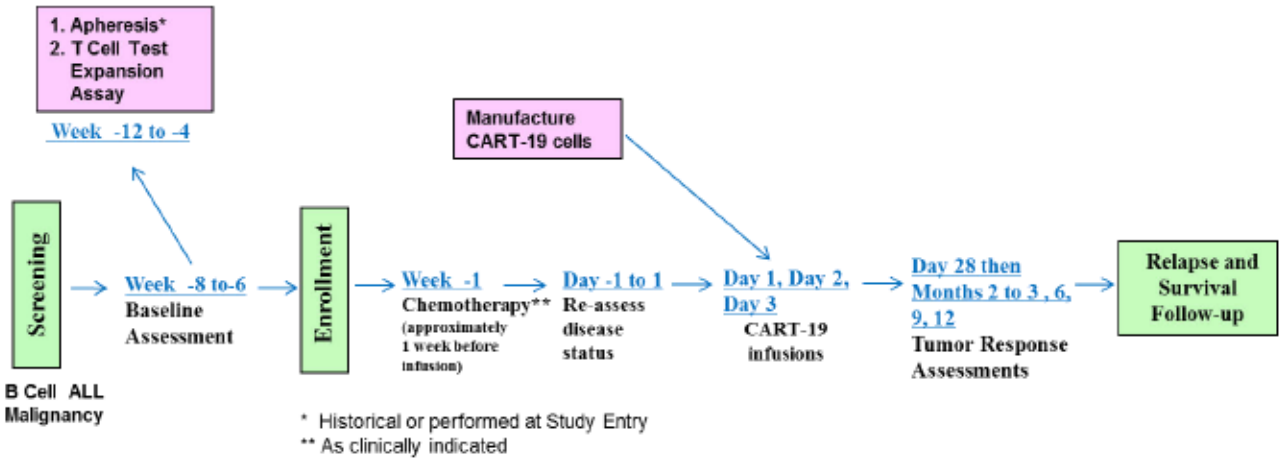
Objectives	Endpoints
marrow, cerebral spinal fluid and other tissues if available)	or until any 2 sequential negative tests documenting loss of CART-19 provided that a specific blood or tissue sample is available at that time point.
Describe the incidence of immunogenicity to CART-19 Assess correlation for immunogenicity with loss of detectable CART-19 (loss of engraftment)	• Host immunity against anti-CD19 as determined by multiparametric flow cytometry.
For patients treated for relapse after allogeneic SCT, describe the risks of GVHD	• Incidence of GVHD
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-CART-19 infusion as determined by multi-parametric flow cytometry.
Patient Reported Outcomes	• Patient's physical, social/family, emotional and functional well-being at baseline and months 3 and 6 as measured by FACT-Leu (Version 4) and EORTC-QLQ C30 questionnaires.
Follow subjects infused with less than protocol-specified target dose	• Exploratory analyses to inform on dose-response activity
Assess safety and efficacy of re-infusion of CART-19 cells in previously treated patients	• Frequency and severity of adverse events and other safety data • Expansion and persistence of CART-19 cells, in comparison to their original infusion • Overall response, time to response, duration of response and time to alternative therapy, in comparison to their original infusion

3. STUDY DESIGN

3.1. General Design

The study will consist of four sequential phases: 1) a screening phase, 2) a manufacturing and pre-treatment phase, consisting of apheresis (if applicable) and chemotherapy, 3) a treatment phase, consisting of a CART-19 transfused cell infusion, and 4) follow up evaluations. The evaluations and infusion schedule are included in **Table 6-1**. The general protocol schema is displayed in **Figure 3-1**.

Figure 3-1 Study Schematic



After signing informed consent, patients will undergo screening tests and procedures to determine eligibility. Once patient eligibility is confirmed, patients will be staged for apheresis (leukapheresed) to obtain peripheral blood mononuclear cells (PBMC) for CART-19 manufacturing. A PBMC sample from the apheresis product will be used for a test expansion to assess the suitability of the patient's T cells for CART-19 manufacturing. The test expansion results will be used for secondary objective analysis, but will not be a criterion for proceeding with CART-19 manufacturing. CD3+ T cells will be purified from the PBMC, transduced with the anti-CD19 TCRζ/4-1BB lentiviral vector, expanded *in vitro* and then frozen for future administration. Due to the highly progressive nature of B-cell ALL, cryopreserved historical apheresis products collected from the patient prior to study entry are usable for CART-19 manufacturing if collected at an appropriately certified apheresis center and the product meets adequate mononuclear cell yields. If the archived sample passes the test expansion, the patient would not have to repeat the baseline apheresis on this study. If a historical apheresis product is not available, an apheresis procedure will be scheduled for cell procurement after study entry.

Unless contraindicated and medically not advisable based on previous chemotherapy, patients will be given conditioning chemotherapy prior to CART-19 cell infusion with the intent of lymphodepletion. Additionally, if the patients WBC $\leq$ 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 CART-19 cells. The chemotherapy start date will vary based on the duration of the selected chemotherapy regimen. If the delayed period from chemotherapy to CART-19 infusion is 4 or more weeks, the patient will need to be re-treated with lymphodepleting chemotherapy prior to CART-19 infusion. Please refer to Section 6.6 for selection guidance of the preferred conditioning chemotherapy regimens.

We will enroll 30 evaluable patients for the primary efficacy endpoint analysis. Primary efficacy evaluable patients are those who have received CART-19 cells at either 1-5x10^8 CART-19 cells or 1-5 x 10^7 CART-19 cells, given as either a single infusion or given via split dosing over 3 days.

For the purposes of the study, primary efficacy non-evaluable patients will be replaced with primary efficacy evaluable patients. Please refer to Tables 3-1 and 3-2 for complete details. Both primary efficacy evaluable and non-evaluable patients will be followed in the same manner

according to the Schedule of Study Procedures for all evaluations, including clinical, research (correlative) and safety.

All patients will have blood tests to assess safety, engraftment and persistence of the CART-19 cells at regular intervals throughout the study (**Table 6-1**). The subsets of circulating T-cells that contain the CART-19 cells will be assessed at various times after infusion. Trafficking of CART-19 cells will be assessed in bone marrow aspirates, cerebral spinal fluid when tested, and other tissues, if available. Follow up is planned at a minimum weekly for 4 weeks, monthly for 6 months, then patients will be followed quarterly for the remainder of the year to obtain a medical history, undergo a physical examination, and blood tests. The trial will be continually monitored for safety.

Following these evaluations, patients will enter a roll-over study for annual follow-up by phone and questionnaire for up to fifteen years to assess for safety assessments per the FDA guidelines.

Table 3-1 Subject Dose, Infusion and Endpoint Analysis Summary- 1-5 x 10^8 CART-19 cells (administered via single infusion or split dosing)

				Included in endpoint analysis? (Y/N)				
Manufactured CART-19 Dose	Released from CVPF*? (Y/N)	Can subject be infused** ? (Y/N)	Total Dose Infused	Primary Efficacy	Secondary Efficacy	Correlative and Safety	Manufacturing Feasibility	Primary Efficacy Evaluable Patient Set; Followed according to SOE (Y/N)?
1-5x10^8	Y	Y	1-5x10^8	Y	Y	Y	Y	Primary Efficacy Evaluable; Y
≥1x10^7-<1x10^8	Y	Y	≥1x10^7-<1x10^8	Y	Y	Y	Y	Primary Efficacy Evaluable; Y
<1x10^7	N	N	<1x10^7	N	N	N	Y	N/A

*Provided that all other manufacturing release criteria are met
**If subject does not receive dose, then they are only assessed for manufacturing feasibility

Table 3-2 Subject Dose, Infusion and Endpoint Analysis Summary- Reduced Dose Level: 1-5 x 10^7 CART19 Cells (administered via single infusion or split dosing)

				Included in endpoint analysis? (Y/N)				
Manufactured CART-19 Dose	Released from CVPF*? (Y/N)	Can subject be infused**? (Y/N)	Total Dose Infused	Primary Efficacy	Secondary Efficacy	Correlative and Safety	Manufacturing Feasibility	Primary Efficacy Evaluable Patient Set; Followed according to SOE (Y/N)?
1-5x10^7	Y	Y	1-5x10^7	Y	Y	Y	Y	Primary Efficacy Evaluable; Y
≥2x10^6-<1x10^7	Y	Y	≥2x10^6-<1x10^7	N	Y	Y	Y	Primary Efficacy Non-Evaluable; Y
<2x10^6	N	N	N/A	N	N	N	Y	N/A

*Provided that all other manufacturing release criteria are met
**If subject does not receive dose, then they are only assessed for manufacturing feasibility

3.2. Primary Efficacy Non-Evaluable Patients

The only difference between the primary efficacy evaluable and non-evaluable patients is that only the primary efficacy evaluable patients will be used for primary efficacy endpoint analysis. Both primary efficacy evaluable and non-evaluable patients will be used in secondary efficacy, safety, manufacturing feasibility, correlative and exploratory analyses. The statistical analysis sets are detailed in Section 7.3.

In Penn's UPCC [redacted] adult CART-19 trial, $1.4x10^7$–15.5×10^8 CART-19 cells have been administered. Complete responses have been achieved at both the highest and lowest dose levels. Though numbers are small over this wide range of cell doses, there is no evidence for either a dose:response or dose:toxicity relationship. Therefore, there is scientific and clinical justification for giving subjects the manufactured cell dose, even if below the protocol-specified dose range.

3.3. Retreatment Cohort

3.3.1. Overview

Based on available clinical trial data as of February 1, 2014, sustained persistence of CART-19 cells are directly correlated with ongoing response. Thus, several subjects who have lost detectable CART-19 cells have subsequently relapsed. Relapsed subjects have been retreated with additional CART-19 cell infusions and, in some cases, the initial responses were reestablished. Given the clinical evidence that will be described in depth below, we would like to allow for the retreatment of subjects who had an initial response to their 1st infusion, lost detectable CART-19 cells and have subsequently relapsed.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Taken together, there have been no severe or unexpected toxicities observed in any subject upon retreatment with CART-19 cells ranging from 2-12 months after the initial infusion(s) retreated with CART-19 cells as of June 1, 2015. Given the currently available safety data, there is no suggestion that retreatment of these targeted subjects will pose risks to the subject greater than that of their original infusion. Additionally, 3 of the 7 subjects retreated have experienced clinical benefit with few alternative treatment options available. Therefore, retreatment with additional CART-19 doses carries potential benefit with no observable increased risk associated with the current data.

[redacted]

3.3.2. Retreatment Objectives

This cohort of subjects will be analyzed separately in order evaluate the following exploratory objectives:

- Assess the frequency and severity of adverse events and other safety data including development of cytokine release syndrome
- Evaluate the expansion and persistence of CART-19 cells, in comparison to their original infusion
- Evaluate overall response, time to response, duration of response and time to alternative therapy, in comparison to their original infusion.

3.3.3. Retreatment Dose

Up to one retreatment dose (split administration) will be allowed per eligible subject. Subjects may receive either murine or humanized CART-19 as part of this retreatment cohort. Preference will be for humanized CART-19 in retreated subjects, if it is clinically, logistically, and financially feasible. If not determined to be feasible and/or a subject has additional cryopreserved murine CART-19 doses available, they may receive retreatment with murine CART-19. The target dose for retreatment is 1-5 x 10^8 CART-19 cells administered via split dosing: 10% on Day 1, 30% on Day 2, 60% on Day 3. The minimum acceptable dose for retreatment is $1x10^7$ CART-19 T-cells. All subjects who receive CART-19 T-cells as part of this retreatment cohort will be considered evaluable.

All prerequisites for eligibility to receive CART-19 outlined in Section 5.2 must be met prior to retreatment. The study drug will be prepared and administered per the guidelines set forth in Section 5. All required study procedures, pre-medications, prophylaxis, monitoring, and follow-up guidelines from the initial infusion outlined in Section 6 will apply for this retreatment cohort. A Retreatment Cohort Visit Evaluation Schedule has also been developed **(Table 6-2).**

Additional information related to retreatment eligibility and procedures, is outlined in Section 6.13 below.

4. PATIENT SELECTION AND WITHDRAWAL

No exceptions to eligibility will be granted for this study.

4.1. Inclusion Criteria

1. Signed informed consent form must be obtained prior to any study procedure
2. Relapsed or refractory B-cell ALL
   a. 1st or greater BM relapse OR
   b. Any marrow relapse after allogeneic HSCT and > 100 days from transplant OR
   c. For patients with refractory disease:
      i. < 60 years old that have not achieved a CR after ≥ 2 or more chemotherapy regimens
      ii. ≥60 years old that have not achieved a CR after 1 prior chemotherapy regimen
   d. Patients with Ph+ ALL are eligible if they have failed tyrosine kinase inhibitor therapy
3. Documentation of CD19 tumor expression in bone marrow or peripheral blood by flow cytometry within 3 months of screening.
4. Adequate organ function defined as:
   a. Creatinine ≤ 1.6 mg/dl
   b. ALT/AST ≤ 3x upper limit of normal range
   c. Direct bilirubin ≤2.0 mg/dl

d. Must have a minimum level of pulmonary reserve defined as ≤ Grade 1 dyspnea, pulse oxygen > 92% on room air, and DLCO ≥ 40% (corrected for anemia if clinically appropriate)
e. Left Ventricle Ejection Fraction (LVEF) ≥ 40% confirmed by ECHO/MUGA

5. Bone marrow with ≥ 5% lymphoblasts
6. ~~Life expectancy > 12 weeks~~ **RETIRED WITH PROTOCOL VERSION 6**
7. Male or female age ≥ 18 years
8. A ECOG Performance Status that is either 0 or 1
9. No contraindications for leukapheresis

4.2. Exclusion Criteria

1. Isolated extramedullary disease relapse
2. ~~Patients with concomitant genetic syndrome: patients with Down syndrome, Fanconi anemia, Kostmann syndrome, Shwachman syndrome or any other known bone marrow failure syndrome~~ **RETIRED WITH PROTOCOL VERSION 9**
3. Active hepatitis B or active hepatitis C
4. Class III/IV cardiovascular disability according to the New York Heart Association Classification (see Appendix 1)
5. HIV infection
6. Active acute or chronic graft-versus-host disease (GVHD) or requirement of immunosuppressant medications for GVHD within 4 weeks of enrollment.
7. Concurrent use of systemic steroids or chronic use of immunosuppressant medications. Recent or current use of inhaled steroids is not exclusionary. For additional details regarding use of steroid and immunosuppressant medications, please see Section 5.6.
8. Active CNS involvement by malignancy. Note: Patients with history of CNS disease that has been effectively treated will be eligible provided that treatment was >4 weeks before enrollment
9. Pregnant or nursing (lactating) women, female study participants of reproductive potential must have a negative serum or urine pregnancy test within 48 hours before infusion
10. Participation in a prior investigational study within 4 weeks prior to enrollment. Participation in non-therapeutic research studies is allowed.
11. 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.

Eligibility criteria for the retreatment cohort are outlined in Section 6.13 below.

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. 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 screening and a negative urine pregnancy test within 48 hours of T cell infusion.

Due to the high risk level of this study, while enrolled, all 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:

- 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:

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

4.4. Early Withdrawal of Patients

4.4.1. When and How to Withdraw Patients

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

Potential reasons for premature discontinuation include:

1. The patient is lost to follow-up.
2. The judgment of the principal investigator that the patient is too ill to continue if this occurs prior to any of the CART-19 T-cell infusions.
3. Pregnancy: Withdraw patient if pregnancy occurs prior to any of the CART-19 T-cell infusions
4. Voluntary withdrawal; a patient may remove himself/herself from the study at any time without prejudice. A patient 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 including, but not limited to, the development of CNS metastasis if this occurs prior to any of the CART-19 T-cell infusions.
6. A serious adverse event that requires the patient's being withdrawn from the trial if the SAE occurs prior to any of the CART-19 T-cell infusions.
7. Technical difficulties are encountered in the T cell genetic modification and expansion procedure that precludes the generation of clinical cell doses that meet all Quality Control release criteria as specified by FDA.
8. Termination of the study by the Principal Investigator, the Sponsor, the study funder, the IRB, or the FDA.

Once a subject has received a CART-19 T-cell infusion, subjects should continue to be followed until the subject withdraws consent, dies or are lost to follow up. Subjects are encouraged to enroll into a 15-year long-term follow-up protocol to evaluate specific long-term adverse events related to the study product.

4.4.2. Data Collection and Follow-up

Follow-up data collection after gene modified cell therapy clinical trials is specified by the FDA. As long as patients have detectable cells transduced with the lentiviral vector, they should be followed for toxicity, immune reactions, and any long-term adverse events.

As part of this study, 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) DFS until there is disease progression or they begin a new cancer therapy, and 3) survival until the time of death; until the patient withdraws consent for clinical data collection or the end of the study (Last Patient/Last Visit).

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.

After subjects complete or prematurely discontinue participation in the Primary Follow-up Phase of the study, subjects will also be asked to participate in a separate 15-year long-term follow-up destination protocol.

5. STUDY DRUG

5.1. Description

CART-19 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 CART-19 cells are cryopreserved in infusible cryomedia and will be administered on Days 1, 2 and 3. Each bag/syringe 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, up to 7.5% DMSO, 1% dextran 40, 5% human serum albumin.

Expected toxicities associated with infusion of CART-19 cells include transient fever, chills nausea, and rigors. In order to minimize these events, patients will receive premedication as instructed below in Section 5.4. Toxicities that could potentially occur but are unprecedented are primarily related to the gene transfer and are described in Section 8.5.2. These include generation of a replication competent lentivirus (RCL), insertional oncogenesis, and uncontrolled proliferation of the CART-19 cells.

5.2. Patient Eligibility to Receive CART-19 Transduced T Cells

Day 1 CART-19 Infusion:

1. All patients must undergo a respiratory virus panel (RVP) within 10 days prior to the first planned CART-19 infusion. If the patient is positive for influenza, Tamiflu® or equivalent should be administered per package insert. The patient must complete treatment **prior** to receiving CART-19. The test does not need to be repeated prior to the first CART-19 infusion; however, if influenza sign and symptoms are present, the CART-19 infusions should be delayed until patient is asymptomatic. If the patient is positive for another virus on the RVP, the CART-19 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. Patient 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. Patients 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 CART-19 T-cells, may have their infusion delayed until both the treating investigator and PI determine it is clinically appropriate to proceed with the CART-19 infusion.

4. Patients experiencing toxicities from their preceding cytoreductive chemotherapy can have their infusion schedule delayed until these toxicities have resolved. **Note:** If patients CART-19 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 evident by positive blood cultures for bacteria, fungus, or virus within 48 hours of CART-19 cell infusion

Day 2 CART-19 Infusion:

1. Patient 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. Patients experiencing new laboratory abnormalities, that in the opinion of the treating investigator or PI may impact subject safety or the subjects' ability to receive CART-19 T-cells, may have their infusion delayed until both the treating investigator and PI determine it is clinically appropriate to proceed with the CART-19 infusion.

Day 3 CART-19 Infusion:

1. Patient 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. Patients experiencing new laboratory abnormalities, that in the opinion of the treating investigator or PI may impact subject safety or the subjects' ability to receive CART-19 T-cells, may have their infusion delayed until both the treating investigator and PI determine it is clinically appropriate to proceed with the CART-19 infusion.

5.3. Treatment Regimen

CART-19 transduced T cells will be administered at a dose of 1 to 5 x 10^8 CART-19 transduced cells given via split dosing on Days 1, 2 and 3. The first CART-19 infusion will be scheduled to occur approximately 1 to 4 days following lymphodepleting chemotherapy but may be delayed as outlined above (Section 5.2).

5.4. Preparation and Administration of Study Drug

Cell manufacturing is done according to [redacted] at the University of Pennsylvania Clinical Cell and Vaccine Production Facility (CVPF). The CART-19 T 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. Upon release, the cells are administered at bedside.

Cell Thawing

The cells will be transported to the patient's bedside on the day of the infusion. The cells will be thawed by trained personnel using a water bath maintained at 36°C to 38°C. The bag will be gently massaged until the cells have just thawed. There should be no frozen clumps remaining at the time it is connected to the i.v site. If the CART-19 cell product appears to be damaged or leaking, 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[115]. It is recommended that the patient be pre-medicated with 650mg acetaminophen and 25-50mg diphenhydramine hydrochloride prior to each infusion of CART-19 cells. 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 CART-19 cell expansion and function.

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 CART-19 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 and available for administration in order to manage suspected toxicities prior to infusion.

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. Vital signs (temperature, pulse, and blood pressure) will be taken before infusion.

Packaging and Labeling

CART-19 transduced T cells will be administered at a dose of 1 to 5 x 10^8 CART-19 transduced cells given via split dosing on Days 1, 2 and 3. Each bag/syringe 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, up to 7.5% DMSO, 1% dextran 40, 5% human serum albumin.

Each infusion bag/syringe will have affixed to it a label containing information regarding the dose, the method of manipulation, and the vector. 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.

5.5. Infusion of CART-19 Product

Trained study staff will administer the CART-19 product via i.v. infusion by gravity or syringe using precautions for immunosuppressed patients. The transduced T cells will be infused at a flow rate of approximately 10 to 20 mL per minute. A leukoreduction filter **must not be used for the infusion of the T cell product**. The duration of the infusion will be based on the total volume to be infused and the recommended infusion rate. Vital signs (temperature, pulse, blood pressure, and oxygen saturation by pulse oximetry) will be measured within 10 minutes prior to the infusion, within 10 minutes after the infusion, every 15 minutes for the first hour and then every hour 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-CART-19 infusion, vital signs will continue to be monitored at a minimum of every hour or as clinically indicated until stable. 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.

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 CART-19 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 are unknown and can be used at the physician's discretion.
- Steroids or other immunosuppressant drugs should NOT be used within 10 days prior to the apheresis procedure.
- Steroids or other immunosuppressant drugs should NOT be used within 24 hours prior to the CART-19 infusion (refer to Section 5.4) or following CART-19 infusion unless under life threatening circumstances or at the physician's discretion to manage CRS.
- 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).

6. STUDY PROCEDURES

Overview:

The schedule of evaluations and study procedures are described in the Visit Evaluation Schedules in **Tables 6-1 (Main Treatment Phase) and 6-2 (Retreatment Cohort)**. Also, refer to Section 6 for further details of the schedule of each assessment, analysis and processing/handling of samples.

Table 6-1: Visit Evaluation Schedule (Main Treatment Phase)

	Screening and Enrollment	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[2]	Quarterly Follow-Up[2]	Secondary Follow-up[26]
Visit Number	1	2	3	4	201	202	5	6	7	8	9	10	501-505	506-507	601+
	-12W to -1W	~ -1W	~ -1D	D1	D2 (+) 1d	D3 (+) 1d	D4 (+) 1d	D7 (+/-) 1d	D11 (+/-) 1d	D14 (+/-) 1d	D21 (+/-) 3d	D28 (+/-) 3d	M2 M3 M4 M5 M6 (+/-) 7d	M9 M12 (+/-) 14d	Every 6Months
Obtain Informed Consent	x														
Patient History															
Demography	x														
Inclusion/exclusion criteria	x														
Relevant medical history/current medical conditions	x														
Diagnosis and extent of cancer	x														
Prior antineoplastic therapy	x														
Prior/concomitant medications	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Antineoplastic therapies post-infusion							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	
Height	x														
Weight	x		x	x	x	x	x	x		x	x	x	x	x	
Vital signs	x		x	x[12]	x[12]	x[12]	x	x	x	x	x	x	x	x	
Laboratory Assessments															
Apheresis[6]	x[27]														

	Screening and Enrollment	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[2]	Quarterly Follow-Up[2]	Secondary Follow-up[25]
Visit Number	1	2	3	4	201	202	5	6	7	8	9	10	501-505	506-507	601+
	-12W to -1W	~ -1W	~ -1D	D1	D2 (+) 1d	D3 (+) 1d	D4 (+) 1d	D7 (+/-) 1d	D11 (+/-) 1d	D14 (+/-) 1d	D21 (+/-) 3d	D28 (+/-) 3d	M2 M3 M4 M5 M6 (+/-) 7d	M9 M12 (+/-) 14d	Every 6Months
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[24]	x[24]	x[24]	x[13]	x	X[13]	x	x	x	x[24]	x[24]	
Serum Pregnancy Test[11] (1 ml SST)	x														
Urine Pregnancy Test[11]			x											x	
T Cell Subsets: CD3/CD4/CD8 (4ml lavender top, EDTA)				x								x	x		
Autoimmune Screen (ANA, ESR) (4 ml SST; 3ml lavender top EDTA)	x														
HIV Test (1ml SST)	x														
Viral Serology (CMV, EBV, Hepatitis B and C) (5ml red top, serum)	x														
Serum Immunoglobulin levels (1ml SST)	x														
HLH/MAS (triglycerides, haptoglobin)(4mL SST; 2.5mL lavender top, EDTA)			x	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	

	Screening and Enrollment	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[2]	Quarterly Follow-Up[2]	Secondary Follow-up[26]
Visit Number	**1**	**2**	**3**	**4**	**201**	**202**	**5**	**6**	**7**	**8**	**9**	**10**	**501-505**	**506-507**	**601+**
	-12W to -1W	**~ -1W**	**~ -1D**	**D1**	**D2 (+) 1d**	**D3 (+) 1d**	**D4 (+) 1d**	**D7 (+/-) 1d**	**D11 (+/-) 1d**	**D14 (+/-) 1d**	**D21 (+/-) 3d**	**D28 (+/-) 3d**	**M2 M3 M4 M5 M6 (+/-) 7d**	**M9 M12 (+/-) 14d**	**Every 6Months**
Ferritin, LDH and CRP for Cytokine Release Syndrome			x	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	
Donor Chimerism [5] (If applicable)	x											x	x[10]	x[10]	
RVP (Respiratory Virus Panel)		x[18]													
CD19 Immunophenotype Results	x														
Research Analysis[2]															
Serum ~5cc (Red top)	x		x	x[19]	x[19]	x[19]	x	x	x	x	x	x		x[7]	
Immunogenicity (e.g. HAMA/HACA)	x											x			
Cytokines			x	x	x	x	x	x	x	x	x	x			
PBMC ~25cc (Lavender, EDTA)	x[27]		x	x[20]	x[20]	x[20]	x	x	x	x	x	x	x	x	
DNA (Q-PCR CTL019 persistence)			x	x	x	x	x	x	x	x	x	x	x	x	
DNA RCL (VSV-G Q-PCR)			x										x[3]	x[3]	
CTL019 Immune-phenotyping, CART-19 and B cell enumeration, functional assays			x				x	x	x	x	x	x	X	x	

	Screening and Enrollment	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[2]	Quarterly Follow-Up[2]	Secondary Follow-up[26]
Visit Number	**1**	**2**	**3**	**4**	**201**	**202**	**5**	**6**	**7**	**8**	**9**	**10**	**501-505**	**506-507**	**601+**
	-12W to -1W	**~ -1W**	**~ -1D**	**D1**	**D2 (+) 1d**	**D3 (+) 1d**	**D4 (+) 1d**	**D7 (+/-) 1d**	**D11 (+/-) 1d**	**D14 (+/-) 1d**	**D21 (+/-) 3d**	**D28 (+/-) 3d**	**M2 M3 M4 M5 M6 (+/-) 7d**	**M9 M12 (+/-) 14d**	**Every 6Months**
Spectra-typing (Clonotyping deep sequencing)			x									x			
Additional Archived Research Blood draw (100cc)												x			
Bone Marrow/LN aspirate[22] (5 cc lavender top, EDTA)	x		x									x[1]	x[1]	x[1]	
DNA (Q-PCR CTL019 homing)	x		x									x[1]	x[1]	x[1]	
CART-19 and B cell enumeration, immunophenotyping	x		x									x[1]	x[1]	x[1]	
Spectra-typing (Clonotyping deep sequencing)	x		x									x[1]			
Marrow Serum (2 cc red top)	x		x									x[1]			
Cytokines	x		x									x[1]			
Disease Monitoring															
Tumor response assessments												x[1]	x[1]	x[1]	
Physical exam (extramedullary disease)	x		x									x[1]	x[1]	x[1]	

	Screening and Enrollment	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[2]	Quarterly Follow-Up[2]	Secondary Follow-up[26]
Visit Number	**1**	**2**	**3**	**4**	**201**	**202**	**5**	**6**	**7**	**8**	**9**	**10**	**501-505**	**506-507**	**601+**
	-12W to -1W	**~ -1W**	**~ -1D**	**D1**	**D2 (+) 1d**	**D3 (+) 1d**	**D4 (+) 1d**	**D7 (+/-) 1d**	**D11 (+/-) 1d**	**D14 (+/-) 1d**	**D21 (+/-) 3d**	**D28 (+/-) 3d**	**M2 M3 M4 M5 M6 (+/-) 7d**	**M9 M12 (+/-) 14d**	**Every 6Months**
Bone marrow aspirate/biopsy (cytogenetics/FISH if appropriate)	x[23]		x[14]									x[1]	x[1]	x[1]	
Lymph node biopsy[22]	x		x									x[1]	x[1]	x[1]	
CSF evaluation[2]	x											x	As clinically indicated		
Mediastinal disease assessment (Chest x-ray → CT/MRI scan	x											x[16]	x[16]		
MRD by flow cytometry	x		x									x	x[1]	x[1]	
BCR-ABL (Ph+ patients only)	x											x	x[1]	x[1]	
Safety															
Adverse events				x	x	x	x	x	x	x	x	x	x	x	
ECHO/MUGA[21]	x														
Pulmonary Function Test (DLCO)/ Pulmonary Reserve[15]	x														
Lymphodepleting Chemotherapy[4]		x													
CART-19 cell infusion				x	x	x									
Prophylactic antibiotics				x[25]											
Relapse and Survival Follow-up															x[26]

	Screening and Enrollment	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[2]	Quarterly Follow-Up[2]	Secondary Follow-up[2,5]
Visit Number	1	2	3	4	201	202	5	6	7	8	9	10	501-505	506-507	601+
	-12W to -1W	~ -1W	~ -1D	D1	D2 (+) 1d	D3 (+) 1d	D4 (+) 1d	D7 (+/-) 1d	D11 (+/-) 1d	D14 (+/-) 1d	D21 (+/-) 3d	D28 (+/-) 3d	M2 M3 M4 M5 M6 (+/-) 7d	M9 M12 (+/-) 14d	Every 6Months
Quality of Life (QoL) questionnaires (FACT-Leu and EORTC-QLQ C30)	x												x[9]		
Total clinical blood draw (mL)	27.5	0	12.5	16	16	16	12.5	12.5	12.5	12.5	12.5	16.5	12	12	
Total research blood draw (mL)	55	0	30	35	35	35	30	30	30	30	30	130	30	30	
Total blood draw (mL)	82.5	0	42.5	51	51	51	42.5	42.5	42.5	42.5	42.5	146.5	42	42	
Total blood draw (Tbsp.; approximately)	5.5	0	3	3.5	3.5	3.5	3	3	3	3	3	10	3	3	

[1] Tumor response assessments will be performed at Day 28, Months 3, 6, 9 and 12 after CART-19 cell infusions (Refer to Section 6.14 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 collections are not to exceed 3 tablespoons of blood twice in one week time window. Marrow/LN collections would not exceed more than one procedure per month. This would be at the PI's discretion.

[3] Months 3, 6 and 12 only

[4] Lymphodepleting chemotherapy prior to CART-19 cell infusion is NOT required if WBC $\leq$ 1,000 /uL

[5] CD3+ lineage-specific chimerism preferred. Chimerism will be performed only in patients with prior allogeneic transplant at the Hospital of the University of Pennsylvania.

[6] Apheresis will be performed in order to obtain a target of $5x10^9$ PBMCs for CART-19 manufacturing (as required). Apheresis can occur anytime after informed consent is obtained, up to 4 weeks prior to CART-19 infusion. Cryopreserved historical apheresis products collected from the patient prior to study entry are usable for CART-19 manufacturing if collected at an appropriately certified apheresis center and the product meets adequate mononuclear cell yields.

[7] Month 12 only.

[8] As clinically indicated if HLH/MAS or CRS is suspected

[9] Months 3 and 6

[10] Months 3, 6, 9 and 12

[11] Pregnancy test (quantitative) for females only

[12] Vital signs will be taken within 10 minutes prior, within 10 minutes after each infusion, every 15 minutes for the first one hour and then every hour for the next two hours until these signs are satisfactory and stable.

[13] D-dimer required on Days 4 and 11. Additional testing to be performed if HLH/MAS or CRS is suspected.

[14] Bone marrow biopsy/aspirate to be performed within 48 hours prior to the first CART-19 T-cell infusion. The results of this baseline bone marrow are not required prior to infusion.

[15] DLCO $\geq$ 40%; Pulse Oxygen > 92% on room air

[16] CT/MRI will only be performed on Day 28 and Month 3 visits if baseline chest x-ray indicates mediastinal disease

[17] Direct bilirubin will be performed at screening only

[18] All patients must undergo a respiratory virus panel (RVP) to test for influenza within 10 days prior to the first planned CART-19 infusion. If the patient is positive for influenza, oseltamivir phosphate (Tamiflu®) or equivalent should be administered (see Tamiflu® package insert for dosing information). The patient must complete this course of preventative treatment prior to receiving the first CART-19 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 CART-19 infusion. If a patient is positive for another virus on the RVP, CART-19 infusions 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 infusions will be delayed until resolution of these symptoms.

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

[20] Research blood (25 cc lavender top) to be taken between 20-120 minutes post-infusion.

[21] ECHO/MUGA must be performed within 8 weeks prior to the first CART-19 infusion.

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

[23] If the results of a historical bone marrow biopsy (obtained at the time of the patient's last relapse) are available at the time of enrollment, this does not need to be repeated for enrollment. 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 first CART-19 infusion. If lymphodepleting chemotherapy is not administered, the pre-infusion bone marrow (to be performed within 48 hours prior to the first CART-19 infusion) is sufficient.

[24] Performed if clinically indicated.

[25] Prophylactic gram negative antibiotics must be initiated beginning on Day 1. The choice of antibiotic will be left to the physician-investigator's discretion and will be administered per institutional guidelines.

[26] For subjects who complete or prematurely discontinue from the Primary Follow-up Phase of the study while in remission, follow-up attempts will be made to assess the subject's relapse and survival status every 6 months post CART-19 infusion until the end of the study (Last Patient/Last Visit). Once subjects relapse they will be followed for survival until the end of the study (Last Patient/Last Visit) only.

[27] Research blood draw (~25cc) will be collected at screening/enrollment and again at the time of apheresis.

Table 6-2: Visit Evaluation Schedule (Retreatment Cohort)

	Screening and Enrollment	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[2]	Quarterly Follow-Up[2]	Secondary Follow-up[26]
Visit Number	101	102	103	104	105	106	107	108	109	110	111	112	551-555	556-557	602+
	-12W to -1W	~ -1W	~ -1D	D1	D2 (+) 1d	D3 (+) 1d	D4 (+) 1d	D7 (+/-) 1d	D11 (+/-) 1d	D14 (+/-) 1d	D21 (+/-) 3d	D28 (+/-) 3d	M2 M3 M4 M5 M6 (+/-) 7d	M9 M12 (+/-) 14d	Every 6Months
Obtain Retreatment Consent	x														
Patient History															
Retreatment Inclusion/exclusion criteria	x														
Current medical conditions	x														
Interim treatment history	x														
Concomitant medications	x	x	x	X	x	x	x	x	x	x	x	x	x	x	
Antineoplastic therapies post-infusion							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	
Height	x														
Weight	x		x	X	x	x	x	x		x	x	x	x	x	
Vital signs	x		x	x[12]	x[12]	x[12]	X	x	x	x	x	x	x	x	
Laboratory Assessments															
Apheresis[6]	X[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	

	Screening and Enrollment	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[2]	Quarterly Follow-Up[2]	Secondary Follow-up[25]
Visit Number	101	102	103	104	105	106	107	108	109	110	111	112	551-555	556-557	602+
	-12W to -1W	~ -1W	~ -1D	D1	D2 (+) 1d	D3 (+) 1d	D4 (+) 1d	D7 (+/-) 1d	D11 (+/-) 1d	D14 (+/-) 1d	D21 (+/-) 3d	D28 (+/-) 3d	M2 M3 M4 M5 M6 (+/-) 7d	M9 M12 (+/-) 14d	Every 6Months
Coagulation [PT, PTT, INR, fibrinogen, D-dimer] (4.5 ml blue top citrate)	x		x	x[24]	x[24]	X[24]	x[13]	x	X[13]	x	x	x	x[24]	x[24]	
Serum Pregnancy Test[11] (1 ml SST)	x														
Urine Pregnancy Test[11]			x											x	
T Cell Subsets: CD3/CD4/CD8 (4ml lavender top, EDTA)				X								x	x		
Autoimmune Screen (ANA, ESR) (4 ml SST; 3ml lavender top EDTA)	x														
HIV Test (1ml SST)	x														
Viral Serology (CMV, EBV, Hepatitis B and C) (5ml red top, serum)	x														
Serum Immunoglobulin levels (1ml SST)	x														
HLH/MAS (triglycerides, haptoglobin)(4mL SST; 2.5mL lavender top, EDTA)			x	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	
Ferritin, LDH and CRP for Cytokine Release Syndrome			x	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	x[8]	

	Screening and Enrollment	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[2]	Quarterly Follow-Up[2]	Secondary Follow-up[26]
Visit Number	101	102	103	104	105	106	107	108	109	110	111	112	551-555	556-557	602+
	-12W to -1W	~ -1W	~ -1D	D1	D2 (+) 1d	D3 (+) 1d	D4 (+) 1d	D7 (+/-) 1d	D11 (+/-) 1d	D14 (+/-) 1d	D21 (+/-) 3d	D28 (+/-) 3d	M2 M3 M4 M5 M6 (+/-) 7d	M9 M12 (+/-) 14d	Every 6Months
Donor Chimerism [5] (if applicable)	x											x	x[10]	x[10]	
RVP (Respiratory Virus Panel)		X[18]													
CD19 Immunophenotype Results	X														
Research Analysis[2]															
Serum 5cc (Red top)			x	x[19]	x[19]	x[19]	X	x	x	x	x	x		X[7]	
Immunogenicity (e.g. HAMA/HACA)			x									x			
Cytokines			x	X	x	x	x	x	x	x	x	x			
PBMC 25cc (Lavender, EDTA)	x[27]		x	x[20]	x[20]	x[20]	x	x	x	x	x	x	X	x	
DNA (Q-PCR CTL019 persistence)			x	X	x	x	x	x	x	x	x	x	X	x	
DNA RCL (VSV-G Q-PCR)			x										x[3]	x[3]	
CTL019 Immune-phenotyping, CART-19 and B cell enumeration, functional assays			x				x	x	x	x	x	x	X	x	
Spectra-typing (Clonotyping deep sequencing)			x									x			

	Screening and Enrollment	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[2]	Quarterly Follow-Up[2]	Secondary Follow-up[26]
Visit Number	101	102	103	104	105	106	107	108	109	110	111	112	551-555	556-557	602+
	-12W to -1W	~ -1W	~ -1D	D1	D2 (+) 1d	D3 (+) 1d	D4 (+) 1d	D7 (+/-) 1d	D11 (+/-) 1d	D14 (+/-) 1d	D21 (+/-) 3d	D28 (+/-) 3d	M2 M3 M4 M5 M6 (+/-) 7d	M9 M12 (+/-) 14d	Every 6Months
Additional Archived Research Blood draw (100cc)												x			
Bone Marrow/LN aspirate[22] (5 cc lavender top, EDTA)			x									x[1]	x[1]	x[1]	
DNA (Q-PCR CTL019 homing)			x									x[1]	x[1]	x[1]	
CART-19 and B cell enumeration, immunophenotyping			x									x[1]	x[1]	x[1]	
Spectra-typing (Clonotyping deep sequencing)			x									x[1]			
Marrow Serum (2 cc red top)			x									x[1]			
Cytokines			x									x[1]			
Disease Monitoring															
Tumor response assessments												x[1]	x[1]	x[1]	
Physical exam (extramedullary disease)	x		x									x[1]	x[1]	x[1]	
Bone marrow aspirate/biopsy (cytogenetics/FISH if appropriate)	x[23]		x[14]									x[1]	x[1]	x[1]	
Lymph node biopsy[22]	x		x									x[1]	x[1]	x[1]	

	Screening and Enrollment	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[2]	Quarterly Follow-Up[2]	Secondary Follow-up[26]
Visit Number	101	102	103	104	105	106	107	108	109	110	111	112	551-555	556-557	602+
	-12W to -1W	~ -1W	~ -1D	D1	D2 (+) 1d	D3 (+) 1d	D4 (+) 1d	D7 (+/-) 1d	D11 (+/-) 1d	D14 (+/-) 1d	D21 (+/-) 3d	D28 (+/-) 3d	M2 M3 M4 M5 M6 (+/-) 7d	M9 M12 (+/-) 14d	Every 6Months
CSF evaluation[2]	x											x	As clinically indicated		
Mediastinal disease assessment (Chest x-ray → CT/MRI scan	x											x[16]	x[16]		
MRD by flow cytometry	x		x									x	x[1]	x[1]	
BCR-ABL (Ph+ patients only)	x											x	x[1]	x[1]	
Safety															
Adverse events				X	x	x	x	x	x	x	x	x	X	x	
ECHO/MUGA[21]	x														
Pulmonary Function Test (DLCO)/ Pulmonary Reserve[15]	x														
Lymphodepleting Chemotherapy[4]		x													
CART-19 cell infusion				X	x	x									
Prophylactic antibiotics				x[25]											
Relapse and Survival Follow-up															x[26]
Quality of Life (QoL) questionnaires (FACT-Leu and EORTC-QLQ C30)	x												x[9]		

	Screening and Enrollment	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[2]	Quarterly Follow-Up[2]	Secondary Follow-up[28]
Visit Number	101	102	103	104	105	106	107	108	109	110	111	112	551-555	556-557	602+
	-12W to -1W	~ -1W	~ -1D	D1	D2 (+) 1d	D3 (+) 1d	D4 (+) 1d	D7 (+/-) 1d	D11 (+/-) 1d	D14 (+/-) 1d	D21 (+/-) 3d	D28 (+/-) 3d	M2 M3 M4 M5 M6 (+/-) 7d	M9 M12 (+/-) 14d	Every 6Months
Total clinical blood draw (mL)	27.5	0	12.5	16	16	16	12.5	12.5	12.5	12.5	12.5	16.5	12	12	
Total research blood draw (mL)	25	0	30	35	35	35	30	30	30	30	30	130	30	30	
Total blood draw (mL)	52.5	0	42.5	51	51	51	42.5	42.5	42.5	42.5	42.5	146.5	42	42	
Total blood draw (Tbsp.; approximately)	3.5	0	3	3.5	3.5	3.5	3	3	3	3	3	10	3	3	

[1] Tumor response assessments will be performed at Day 28, Months 3, 6, 9 and 12 after CART-19 cell infusions (Refer to Section 6.14 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 collections are not to exceed 3 tablespoons of blood twice in one week time window. Marrow/LN collections would not exceed more than one procedure per month. This would be at the PI's discretion.

[3] Months 3, 6 and 12 only

[4] Lymphodepleting chemotherapy prior to CART-19 cell infusion is NOT required if WBC $\leq$ 1,000 /uL

[5] CD3+ lineage-specific chimerism preferred. Chimerism will be performed only in patients with prior allogeneic transplant at the Hospital of the University of Pennsylvania.

[6] Apheresis will be performed in order to obtain a target of $5x10^9$ PBMCs for CART-19 manufacturing (as required). Apheresis can occur anytime after informed consent is obtained, up to 4 weeks prior to CART-19 infusion. Cryopreserved historical apheresis products collected from the patient are usable for CART-19 manufacturing if collected at an appropriately certified apheresis center and the product meets adequate mononuclear cell yields.

[7] Month 12 only.

[8] As clinically indicated if HLH/MAS or CRS is suspected

[9] Months 3 and 6

[10] Months 3, 6, 9 and 12

[11] Pregnancy test (quantitative) for females only

[12] Vital signs will be taken within 10 minutes prior, within 10 minutes after each infusion, every 15 minutes for the first one hour and then every hour for the next two hours until these signs are satisfactory and stable.

[13] D-dimer required on Days 4 and 11. Additional testing to be performed if HLH/MAS or CRS is suspected.

[14] Bone marrow biopsy/aspirate to be performed within 48 hours prior to the first CART-19 T-cell infusion. The results of this baseline bone marrow are not required prior to infusion.

[15] DLCO $\geq$ 40%; Pulse Oxygen > 92% on room air

[16] CT/MRI will only be performed on Day 28 and Month 3 visits if baseline chest x-ray indicates mediastinal disease

[17] Direct bilirubin will be performed at screening only

[18] All patients must undergo a respiratory virus panel (RVP) to test for influenza within 10 days prior to the first planned CART-19 infusion. If the patient is positive for influenza, oseltamivir phosphate (Tamiflu®) or equivalent should be administered (see Tamiflu® package insert for dosing information). The patient must complete this course of preventative treatment prior to receiving the first CART-19 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 CART-19 infusion. If a patient is positive for another virus on the RVP, CART-19 infusions 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 infusions will be delayed until resolution of these symptoms.

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

[20] Research blood (25 cc lavender top) to be taken between 20-120 minutes post-infusion.

[21] ECHO/MUGA must be performed within 8 weeks prior to the first CART-19 infusion.

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

[23] If the results of a historical bone marrow biopsy (obtained at the time of the patient's last relapse) are available at the time of enrollment, this does not need to be repeated for enrollment. 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 first CART-19 infusion. If lymphodepleting chemotherapy is not administered, the pre-infusion bone marrow (to be performed within 48 hours prior to the first CART-19 infusion) is sufficient.

[24] Performed if clinically indicated.

[25] Prophylactic gram negative antibiotics must be initiated beginning on Day 1. The choice of antibiotic will be left to the physician-investigator's discretion and will be administered per institutional guidelines.

[26] For subjects who complete or prematurely discontinue from the Primary Follow-up Phase of the study while in remission, follow-up attempts will be made to assess the subject's relapse and survival status every 6 months post CART-19 infusion until the end of the study (Last Patient/Last Visit). Once subjects relapse they will be followed for survival until the end of the study (Last Patient/Last Visit) only.

[27] Research blood draw (~25cc) will be collected at the time of apheresis (if performed).

6.1. Screening and Enrollment Assessments

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 (**Table 6-1**).

- 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- including blinatumomab
- 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
- Screening ECHO/MUGA must be performed within 8 weeks prior to the first CART-19 infusion.
- Blood will be taken for Hematology, Coagulation, and Biochemistry analysis. Viral serologies (HIV, CMV, EBV, Hepatitis B/C). If the HCV antibody is positive, a screening HCV RNA by any RT-PCR or bDNA assay must be performed. Eligibility will be determined based on the screening value. The test is not required if documentation of a negative result of a HCV RNA test performed within 60 days prior to screening is provided.
- Serum pregnancy test for females of child bearing potential
- Autoimmune screening: antinuclear antibody (ANA) and Erythrocyte sedimentation rate (ESR)
- Donor chimerism assessment for those patients that have had previous allogeneic HSCT at the Hospital of the University of Pennsylvania
- Serum immunoglobulin levels
- BCR-ABL (Ph+ ALL patients only)
- Bone marrow and peripheral blood samples will be taken for research analysis as described in **Table 6-1**.
- Bone marrow aspirate and lymph node biopsy (if accessible) for disease and MRD assessment as described in **Table 6-1**. If the results of a historical bone marrow biopsy (obtained at the time of the patient's last relapse) are available at the time of enrollment, a bone marrow does not need to be repeated for enrollment.

- Chest x-ray for mediastinal disease assessment. If the chest x-ray reveals mediastinal widening, then a CT or MRI scan is required at baseline.
- CSF evaluation- If CSF evaluation indicates that they have CNS involvement, brain imaging by MRI or CT will be performed to further assess CNS leukemic involvement.
- Pulmonary function test (DLCO)
- Quality of life questionnaires (FACT-Leu version 4 and EORTC-QLQ C30)

In the event that the time between the baseline assessment and the infusion of CART-19 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 8 weeks prior to the first CART-19 infusion.

6.2. Apheresis and Test expansion

After the patient has been enrolled, patients will be scheduled for apheresis (leukapheresed) to obtain a target of $5x10^9$ PBMCs for CART-19 manufacturing. A PBMC sample from the apheresis product will be used for a test expansion to assess the suitability of the patient's T cells for CART-19 manufacturing. The test expansion results will be used for secondary objective analysis, but will not be a criterion for proceeding with CART-19 manufacturing. $1x10^8$ cells from the apheresis product will be delivered to TCSL by the CVPF.

As noted in **Table 6-1**, the apheresis can occur between 12 to 4 weeks prior to CART-19 infusion. Apheresis should be scheduled prior to any planned chemotherapy administration. After completion of apheresis, cells should be cryopreserved by standard temperature controlled procedure and then shipped to the CVPF. 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 CART-19 manufacturing if collected at an appropriately certified apheresis center and the product meets adequate mononuclear cell yields. If this is the case, the patient therefore would not have to repeat the baseline apheresis on this study and this historical sample will also be used to determine the feasibility for the T-cell test expansion assay. 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. Refer to section 6.5 for detailed procedures regarding apheresis and analysis.

6.3. Assessment Types

6.3.1. 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. For subjects enrolling into the retreatment cohort, demographic information and detailed medical history will not be re-collected. An interval medical history will be collected as appropriate per subject status.

6.3.2. Physical Exam

A complete physical examination will be performed by the investigator according to **Table 6-1**. Height will be measured in centimeters and recorded in the source documents. Weight will be measured in kilograms and recorded in the Vital Signs source documents.

Significant findings that are present prior to the start of study drug must be included on the Relevant Medical History/Current Medical Condition pages of the CRF. Significant findings made after the start of study drug which meet the definition of an Adverse Event must be recorded on the Adverse Event part in the CRF.

6.3.3. Vital Signs

Blood pressure, body temperature, oxygen saturation by pulse oximetry, and heart rate will be measured as indicated in the **Table 6-1** and will be recorded on source documents, and transcribed into the appropriate CRF pages. Blood pressure and pulse should be measured on patients after at least 3 minutes in the sitting position. Vital signs will be taken 10 minutes prior to and 10 minutes immediately after each infusion and then every 15 minutes for at least one hour and then every hour for the next two hours until these signs are satisfactory and stable. If the subject's vital signs are not satisfactory and stable three hours post-CART-19 infusion, vital signs will continue to be monitored at a minimum of every hour or as clinically indicated until stable.

If high fevers ($\geq$ 101.5° F / 38.6° C) occur in the days to weeks following the CART-19 infusions, additional assessments are required to more closely monitor the patient until resolution of the fever (below 101.5° F / 38.6° C). Please refer to Sections 6.3.2.1, 6.3.2.2 and 6.3.3 for details.

6.3.4. ECOG Performance status

At Visits according to **Table 6-1**, the ECOG performance scale index will be used to evaluate the performance status of the patients.

Table 6-2: 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

Grade	ECOG
5	Dead

6.3.5. Cardiac Assessment: ECHO/MUGA

An ECHO/MUGA test is required to be completed within 8 weeks prior to the first CART-19 infusion to confirm protocol eligibility. Any abnormalities that are clinically significant should be recorded on the Patient's Medical History CRF. Patients must have a LVEF ≥40% to be included into the study.

6.3.6. Local Laboratory Evaluations

Pre-entry, screening and other laboratory assessments will be performed accordingly to **Table 6-1**. Note: Additional assessments should be performed between visits as clinically required to follow AEs or CART-19 expected events. For all laboratory assessments that occur on Days 1, 2 and 3, these should be performed **prior** to CART-19 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 CART-19 will be noted. The Investigator may choose to repeat any abnormal result once, in order to rule out laboratory error. "NCS" will be entered on the original laboratory sheet of all laboratory values which are outside the reference range, but are judged "not clinically significant." The physician making these assessments shall date and initial each form. Further details on recording abnormal laboratory values as AEs are described in Section 8.1.

Table 6-3 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
Autoimmune Screen	Antinuclear antibody (ANA) and Erythrocyte sedimentation rate (ESR)

Test Category	Test Name
Serology	Viral Serology (CMV, EBV), HCV antibody, HCV RNA-PCR (if applicable), HbsAg
RVP	Respiratory Virus Panel: Includes 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

6.3.6.1. Hematology, Coagulation and T-cell Subsets

Hematology & Coagulation safety assessments will be performed at screening, pre-infusion (Day-1), prior to the CART-19 infusions on Days 1, 2 and 3, and at each study visit according to **Table 6-1**. 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 CART-19 infusion on Day 1 and again on Day 28, Month 2, 3, 4, 5 and 6.

Additional assessment of D-dimer for CRS

As noted, side effects following CART-19 cell infusions can induce high fevers and should be expected. If high fevers (≥ 101.5° F / 38.6° C) occur following CART-19 infusion, every attempt will be made to monitor D-dimer levels **daily** at fever onset and until resolution of the fever (below 101.5° F / 38.6° C).

6.3.6.2. Chemistry

Biochemical safety assessments will be performed at screening, pre-infusion (Day-1), prior to the CART-19 infusions on Days 1, 2 and 3, and at each study visit according to Table 6-1. 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.

Additional assessment of Ferritin, LDH and CRP levels for CRS

As noted, side effects following CART-19 cell infusions can induce high fevers and should be expected. If high fevers (≥ 101.5° F / 38.6° C) occur following CART-19 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 **Table 6-1** 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 (Day -1) and should be monitored per **Table 6-1** or as clinically indicated if HLH/MAS is suspected.

6.3.6.3. Viral Serology

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

6.3.6.4. Serum Immunoglobulin Levels

Peripheral blood will be sampled at screening and accordingly to **Table 6-1**, for analysis of serum immunoglobulin.

6.3.6.5. Donor chimerism

Due to the possibility of some degree of residual donor engraftment, which will include T cells of donor origin, patients that received a previous allogeneic HSCT transplant at the Hospital of the University of Pennsylvania will be assessed for donor chimerism at screening and then at Day 28, Month 3, 6, 9 and 12. This assessment will be used to identify risk for potential GVHD. Whole blood and CD3+ lineage-specific chimerism is preferred.

6.3.6.6. 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 **Table 6-1** at screening. During treatment, an additional test will be performed prior (within 48 hours) to the first CART-19 infusion and again at the end of study visit (Month 12). Additionally, if menses is delayed for more than 7 days, the patient should be instructed to conduct a urine pregnancy to rule out any possibility of pregnancy. 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 CART-19 T-cell infusion, patients must be withdrawn from the study.

6.3.7. Research Assessments to Assess Engraftment, Persistence and Bioactivity

The following assessments will be collected according to **Tables 6-1 and 6-2** and will be analyzed as described below.

- Immunogenicity: Human Anti-Murine Antibody (HAMA) and Human Anti-CAR Antibody (HACA)

- Serum Cytokines
- DNA Q-PCR CTL019 persistence
- DNA RCL (VSV-G Q-PCR)
- CTL019 Immune phenotyping (flow cytometry)
- Spectra-typing (clonotyping and MRD by deep sequencing)

For molecular studies (Q-PCR and Q-RT-PCR), 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. Samples will be delivered, processed, and frozen as per SOP to the Translational and Correlative Studies Laboratory (TCSL) (University of Pennsylvania). Samples will be stored in the TCSL at the University of Pennsylvania 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
University of Pennsylvania Perelman School of Medicine
Translational Research Center, 9-188
3400 Civic Center Boulevard, Building 421
Philadelphia, PA 19104-5157

Additional assessment of Serum Cytokines and CART-19 levels

As noted, side effects following CART-19 cell infusions can induce high fevers and should be expected. If high fevers (≥ 101.5° F / 38.6° C) occur following CART-19 infusion until Month 2 visit, additional blood draws to assess Serum Cytokine and CART-19 levels are required to be monitored on the onset of fevers and **every 3-4 days** until resolution of the fever (below 101.5° F / 38.6° C).

An additional larger blood draw (~100cc) on Day 28 will be drawn and archived for future research purposes. This blood sample will also be sent to TCSL as indicated above.

6.3.8. Cytogenetics/FISH

Cytogenetics/FISH assessments will be performed at each time point a bone marrow aspirate/biopsy is sampled (**per Table 6-1**).

6.3.9. Quality of Life (QoL)

Quality of life questionnaires will be administered at screening and again at Months 3 and 6. If the patient discontinues the study prior to Month 3 (or after Month 3 but before Month 6), all attempts should be made to obtain a final QOL questionnaire prior to patient discontinuing the study. The FACT-Leu (Version 4) [98] and EORTC-QLQ C30[99] questionnaires will be used to assess the patient's health as well as physical, social/family, emotional and functional well-being. If site staff is administering to the patient, the questions should be described to elicit patient experiences, opinions and observations on how their disease is affecting them.

6.4. Patient Enrollment

To enroll a patient on this study, provide the documents listed below to:

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

Documents required:

1. Complete Enrollment Form (including patient past medical history, laboratory, radiological reports, physical exam, concomitant medications and any other source documentation to support patient meets eligibility criteria and has completed all required screening assessments)
2. Copy of signed patient consent and HIPAA form

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

Each patient is identified in the study by a Subject No. that is assigned when the subject is first enrolled for pre-screening and is retained as the primary identifier for the subject throughout his/her entire participation in the trial. The Subject No. consists of the Center Number (Center No.) (as assigned by Sponsor to the investigative site) with a sequential subject number suffixed to it, so that each subject is numbered uniquely across the entire database. After a subject has passed all screening procedures and is ready to be enrolled, the subject is enrolled using the same Subject No. provided at pre-screening. Once assigned, the Subject No. must not be reused for any other subject and the Subject No. 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 Screening Disposition page.

6.5. Apheresis Visit Procedure

For patients that do not have a historical apheresis available, a 4-6 blood volume apheresis procedure will be carried out at the apheresis center during the screening procedures. PBMC are obtained for CART-19 during this procedure. From a single leukapheresis, the intention is to harvest at least 5×10^9 white blood cells to manufacture CART-19 T cells. Baseline blood leukocytes for FDA requirements and for research are also obtained and cryopreserved. After the PBMC is sent for CART-19 manufacturing, the cell product is expected to be ready for release approximately 4 weeks later.

As mentioned in Section 6.1.3, for those patients that do have a historical apheresis available, this product may be able to be used. If this is the case, the patient therefore would not have to repeat the baseline apheresis on this study.

6.6. Cytoreductive chemotherapy

Prior to CART-19 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 CART-19 cell infusion is **NOT needed if the patients WBC $\leq$ 1,000 /uL. Additionally, if the delayed period from chemotherapy to CART-19 infusion is 4 or more weeks, the patient will need to be re-treated with lymphodepleting chemotherapy prior to CART-19 infusion.

For B-cell ALL patients, the cytoreduction/conditioning regimen administered prior to CART-19 will be at the discretion of the physician. The suggested regimens are as follows:

a. Clofarabine 40 mg/m^2/d x 5 d
b. High dose methotrexate 3 gm/m^2
c. AraC 1.5-3 gm/m^2 q12 hr x 6-12 doses
d. Methotrexate 1mg/m^2 with AraC 1-3gm/m^2 x 4 doses
e. CVAD (cytoxan, vincristine, adriamycin, decadron)
f. Cyclophosphamide 1.5-3gm/m^2 over 1-3 days
g. ICE (ifosphamide, carboplatin, etoposide)
h. Daunorubicin/AraC
i. Fludarabine (30mg/m^2/d x 4 days) and Cyclophosphamide (500mg/m^2/d x 2 days)

The chemotherapy will be planned so that the last dose is completed 1-4 days BEFORE the first planned infusion of CART-19 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 CART-19 cells. In addition, chemotherapy can potentiate the ability of T cells to kill tumor cells[116, 117]. The chemotherapy is not investigational and may be given by a patient's local oncologist within the specified time frame.

All patients must undergo a respiratory virus panel (RVP) to test for influenza within 10 days prior to the first planned CART-19 infusion. If the patient is positive for influenza, oseltamivir phosphate (Tamiflu®) or equivalent should be administered (see Tamiflu® package insert for dosing information). The patient must complete this course of treatment prior to receiving the first CART-19 infusion. If the patient is positive for influenza and is also experiencing flu-like symptoms, all clinical symptoms must be resolved prior to the first CART-19 infusion. If the patient is positive for another test on the RVP, the CART-19 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.

6.7. CART-19 Infusion

The first CART-19 infusion will begin 1 to 4 days after completion of chemotherapy as indicated in Section 6.6. On Days 1, 2 and 3, prior to each infusion, patients will have a CBC with

differential, and assessment of CD3, CD4 and CD8 counts since chemotherapy is given in part to induce lymphopenia.

The dose will be administered as a split infusion of 1 to 5 x 10^8 total CART-19 transduced cells: 10% on Day 1, 30% on Day 2, 60% on Day 3. Patients will be infused and premedicated as described in Section 5.4.

A blood sample for determination of a baseline CART-19 level is obtained prior to each infusion and 20 minutes to 2 hours post each infusion (and sent to TCSL) per Table 6-1.

Patients experiencing toxicities from their preceding cytoreductive chemotherapy will have their infusion delayed until the following toxicities have resolved:

1) Pulmonary: Requirement for supplemental oxygen to keep saturation greater than 92% or presence of radiographic abnormalities on chest x-ray that are progressive
2) Cardiac: New cardiac arrhythmia not controlled with medical management.
3) Hypotension requiring pressor support.
4) Active Infection: Positive blood cultures for bacteria, fungus, or virus within 48 hours of T cell infusion.

Prophylactic gram negative antibiotics must be initiated beginning on Day 1. The choice of antibiotic will be left to the physician investigator's discretion and will be administered per institutional guidelines.

6.8. Day 28: Follow Up

At the Day 28 visit, patients will undergo the following: physical exam, documentation of adverse events and blood draws for hematology, chemistry, engraftment and persistence of CART-19 cells and research labs. In addition, restaging is done in order to provide tumor burden measurements. Restaging testing is determined by the patients' baseline disease assessment and may include imaging, MRD assessments, CSF assessments, bone marrow aspirate and biopsy and/or optional lymph node biopsy as necessary. Tumor response assessments will be done according to National Comprehensive Cancer Network (NCCN) v1 2013 guidelines (Sections 6.14 and 6.15)

6.9. Monthly Evaluations 2 to 6 Months Post Infusion

Patients will return to the clinic on a monthly basis during months 3 to 6 post CART-19 cell infusion. At these study visits, patients will undergo the following: concomitant medication, physical exam, documentation of adverse events and blood draws for hematology, chemistry, engraftment and persistence of CART-19 cells and research labs. Tumor response will be measured accordingly to Sections 6.14 and 6.15 at Months 3 and Month 6.

6.10. Quarterly Evaluations for up to 1 Year Post Infusion

Patients will be evaluated on a quarterly basis until 1 year post infusion. At these study visits, patients will undergo the following: concomitant medication, physical exam, documentation of adverse events and blood draws for hematology, chemistry, engraftment and persistence of CART-19 cells and research labs. The DNA RCL (VSV-G Q-PCR) assay will be performed at 3, 6 and

12 months post CART-19 cell infusion to exclude the presence of detectable RCL. Tumor response will be measured accordingly to Sections 6.14 and 6.15 at Months 9 and 12.

6.11. Secondary Follow-up

For subjects who complete or prematurely discontinue from the Primary Follow-up Phase of the study while in remission, follow-up attempts will be made to assess the subject's relapse and survival status every 6 months post CART-19 infusion until the end of the study (Last Patient/Last Visit). Once subjects relapse they will be followed for survival until the end of study (Last Patient/Last Visit) only. Last Patient/Last Visit (LPLV) is defined as the last primary follow-up visit of the last subject remaining in in primary follow-up.

6.12. Long-term Follow-up Protocol

After subjects' complete or prematurely discontinue participation in the Primary Follow-up Phase of the study, subjects will be asked to participate in a separate 15 year long-term follow-up destination protocol.

6.13. Re-treatment Cohort Procedures

6.13.1. Eligibility for Retreatment

Up to one retreatment dose (split administration) will be allowed per eligible subject.

1. Subjects previously infused with murine CART19 cells as part of this protocol and who have relapsed with CD19+ disease.
2. Subjects have undergone the 28 day efficacy endpoint evaluation.
3. Subjects have <5% CART19 cells in the CD3+ population by flow cytometry on PBMCs
4. Subjects have recovered from any toxicity attributed to the initial CART-19 infusion, such as CRS.

In addition, subjects must also meet the following:

Retreatment Inclusion Criteria

1. Performance Status 0-1
2. Adequate organ system function including:
    - Creatinine < 1.6 mg/dl
    - ALT/AST < 3x upper limit of normal
    - Total Bilirubin < 2.0 mg/dl
    - Must have a minimum level of pulmonary reserve defined as ≤ Grade 1 dyspnea, pulse oxygen > 92% on room air, and DLCO ≥ 40% (corrected for anemia if clinically appropriate)
3. Left Ventricular Ejection Fraction ≥ 40%
4. No contraindications for leukapheresis (if required for retreatment)
5. Gives voluntary informed consent for retreatment

Retreatment Exclusion Criteria

1. Pregnant or lactating women.
2. Active hepatitis B or hepatitis C

3. Concurrent use of systemic steroids or chronic use of immunosuppressant medications. Recent or current use of inhaled steroids is not exclusionary. For additional details regarding use of steroid or immunosuppressant medications, please see Section 5.6.
4. HIV infection
5. Patients with active CNS involvement with malignancy. Patients with prior CNS disease that has been effectively treated will be eligible providing treatment was >4 weeks before enrollment on the retreatment cohort.
6. Class III/IV cardiovascular disability according to the New York Heart Association Classification (see Appendix 1).
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.
8. Active acute or chronic graft-versus-host disease (GVHD) or requirement of immunosuppressant medications for GVHD within 4 weeks of enrollment.

6.13.2. Retreatment Procedures

Subjects eligible for retreatment who consent to participate in this cohort will be enrolled on the retreatment cohort. To enroll a subject on the retreatment cohort, please provide the documents listed below to:

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

Documents required:

- Copy of signed retreatment consent
- Completed Retreatment Cohort Enrollment Form- including documentation of retreatment consent, current physical examination, laboratory and radiological reports, current/past medical history and concomitant medications, and any other documentation to support the subjects' enrollment onto the retreatment cohort and has completed all required assessments.

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

The same Subject Number previously assigned will be used to identify participants. Enrollment on the retreatment cohort will be tracked by adding "r" to the end of this existing subject identification number (i.e. 1000-00001R), however the same Subject Number previously assigned will be used in the clinical database.

All prerequisites for eligibility to receive CART-19 outlined in Section 5.2 must be met prior to retreatment. The study drug will be prepared and administered per the guidelines set forth in Section 5. All required study procedures, pre-medications, prophylaxis, and monitoring guidelines

from the initial infusion outlined in Section 6 will apply for this retreatment cohort. Subjects will also be followed per the Retreatment Cohort Visit Evaluation Schedule in Table 6-2.

6.14. Efficacy Assessments

Efficacy assessments will be performed according to the Guidelines for efficacy evaluation in Acute Lymphoblastic Leukemia studies. This document is based on the standardized response criteria defined by National Comprehensive Cancer Network (NCCN Guidelines (NCCN, 2013 v1) and further supported by the workshop report on acute leukemia from American Society of Hematology (ASH) (Appelbaum et al 2007) and the International Working Group (IWG) guideline for acute myeloid leukemia (AML) (Cheson et al 2003).

Tumor response assessments will be done at baseline (prior to CART-19 infusion) and then at Day 28 and Months 3, 6, 9 and 12 after CART-19 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 6-4**.

Table 6-4: Disease Assessment Collection Plan

Procedure	Screening/Pre-infusion	Post Infusion Assessments
Bone marrow aspirate and biopsy	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	If accessible	Optional if accessible/necessary at Day 28, Months 3, 6, 9 and 12
CSF Assessment for CNS disease	Mandated	Performed 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	Mandated	As clinically indicated
Chest CT/MRI scan for mediastinal disease	If the screening chest x-ray suggests mediastinal enlargement	Mandated only if abnormal at or before screening: Day 28 and Month 3

Procedure	Screening/Pre-infusion	Post Infusion Assessments
Evaluation for extramedullary disease	Mandated	Mandated Day 28, Months 3, 6, 9 and 12 (See Section 6.14.1 for additional information)
MRD assessment of bone marrow by flow cytometry (every patient)	Mandated	Mandated Day 28, Months 3, 6, 9 and 12
BCR-ABL Q-PCR of blood and bone marrow aspirate for patients with Ph+ ALL	Mandated	Mandated Day 28, Months 3, 6, 9 and 12
Transfusion dates	Assess dependency	Record as needed during the course of the trial

6.14.1. Evaluation for extramedullary disease

An evaluation will be performed to assess evidence of extramedullary disease at the timepoints identified in Table 6-4 above. The scope of assessments performed as part of this evaluation is based on the results of the pre-infusion assessment and the physician's clinical discretion. This may include assessments of the liver, spleen, lymph nodes, skin, gum infiltration, testicular involvement, and other sites as applicable.

6.14.2. Bone Marrow Aspirate/Biopsy and Peripheral Blood

Bone marrow biopsies and aspirate will be measured for tumor evaluations and efficacy analysis per Table 6-5.

6.14.3. Cerebrospinal Fluid (CSF) Assessment

CSF will be assessed at screening. Subsequent CSF assessments after CART-19 infusion will be performed as clinically indicated (i.e. when new neurological symptoms are present). CSF will be analyzed for cell count and differential, cytology, and for the presence of CART-19 cells.

6.14.4. 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 6-4**).

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).

6.14.5. Minimal Residual Disease (MRD)

All patients will have multiparameter flow cytometry on bone marrow aspirate for MRD status at each time point a bone marrow aspirate is performed (per Table 6-1).

6.14.6. Quantitative BCR-ABL: Ph+ ALL Patients

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

6.14.7. 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[100]. 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.

6.15. ALL Response Criteria

The response criteria will be evaluated accordingly to the Guidelines for efficacy evaluation in Acute Lymphoblastic Leukemia studies. This document provides the working definitions, specifications, components and timing of overall disease response evaluation for a consistent and efficient analysis of efficacy assessing antineoplastic activity in ALL. 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)[101] and the International Working Group (IWG) guideline for acute myeloid leukemia (AML)[102]. 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 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 6-5**.

Table 6-5: 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* **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 of 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 (i.e. CRu or PR) is present (See Note Below):
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 be assessed as relapse with the relapsed component alone.

* Blast percentage assessment is based on a bone marrow aspirate differential count. Occasionally, a precise aspirate differential count is precluded by hemodilution or other technical factors. Such cases may still be scored as compatible with complete remission (CR) if no other morphologic or ancillary (e.g flow cytometry, molecular, cytogenetic) evidence of residual/recurrent disease is identified by the pathologist.

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 (CRu or PR), 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) guideline[102]. The difference between PD and "No response" in ALL is not believed to be clinical meaningful.

6.15.1. Confirmation of Response

For analysis purposes, a patient will be considered to have achieved CR only if the patient satisfies all criteria for CR at two consecutive assessments that are at least 4 weeks apart. The date of CR will then be derived as the earlier of the two assessment dates.

Overall disease response evaluation, including blood, bone marrow, extramedullary disease (per Section 6.14.1), CT/MRI (if needed), must be performed at Day 28, and then at Month 2 to 3 to confirm response status assessed at Day 28. This response confirmation cannot be performed earlier than 4 weeks following the Day 28 assessment. Therefore, if necessary the Month 3 response evaluation may be performed earlier, but must be more than 28 days after the last component of the Day 28 evaluation. CSF will be performed at baseline and at post-infusion time points as clinically appropriate.

The same principle applies when the initial disease response occurs beyond Day 28 (e.g. Month 3, Month 6, etc.). A confirmation is required more than 28 days later than last component of the initial response evaluation.

If a patient satisfied CRi at one assessment and later confirmed as a CR in the next assessment more than 4 weeks apart, the patient will be considered as having confirmed CR. However, the date of CR will be derived as the latter (confirmed) assessment dates.

7. STATISTICAL PLAN

7.1. Design Overview

This is a single center, single arm, open-label phase II study to determine the efficacy and safety of autologous T cells expressing CD19 chimeric antigen receptors expressing tandem TCRζ and 4-1BB (TCRζ/4-1BB) co-stimulatory domains (referred to as "CART-19" cells) in adult patients with relapsed or refractory B-cell acute lymphoblastic leukemia.

7.2. Sample Size Justification

The study will target a total of 30 primary efficacy evaluable relapsed/refractory ALL patients treated with CART-19 cells in the study. The sample size was increased to 30 for amendment 11 which will yield more experience, a total of 15 subjects, with the targeted fractionated 1-5x10^8 dose.

The primary objective in this study is to determine overall complete remission rate (ORR) which includes complete remission (CR) and CR with incomplete blood count recovery (CRi) at Day 28.

In a recent study of vincristine sulfate liposome injection in adult ALL patients in second or greater relapse or whose disease has progressed following two or more anti-leukemia therapies, the observed ORR was 15.4% (95% CI [7.6, 24.5]) (Marqibo® US label). The half-width of the 95% exact confidence interval for ORR will be no larger than 19% for a cohort of size 30 and no larger than 26% for a cohort of size 15.

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 **Efficacy Evaluable Set** comprises all patients who receive the CART-19 cells at the intended dose range and completed the response assessments for the primary efficacy endpoint as planned by the protocol. Efficacy evaluable patients also include those with disease progression or death prior to the primary efficacy endpoint response assessment. These are the primary efficacy evaluable patients as defined below. The Efficacy Evaluable Set will be used for the primary efficacy endpoint analysis.
- The **Full Analysis Set (FAS)** comprises all patients who received the CART-19 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:

- **Screening failure** - Any patient who fails to meet the inclusion/exclusion criteria specified by the protocol.
- **Manufacturing failure** – Any patient who has manufactured CART-19 cells that do not meet the manufacturing release criteria.
- **Primary efficacy evaluable patient** – Any patient who is infused with at least 1-5x10^7 CART-19 cells and completed the response assessments for the primary efficacy endpoint as planned by the protocol. Efficacy evaluable patients also include those with disease progression or death prior to the primary efficacy endpoint response assessment.
- **Primary efficacy non-evaluable patient** – Any patient who is infused with the CART-19 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 the overall complete remission rate (ORR) at Day 28. The overall complete remission rate is computed as the proportion of patients with CR or CRi according to the response criterion described in Section 6.15. Analyses of subgroups by dose-level will also be considered.

The two-sided exact Clopper-Pearson 95% confidence intervals for Day 28 ORR will be computed. The study would indicate meaningful efficacy if the lower bound of the 2-sided 95% exact confidence interval is greater than 15%, so that the null hypothesis that the ORR is less than or equal to 15% can be rejected.

7.5. Analysis of Secondary Efficacy Objectives

For the secondary efficacy objectives for this study, the proportion of patients 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 patients achieving CR or CRi before or at Month 6 (prior to receiving other anticancer therapy if any), and the proportion of patients with a minimal residual disease (MRD) negative bone marrow as determined according to Section 6.14 will also be computed. Two-sided exact Clopper-Pearson 95% confidence intervals for the proportions will be provided. A subset analysis of specific dosing subgroups (e.g. the fractionated 1-5x10^8 dose group) may also be considered for secondary endpoints.

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. 95% 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 95% confidence intervals will be presented if appropriated. **Overall survival (OS)** is defined as the time from the date of CART-19 infusion to the date of death due to any reason. In case a patient 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 patients with the best overall response of CR or CRi.

In case a patient 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 patients 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 CART-19 cells in the patient thus outcomes after HSCT cannot be solely attributed to the CART-19 treatment. If a substantial number of patients choose to receive HSCT while in CR or CRi, sensitivity analysis will be performed in which patients 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 CART-19 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 which 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 patients with the best overall response of CR or CRi.

In case a patient 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 CART-19 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 patient 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 Secondary Safety objectives

For all safety analyses, the Full Analysis Set (FAS) will be used. All adverse events (AE) will be collected in accordance with section 8.1, starting at the time of CART-19 infusion, including, but not limited to, cytokine release syndrome (CRS) and macrophage activation syndrome (MAS) will be listed. Incidence and severity of AEs will be summarized and tabulated by system organ class, preferred term and maximum toxicity grade (based on CTCAE v4.03). Exact confidence interval for proportions will be computed if appropriate. Changes in laboratory values and vital signs from time of infusion will be summarized descriptively for each scheduled and unscheduled time points.

Laboratory values outside normal limits will be identified in data listing and will include flags for high and low values. A patient with multiple CTC grades for an AE will be summarized under the maximum CTC grade recorded for the event. The frequency of CTC grade 3 and 4 AEs will be summarized separately. In addition, AEs related to study drug will be presented by primary system organ class, preferred term, and maximum CTC grade. Serious adverse events (SAEs) will be summarized separately. A subset analysis of specific dosing subgroups (e.g. the fractionated 1-5x10^8 dose group) may also be considered for safety endpoints.

7.7. 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 CART-19 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., CART-19 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. The proportion of patients that meet successful test expansion criteria will be computed. 95% confidence interval appropriate for each statistic will be used.

7.8. Retreatment Cohort Analysis

Safety and efficacy endpoints for those subjects participating in the retreatment cohort will include frequency and severity of adverse events, overall response, time to response, duration of response and time to alternative therapy, expansion and persistence of CART-19 cells. Definition and statistical methods used for those endpoints will be the same as described in Sections 7.4 and 7.5 but with the start of the observation time as time of the re-infusion. Data analyses for the retreatment cohort will be exploratory and performed separately from the analyses for the initial infused subjects. In particular, we will use statistical methods appropriate for paired data (e.g., paired t-test, McNemar test) to compare endpoints from the same subject between the first and re-infusion. Results from these analyses will be used to generate hypotheses for future studies.

7.9. Monitoring of Safety

On July 14, 2014 and November 20, 2014, the CART-19 research team met with the FDA to discuss the study stopping/pausing rules. At that time, they advised us to treat 3 additional subjects at the reduced dose level of 1-5x10^7 CART-19 T-cells and to stagger these infusions by at least 7 days to evaluate safety. On May 5, 2015, after the 3rd subject reached Day +14, the research team met with the FDA to review the outcomes/safety of these subsequent 3 subjects. The FDA agreed with the proposal to dose escalate to 1-5x10^8 CART-19 T-cells administered via split dosing, and to treat 6 additional subjects staggered at least 7 days apart to evaluate safety.

After the 6th subject infused with 1-5x10^8 CART-19 T-cells administered via split dosing reached Day +14, the CART-19 research team met with the FDA to review the safety of these subjects and

clinical outcomes. As the safety of the CART-19 T-cells had been established at this dose level, the FDA agreed with the proposal to continue to enroll subjects per protocol.

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
- 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 (in the absence of an investigator brochure). 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

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, adverse events are reported starting on Day 1 (from the start of the first CART-19 infusion) until the subject discontinues primary follow-up or until the 12 month study follow-up visit. Patients experiencing toxicity from their preceding cytoreductive chemotherapy will have their schedule delayed until these toxicities have resolved.

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

Preexisting Condition/General Physical Examination Findings

A preexisting condition is one that is present at the start of the 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. A Grade 3 or 4 event

(severe) as per CTCAE does not automatically indicate a SAE unless it meets the definition of serious as defined below 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. Subjects 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 far as possible, each adverse event should be evaluated to determine:

1. The severity grade (CTCAE v4.03 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 or protocol (in the absence of an investigator brochure)].
5. Action taken with respect to study or investigational treatment (none, dose adjusted, temporarily interrupted, permanently discontinued, unknown, not applicable)
6. Whether medication or therapy taken (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
7. Whether it is serious, where a serious adverse event (SAE) is defined as in Section 8.1.

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

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.

Grading System of Cytokine Release Syndrome (CRS)

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 for additional detail on CRS in CAR T-cell therapy.

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 100.4^0 F/38^0 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 with associated neutropenia.	More severe reaction: Hospitalization required for management of symptoms related to organ dysfunction including grade 4 LFTs or grade 3 creatinine related to CRS and not attributable to any other conditions. This excludes management of fever or myalgias. Includes hypotension treated with IVFs* or low-dose pressors, coagulopathy requiring fresh frozen plasma (FFP) or cryoprecipitate, and hypoxia requiring supplemental oxygen (nasal cannula oxygen, high flow oxygen, Continuous Positive Airway Pressure [CPAP] or Bilateral Positive Airway Pressure [BiPAP]. Patients admitted for management of suspected infection due to fevers and/or neutropenia may have grade 2 CRS.	Life-threatening complications such as hypotension requiring high dose pressors (See Table 8-2), or hypoxia requiring mechanical ventilation	Death

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

Table 8-2 High Dose Vasopressor Use

Definition of "High-Dose" Vasopressors	
Vasopressor	Dose for ≥ 3 hours
Norepinephrine monotherapy	≥ 0.2 mcg/kg/min 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 10 mcg/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[106].

Pregnancies

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

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.

8.3. Reporting of Serious Adverse Events

Every SAE, **regardless of suspected causality**, occurring during the adverse event reporting period defined in Section 8.1 above must be reported to the sponsor within 24 hours of learning of its occurrence. The original SAE notification may take place by email to meet the 24-hour reporting window. However, within 3 business 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: Sponsor Clinical Safety Manager or designee
Center for Cellular Immunotherapies (CCI)
[redacted]

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

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

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 10 working 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 is deemed probably or definitely related to the investigational product or a 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 72 hours, when the death is unforeseen (unexpected) and indicates participants or others are at increased risk of harm (including death of subjects off-study).

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.

Follow-up report
If an SAE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) should be submitted to the IRB. The investigator is responsible for ensuring that all SAEs are followed until either resolved or stable.

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 reported to the ACC DSMC through 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 the 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 24 business 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 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

Any study event occurring after retreatment with either humanized or murine CART19 T-cells that is:

- *Unexpected* fatal or life-threatening *suspected adverse reaction.*
- Expected and unexpected Grade 3 or higher events of cytokine release syndrome per the modified CRS grading scale in Table 8-1
- All grade 4 neurologic events
- All fatal events occurring within 30 days of T-cell infusion, regardless of attribution and expectedness
- Any adverse event requiring the study to be paused and/or stopped

- ***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 patients 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 or investigator brochure.

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 as in the IND Annual Report

8.4. 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 CART-19 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, the DSMB, Medical Monitor, 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.

8.4.1. Criteria for stopping or pausing 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 CART-19 infusion:
  - life-threatening (grade 4) toxicity attributable to protocol therapy 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.

If the study is paused for the reasons above, the PI, members of the study team and Protocol Advisor will meet in person or by teleconference within 24 hours of the event to have a thorough discussion of the event. These types of events will not be vetted via e-mail. The Regulatory Sponsor or designee should be present, but should not be involved in any discussions related to attribution. Meeting minutes capturing the review of any ongoing investigations, including next steps in the management of subjects and any proposed changes to the protocol will be forwarded to the FDA, IRB, ACC DSMC, Medical Monitor and DSMB. If all parties are in agreement as to the event resolution and any proposed modifications, 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 CART-19 cells).

If the study is paused for manufacturing reasons, the PI, members of the study team, Protocol Advisor, Clinical Operations and Cell Manufacturing will meet to identify 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.

8.4.2. General Toxicity Management Considerations

Replication-competent lentivirus (RCL) may be generated during the CART-19 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. The development of RCL could pose a risk to both the patient and their close contact(s), and therefore, monitoring for RCL will be conducted during the course of the trial.

Regulatory agencies and the gene therapy community have previously discussed measures to be taken should an RCL be confirmed in a patient. However, because the probability of developing, and characteristics of, an RCL are unknown, no guidelines have been put in place.

Nevertheless, all agree that the patient must be isolated until an understanding of how to manage the patient becomes clear. Some considerations are

- Intensive follow-up of patient in consultation with gene therapy experts, study investigators, FDA and NIH.
- Inform local public health officials and CDC.
- Identify sexual partners and provide appropriate counseling and intervention.

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 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 RCR/L testing for detection and/or characterization of the RCRL.

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[118]. To date, clinically evident insertional mutagenesis has not been reported following adoptive of engineered T cells. Lentiviral vectors may have a lower risk than oncoretroviral vectors based on several considerations[119]. Monitoring for T cell clonal outgrowth will be performed by flow cytometric analysis for CAR-expressing cells, and by CBC count. If the number of chimeric immune receptor cells continues to increase after 6 weeks, a Vβ repertoire analysis will be performed to evaluate clonality, or if the CBC analysis reveals abnormal T cell counts, then the Vβ analysis will be performed earlier. If a patient's Vβ repertoire is found to be monoclonal or oligoclonal, the patient's T cells will be evaluated for the pattern of vector insertion. If the pattern of insertion is found to favor a single dominant insertion site pattern the clinical trial will be placed on hold for dosing to allow evaluation of the patient in consultation with gene therapy experts, study investigators, DSMB, FDA and NIH. Further evaluation of the patient will comprise confirmation of the persistence of the clonality within a 3 month period, and monitoring of the patient for hematologic malignancies. Optionally, a sequence analysis of the dominant insertion site(s) will be performed, in order to locate any association of the insertion sites with known human oncogenes.

Uncontrolled T cell proliferation: CART-19 cells could proliferate without control of normal homeostatic mechanisms. In pre-clinical studies, CART-19 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[75, 103]. If any patient develops excessive CART-19 cell accumulation, corticosteroids will be administered to eradicate the infused cells.

Toxicity associated with allogeneic or autologous T cell infusions has been managed with a course of pharmacologic immunosuppression. T body associated toxicity has been reported to respond to systemic corticosteroids[104]. If uncontrolled T cell proliferation occurs (grade 3 or 4 toxicity related to CART-19 cells), patients may be treated with corticosteroids. Patients will be treated with pulse methylprednisolone (2 mg/kg i.v. divided q8 hr x 2 days), followed by a rapid taper.

B cell depletion: It is possible that B cell depletion and hypogammaglobulinemia will occur. This is common with anti-CD20 directed therapies[105]. 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 diphenhydramine hydrochloride may be repeated every 6 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. 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 CART-19 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 CART-19 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):
CRS has been observed in patients after treatment with CART-19. Patients with clinical responses exhibited some level of CRS that ranged from mild to severe consisting of fevers, hypotension, capillary leak, hypoxia or other symptoms (See Section 1.5.2 and 8.2). All patients who have responded to CART-19 cells have experienced a CRS.

Cytokine production is required for the activation, expansion and cytolytic function of T cells and for CART-19 T cells. Therefore, some degree of CRS may be a desired clinical outcome. Premature or early intervention with anti-cytokine therapy may therefore abrogate the anti-tumor efficacy of CART-19. Subsequent to this experience, selective tocilizumab (an anti-IL6-receptor antibody) therapy has been utilized (described below) with effective toxicity management and successful ongoing CART-19 T cell expansion in patients. Please note, steroids or other immunosuppressant drugs should **NOT** be used as pre-medication for CART-19 therapy but may be considered in the management of CRS.

The moderate to severe cases of CRS observed required intervention with tocilizumab, with or without high dose corticosteroids, between 2 and 9 days after T cell infusion. This resulted in rapid reversal of the high persistent fevers associated with CRS in most but not all patients.

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 CART-19 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 Principal Investigator and/or expert consultants for the trial. 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 CART-19 function and efficacy, if used, they should be rapidly tapered.

Upon developing the prodrome of high-persistent fevers following CART-19 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 O2, and/or need for mechanical ventilation.
- Any other signs or symptoms of rapid deterioration despite medical management

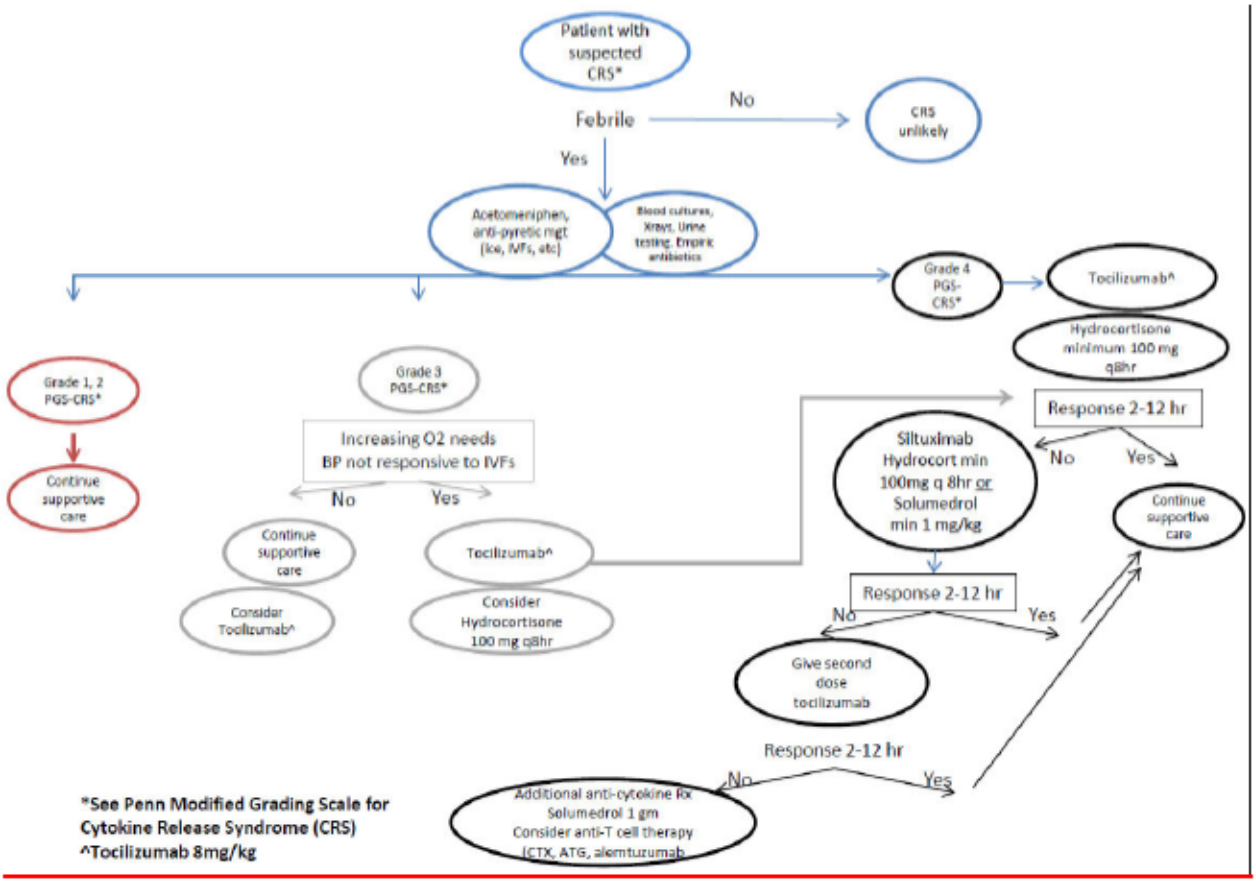
The recommended dosing for tocilizumab is 8 mg/kg i.v. single dose. Not all Grade 4 CRS reactions following CART-19 have been immediately treated with tocilizumab and decisions are, in part, based upon the rapidity of the syndrome onset and underlying patient reserve.

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 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 CART-19 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 CART-19 therapy is not acute, but rather delayed. Refer to Section 8.2 and **Table 8-1** for modified definitions of grading of CART-19 delayed CRS events.

Figure 8-1 CRS Management Algorithm



Tumor lysis syndrome: Patients will receive allopurinol prophylactically for 28 days 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.

GVHD: The chance of GVHD occurring is low, but it is a potential risk with CTL019 therapy. A prior study of activated donor lymphocyte infusions (ex vivo activated cells collected from the donor and grown in the same fashion as CART-19 but without the CAR introduction) did not show high rates of GVHD (2/18 patients with grade 3 GVHD and none with grade 4)[97]. Eight ALL patients treated to date with autologous CART-19 therapy had prior allogeneic hematopoietic SCT with residual donor chimerism. None of these patients have developed GVHD after autologous CART-19 infusion

8.4.3. Criteria for Discontinuing a Patient's Participation in the Study

If a patient develops a condition that precludes CART-19 infusion after enrollment but before infusion, the patient will be prematurely discontinued. This will be done at the judgment of the PI, and could include for example, the occurrence of an intercurrent illness requiring the institution of systemic immunosuppression

8.5. 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 IRB and DSMC approval is required.

No exception would be granted 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

Exceptions will be approved by the Medical Monitor and Regulatory Sponsor prior to submission to the IRB and DSMC. Documentation of approval by the Medical Monitor and Regulatory Sponsor will be submitted with the initial request for an exception to the IRB and DSMC. All exceptions require **advance** documented IRB, ACC DSMC, and other applicable 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 IRB and DSMC 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 DSMC within 5 business days and the IRB within 10 business days.

Any departure from the protocol that meets the following criteria should be submitted to the regulatory sponsor, ACC DSMC and IRB:

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

Other deviations should be explained in a memo to file (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).

Include the following information on the Sponsor supplied exception/deviation form: Protocol number, subject study number, description of the exception/deviation from the protocol, 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
Center for Cellular Immunotherapies (CCI)
University of Pennsylvania
[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.

8.6. 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, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

A protocol-specific independent Medical Monitor with appropriate expertise has been recruited in addition to the DSMB to review subjects' safety data and ensure the safety of participants. The Medical Monitor will receive real-time reporting of any event that could potentially impact subject safety (including dose-limiting toxicities). The Medical Monitor will also receive all of the following:

- All Serious Adverse Events (regardless of expectedness/relatedness). The SAE will be reported to the Medical Monitor within 24 hours of becoming aware of the SAE.
- Deviations reported to the Regulatory Sponsor, ACC DSMC and IRB as they occur
- Exceptions prior to submission to the IRB and ACC DSMC
- IRB Continuing Reviews
- Monthly listing of SAE's which occur after T cell infusion
- All queries issued by the DSMC (including those related to grading, attribution and expectedness of adverse events).

The Medical Monitor will correspond via email to communicate:

- SAE acknowledgement and inquiries
- Deviation acknowledgement, inquiries, recommendations
- Exception acknowledgement, inquiries and approval/disapproval
- Continuing review acknowledgement, inquiries

The Medical Monitor will review the above and make recommendations to continue with the study, amend the study, and/or stop/pause the study as needed.

Documentation of Medical Monitor review will be maintained in the study regulatory binder. Documentation of Medical Monitor review will also be sent to the ACC DSMC and Regulatory Sponsor as soon as a response is received from the Medical Monitor.

8.6.1. Independent Data and Safety Monitoring Board

An Independent Data and Safety Monitoring Board (DSMB) will be constituted prior to enrollment of the first patient. Please note that the DSMB is separate from the ACC DSMC (refer to section 9.3.3). The DSMB will be comprised of a minimum of four individuals, including physicians with

experience in oncology and/or gene transfer therapy and a statistician and will work under a charter specifically developed for safety oversight of this study. The DSMB will provide guidance/advice to the Regulatory 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 local regulatory review committees as required per institutional policy.

9. DATA HANDLING AND RECORDKEEPING

9.1. Confidentiality

The investigator must ensure anonymity of the patients; patients must not be identified by names in any documents submitted to the sponsor. Signed informed consent forms and patient enrollment log must be kept strictly confidential to enable patient identification at the site.

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

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

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

The data collection system for this study uses built-in security features to encrypt all data for transmission in both directions, preventing unauthorized access to confidential participant information. Access to the system will be controlled by a sequence of individually assigned user identification codes and passwords, made available only to unauthorized personnel who have completed the prerequisite training.

If country rules or ethics committee standards do not permit collection of patient initials and the exact date of birth, generic initials will be used and only the year of birth will be collected.

9.2. Source Documents

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

The investigator must maintain source documents for each patient in the study, consisting of case and visit notes (hospital or clinical medical records) containing demographic and medical information, laboratory data, electrocardiograms and the results of any other tests or assessments. All information recorded on the eCRFs must be traceable to source documents in the patient's file. The investigator must also keep the original signed informed consent form, and a signed copy must be given to the patient.

9.3. Case Report Forms

For studies using electronic data capture (EDC), the designated investigator staff will enter the data required by the protocol into the electronic case report form (eCRFs). The eCRFs have been built using fully validated secure web-enabled software that conforms to 21 CFR Part 11 requirements. Investigator site staff will not be given access to the EDC system until they have been fully trained. Automatic validation programs check for data discrepancies in the eCRFs and allow modification or verification of the entered data by the investigator staff.

The Principal Investigator is responsible for assuring that the data entered into the eCRF is complete, accurate, and that entry and updates are performed in a timely manner. The designated CRO will review the data entered by investigational staff for completeness and accuracy. Electronic data queries stating the mature of the problem and requesting clarification will be created for discrepancies and missing values and sent to the investigational site via the EDC system. Designated investigator site staff is required to respond promptly to queries and to make any necessary changes to the data.

Concomitant treatments entered into the database will be coded using the WHO Drug reference list. Medical history/current medical conditions and adverse events will be coded using the Medical dictionary for regulatory activities (MedRA) terminology.

At the conclusion of the study the occurrence of any protocol violations will be determined, and all unused supplies are to be returned. After this has been completed and the data has been verified to be complete and accurate, the database will be declared locked. After database lock, all investigators will receive a CD-ROM or paper copies of the patient data for archiving at the investigational site.

10. STUDY MONITORING, AUDITING, AND INSPECTING

10.1. Site Monitoring

All monitoring will be conducted by representatives of the Sponsor as described in the Monitoring Plan. At the site initiation visit, monitors will assure that proper study related documentation exists, provide training to investigators and other site personnel in the GCP guidelines, and assure that acceptable facilities and adequately trained staff are available to conduct the study.

During the study, the sponsor monitor will visit the site regularly to check the completeness of patient records, the accuracy of entries on the CRFs, the adherence to the protocol to Good Clinical Practice, the progress of enrollment, and to ensure that study treatment is being stored, dispensed, and accounted for according to specifications. Key study personnel must be available to assist the sponsor monitor during these visits.

The investigator must maintain source documents for each patient in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, electrocardiograms, and the results of any other tests or assessments. All information recorded on CRFs must be traceable to source documents in the patient's file. The investigator must also keep the original signed informed consent form (a signed copy is given to the patient).

The investigator must give the monitor access to all relevant source documents to confirm their consistency with the CRF entries. The monitoring standards for this study will require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria and documentation of SAEs. Additional checks of the consistency of the source data with the CRFs are performed according to the study-specific monitoring plan.

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 of all study-related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). 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.

Please notify the Sponsor in real-time if an audit/inspection notification is received.

11. ETHICAL CONSIDERATIONS

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

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

All patients for this study will be provided a consent form describing this study and providing sufficient information for patients to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a patient, using the IRB-approved consent form, must be obtained before that patient is submitted to any study procedure. This consent form must be signed by the patient and the investigator-designated research professional obtaining the consent.

The protocol is listed under clinicaltrials.gov.

12. STUDY FINANCES

12.1. Funding Source

This study will be funded by Novartis Pharmaceuticals.

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

12.4. Study Discontinuation

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

Appendix 2. Classification of Graft-Versus-Host-Disease

Classification of Chronic Graft-Versus-Host Disease

Limited Chronic GVHD

Either or Both:

1. Localized skin involvement

2. Hepatic dysfunction due to chronic GVHD

Extensive Chronic GVHD

Either:

1. General skin involvement

Or

2. Localized skin involvement and/or hepatic dysfunction due to chronic GVHD

Plus:

3a). Liver histology showing aggressive hepatitis, bridging necrosis or cirrhosis

Or

3b). Involvement of eyes: Schirmer's test with less than 5mm wetting

Or

3c). Involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy specimen

Or

3d). Involvement of any other organ

Classification of Acute Graft-Versus-Host Disease

Acute GVHD will be graded using the consensus conference criteria. The first day of acute GVHD onset at a certain grade will be used to calculate cumulative incidence curves. This end-point will be evaluated through day 180 post-transplant to account for delayed onset.

	Extent of organ involvement		
	Skin[a]	**Liver[b]**	**Gut[c]**
Stage			
1	Rash on <25% of skin	Bilirubin 2-3 mg/dL	Diarrhea > 500 ml/day or persistent nausea[d]
2	Rash on 25-50% of skin	Bilirubin 3-6 mg/dL	Diarrhea > 1000 ml/day
3	Rash on >50% of skin	Bilirubin 6-15 mg/dL	Diarrhea > 1500 ml/day
4	Generalized erythroderma with bullous formation	Bilirubin > 15 mg/dL	Severe abdominal pain with or without ileus
Grade[e]			
I	Stage 1-2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	-	Stage 2-3 or	Stage 2-4
IV[f]	Stage 4 or	Stage 4	-

[a] Use 'Rule of Nines' or burn chart to determine extent of rash.

[b] Range given as total bilirubin. Downgrade one stage if an additional cause of elevated bilirubin has been documented.

[c] Volume of diarrhea applies to adults. Downgrade one stage if an additional cause of diarrhea has been documented.

[d] Persistent nausea with histologic evidence of GVHD in the stomach or duodenum.

[e] Criteria for grading given as minimum degree of organ involvement required to confer that grade.

[f] Grade IV may also include lesser organ involvement but with extreme decrease in performance status.