CHP959 – A Phase I/IIA Study of Redirected Autologous T Cells Engineered to Contain Anti-CD19 Attached to TCR zeta and 4-1BB Signaling Domains in Patients with Chemotherapy Resistant or Refractory CD19+ Leukemia and Lymphoma

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Regulatory Sponsor: [redacted]

Scientific Advisor: [redacted]

Study Product: *CD19 redirected autologous T cells (CART-19 T Cells)*

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LIST OF ABBREVIATIONS

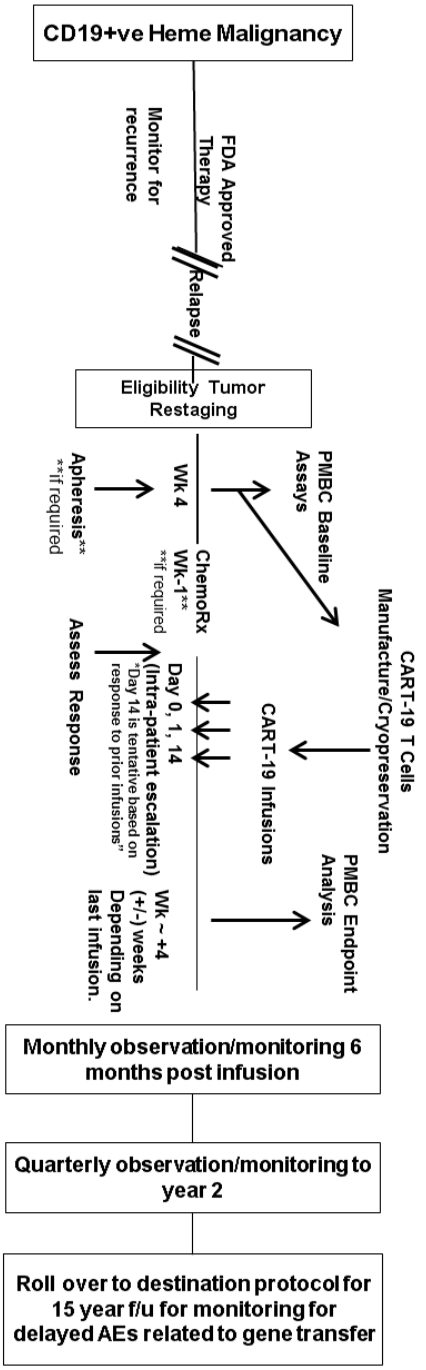
ACC	Abramson Cancer Center
(a)APC	(artificial) antigen presenting cell
AE	adverse event
CART-19 cells	CD19-targeted, redirected autologous T cells
CIR	chimeric immune receptor
CFR	code of federal regulations
CHOP	Children's Hospital of Philadelphia
CMV	Cytomegalovirus
CRF	case report form
CTC	common toxicity criteria
CRQA	Clinical Research Quality Assessment Committee
CRS	Cytokine Release Syndrome
CTL	cytotoxic T lymphocyte
[redacted]	[redacted]
CTL	cytotoxic T lymphocyte
CD137	4-1BB costimulatory molecule
DFS	disease free survival
DSMB	data safety and monitoring board
FDA	food and drug administration
GCP	good clinical practices
GMP	good manufacturing practices
GVHD	graft versus host disease
IBC	Institutional Biosafety Committee
IRB	Institutional Review Board
MRD	minimal residual disease
PBMC	peripheral blood mononuclear cells
RAC	NIH Office of Biotechnology Recombinant DNA Advisory Committee
RCL	replication competent lentivirus
scFv	single chain Fv fragment
T-body	fusion protein expressed in T cells that combines an extracellular antibody domain and an intracellular signaling domain. Interchangeable with CIR
TCR	T cell receptor
TCR-ζ	signaling domain found in the intracellular region of the TCR zeta, gamma and epsilon chains
Vβ	a rearranged T cell specific gene that can be used to determine clonality of a T cell population
UPENN	University of Pennsylvania

STUDY SUMMARY AND STUDY SCHEMA

Title	A PHASE I/IIA STUDY OF REDIRECTED AUTOLOGOUS T CELLS ENGINEERED TO CONTAIN ANTI-CD19 ATTACHED TO TCR ZETA AND 4-1BB SIGNALING DOMAINS IN PATIENTS WITH CHEMOTHERAPY RESISTANT OR REFRACTORY CD19+ LEUKEMIA AND LYMPHOMA
Short Title	CD19 redirected autologous T cells
Phase	Phase I/IIa
Methodology	Open-label approach
Study Duration	Approximately 4-5 years
Study Center(s)	CHOP [redacted]
Objectives	The primary objective is to determine the safety and survival of the redirected autologous T cells transduced with the anti-CD19 lentiviral vector (referred to as "CART-19" cells) given in the context of an adult and a pediatric protocol targeting CD19+ malignancies.
Number of Subjects	72 infused, up to 86 enrolled
Diagnosis and Main Inclusion Criteria	Inclusion criteria are designed to include pediatric patients aged 1-24 years with CD19+ B cell malignancies with no available curative treatment options (such as autologous or allogeneic stem cell transplantation) who have limited prognosis (several months to <2 year survival) with currently available therapies.
Study Product, Dose, Route, Regimen	CART-19 cells transduced with a lentiviral vector to express either anti-CD19 scFv:TCRζ or anti-CD19 scFv TCRζ:41BB, administered by i.v. injection using an intra-patient dose escalation approach: 10% on day 0, 30% on day 1, possibly followed by 60% on day 14 (or later) with a total dose goal of ~1.5 $x10^7$ – 5 $x10^9$ (~0.3$x10^6$ - 1.0$x10^8$/kg) T cells.
Duration of administration	Approximately 10-15 minutes; drug is expected to persist at detectable levels in circulation for weeks to months.
Reference therapy	None. This protocol will be given to subjects with unmet medical needs for which there are no current curative therapeutic options.
Statistical Methodology	The statistical analysis will be primarily descriptive in keeping with the exploratory nature of the study. Descriptive statistics will be applied to determine the relative engraftment and persistence. All adverse events will be described and exact 95% confidence intervals will be produced for adverse event rates, both overall and within major categories. The change in the ratio of CART-19 cells over time will be compared using a Wilcoxon signed-rank test for paired data. This nonparametric test is very efficient (>95%) compared to the t-test if the underlying data are normally distributed. Analysis of other secondary endpoints such as anti-tumor activity will also be primarily descriptive and may include summary statistics such as means and standard deviations or Kaplan-Meier curves for survival information.

STUDY SCHEMA (Figure 1)

Figure 1: Conduct of study. Initial leukapheresis (week -4) will be a 3-4 volume leukapheresis in smaller children.



1. INTRODUCTION

This document is a protocol for a human research study. 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.

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 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]. However, several problems remain to be solved before this therapy becomes routine; see our reviews for details[10, 11].

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[12-14]. 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[14-16]. 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, 17]. Expression of CD19 is restricted to B lineage cells and is not expressed by pluripotent blood stem cells[18]. CD19 is also expressed by most B cell lymphomas, mantle cell lymphoma, ALLs, CLLs, hairy cell leukemias, and a subset of acute myelogenous leukemias[12, 19, 20]. CD19 thus represents a highly attractive target for immunotherapy[18]. Furthermore, CD19 is not present on most normal tissues, other than normal B cells, including pluripotent blood stem cells[18], 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[21-25] and human and non-human primates[26-36].

Engineered T cells with redirected specificity: chimeric immune receptors (CIR). The daunting task of breaking tolerance to self antigens is the major obstacle facing the field of cancer immunotherapy. This can be difficult or impossible if the TCR repertoire has been deleted or rendered non functional by various post thymic tolerance mechanisms[37, 38]. One strategy is to identify therapeutically effective T cell clones, clone the heterodimeric TCR, and express it in other T cells, creating bispecific T cells with reactivity against the original TCR and the cloned TCR (reviewed in[39-41]). This was first carried out using protoplast fusion to transfer the TCR genes from a mouse T cell into another mouse T cell clone with a different specificity[42]. Other labs used cell fusion or electroporation to transfer TCR genes[43, 44], and in these cases demonstrated that MHC restricted specificity could be transferred. The advent of retroviral vectors made it possible to make this process more efficient. The Pease laboratory first used a retroviral vector with the LTR directing expression of TCRζ and an internal CMV promoter driving TCRα expression in mouse T cells[45, 46]. In 1999 Clay used a retroviral vector to transfer an HLA-A2 restricted TCR with specificity for MART-1, derived from tumor infiltrating lymphocytes, from a melanoma patient to T cells from 3 normal donors[47]. Recently Kessels and coworkers demonstrated that redirected mouse T cells were fully functional, could protect against tumor challenge, and could treat established metastasis[48]. The T cells were shown to expand dramatically (by more than 3 logs) after in vivo antigen encounter, and they trafficked to tumor sites.

An alternative strategy to produce genetically engineered T cells is the 'T-body' or chimeric antigen receptor (CAR) approach, which uses genetically programmed, patient-derived lymphocytes transfected with chimeric receptor genes to combine the effector functions of T lymphocytes with the ability of antibodies to recognize predefined surface antigens with high specificity in a non-MHC restricted manner[49, 50]. These receptors have the ability to recognize intact membrane proteins independent of antigen processing. CARs or T-bodies typically encode an extracellular domain to bind tumor or virus linked to an intracellular signaling domain that mediates T cell activation (reviewed in[41, 51]). In principle, universal targeting vectors can be constructed because the scFv bind to native cell surface epitopes and bypass the requirement for MHC restriction. The tumor binding function of CAR is usually accomplished by the inclusion of a single chain variable fragment (scFv) antibody, containing the V_H and V_L chains joined by a peptide linker of about 15 residues in length[52].

A large number of CARs targeting diverse tumors have been developed[41], however, clinical pilot tests are just beginning.

Lymphocyte costimulation. Extensive research in the past two decades has documented that maximal activation, proliferation and persistence of T cells responding to antigenic stimuli is dependent on receipt of two discrete signals mediated by cell surface receptors. The primary "activation" signal is generated by ligation of the TCR with antigen (typically in the form of peptides presented in the groove of HLA class I molecules) and the second signal by ligation of a costimulatory molecule with its cognate ligand. T cell costimulatory molecules which have been identified to date include members of the immunoglobulin super-family (CD28), members of the tumor necrosis factor (TNF) super-family (e.g. CD40L, CD134 [OX-40], CD137 [4-1BB][53].

One issue that needs to be addressed with CARs is that signaling through the cytosolic domain of the usual scFv-TCRζ single chain construct does not fully replicate the multichain TCR signaling complex[54, 55]. Chimeric receptors bearing TCRζ signaling modules are sufficient to trigger sustained proliferation in T cell hybridomas and clones but are not sufficient to drive

proliferation or cytokine production in peripheral T cells [54]. 4-1BB is a T cell co-stimulatory receptor induced by TCR activation, and evokes various T cell responses[56]. We and others have observed that 4-1BB signals are critical for long term proliferation of CD8 cells, and that CD28 is essential for sustained CD4 cell proliferation [57, 58]. We have begun to investigate the requirement for costimulation in our CD19-CAR by incorporating additional signaling modules in the cytoplasmic domain of the chimeric receptor. Our preclinical data are in accordance with the results from other laboratories [59, 60], and indicate that "bipartite receptors" comprised of TCRζ and either CD28 or 41BB signaling modules substantially improve the function and proliferation of T cells. The coalescence of improved T cell culture methods and lentiviral vector technology have made it possible to test this novel concept, which is now ready for clinical evaluation, and thereby advance the field of cancer gene therapy by adoptive immunotherapy.

Rationale for lymphodepletion. Adoptive immunotherapy strategies may be able to capitalize on homeostatic T cell proliferation [61], 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 [62, 63]. Host lymphodepletion may enhance the effectiveness of adoptively transferred T cells[61]. Homeostatic T cell proliferation can result in the induction of autoimmunity [64], providing a clue to improved antitumor strategies. T cells can undergo up to seven rounds of cell division after being deprived of contact with APC [65, 66]. This homeostatic response of T cells is directed largely to self antigens [67].

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 [68]. This hypothesis has been tested clinically in patients with metastatic melanoma refractory of conventional treatments [69]. The patients received a lymphodepleting conditioning regimen consisting of cyclophosphamide (60 mg/kg x 2 days) and fludarabine (25 mg/m2 x 5 days) prior to adoptive transfer of T cells. We have treated patients with myeloma and lymphopenia after lymphodepleting chemotherapy, and observed improved engraftment[70, 71]. In this protocol we propose to transfer RAT-19 T cells into subjects that are rendered lymphopenic as a result of cytotoxic chemotherapy. 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 [72].

Competitive repopulation trials. As a secondary endpoint, we propose a competitive repopulation strategy for this trial. Competitive repopulation strategies have been used previously to test the relative performance of vector constructs. In a 3 patient pilot trial[73], Nabel and colleagues tested the utility of a transdominant Rev protein, Rev M10. Autologous T cells were separately transfected with either RevM10 or a control protein and were then returned to each patient, and toxicity, gene expression, and survival of genetically modified cells assessed. The absolute engraftment of the gene marked cells was poor, due to the low transduction efficiency and the cell culture process. However, the cells that expressed Rev M10 were more resistant to HIV infection than those with Delta Rev M10 *in vitro* [73], as the Rev M10-transduced cells showed preferential in vivo survival compared to Delta Rev M10 controls. More recently, Morgan and colleagues have confirmed and extended these findings in 10 subjects with a more sophisticated series of anti-HIV vectors[74].

[redacted]

1.2 Investigational Agent

The investigational agent in this protocol is CART-19 cells [redacted]. Autologous T cells will be engineered 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 CD19 scFv, the cells will be transduced to express an intracellular signaling molecule comprised of either the TCRζ chain or a tandem signaling domain comprised of 4-1BB and TCRζ signaling modules. The scFv is derived from a mouse monoclonal antibody, and thus contains mouse sequences, and the signaling domains are entirely of the native human sequences. The CART-19 scFv plasmid was previously reported[30] and was generously provided [redacted]. CART-19 T cells are manufactured by isolating the T cells by apheresis, and using lentiviral vector technology[75-77] to introduce the scFv:TCRζ:4–1BB into CD4 and CD8 T cells. In some patients, a control scFv:TCRζ: will be introduced into a portion of the cells for the competitive repopulation experiment described below. These receptors are "universal" in that they bind antigen in an MHC-independent fashion, thus, one receptor construct can be used to treat a population of patients with CD19 antigen-positive tumors.

The CAR constructs were developed at the [redacted], and the clinical grade vector will be manufactured by [redacted] or another facility in accordance with the sponsor's specifications, as outlined in the FDA-approved [redacted]. The CART-19 cells will be manufactured in the [redacted] according to the process shown in **figure 3**. At the end of cell cultures, the cells are cryopreserved in infusible cryomedia. The maximium total dose of CART-19 transduced T cells will consist of the infusion of $1.5x10^7$-$5x10^9$ ($0.3x10^6$-$1.0x10^8$/kg) total cells. Each infusion will contain an aliquot (volume dependent upon dose) of cryomedia containing the following infusible grade reagents: plasmalyte-A, dextrose (5%), NaCl, up to 7.5% DMSO, dextran 40, human serum albumin with up to $5x10^9$ autologous T cells per bag. The initial dose will be decreased 10-fold, with intra-patient dose escalation (see Section 5.3 for details).

[redacted]

General description of the lentiviral vector (modified from Manilla et al[125]). The lentiviral vector is derived from the NL4-3 clone of wildtype HIV. It is maximally gutted so that its backbone is constructed only from conserved regions of HIV (5' and 3' long terminal repeats, central polypurine tract/central termination sequence, and Rev-responsive element). The final transgene constructs were cloned into a SIN vector under the control of the mammalian elongation factor 1α promoter. The vector is pseudotyped with a VSV-G envelope coat. The VSV-G DNA and the vector DNA are encoded on separate plasmids and are then transfected into cells for production of viral vector. The VSV-G envelope facilitates vector attachment to cell membranes for efficient vector entry into the cells, and helps to retain the infectivity of the viral vector during the stringent purification required for a clinical-grade vector.

Mechanism of action of transduced T cells. 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[41, 78]. In the case of CART-19 cells, retrovirally transduced primary human T cells containing a TCRζ cytosolic signaling domain were shown to engraft and to eradicate Raji Burkitt lymphoma tumor cells in immunodeficient mice[34]. Human T cells that express the CD19 scFv:TCRζ or CD19 scFv:TCRζ:4-1BB can kill B-ALL and lymphoma cells in vitro[35, 79-81]. 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 can 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 of T cells. 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[82]. 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[83]. In patients with HIV infection, we found 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[69, 83-85].

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

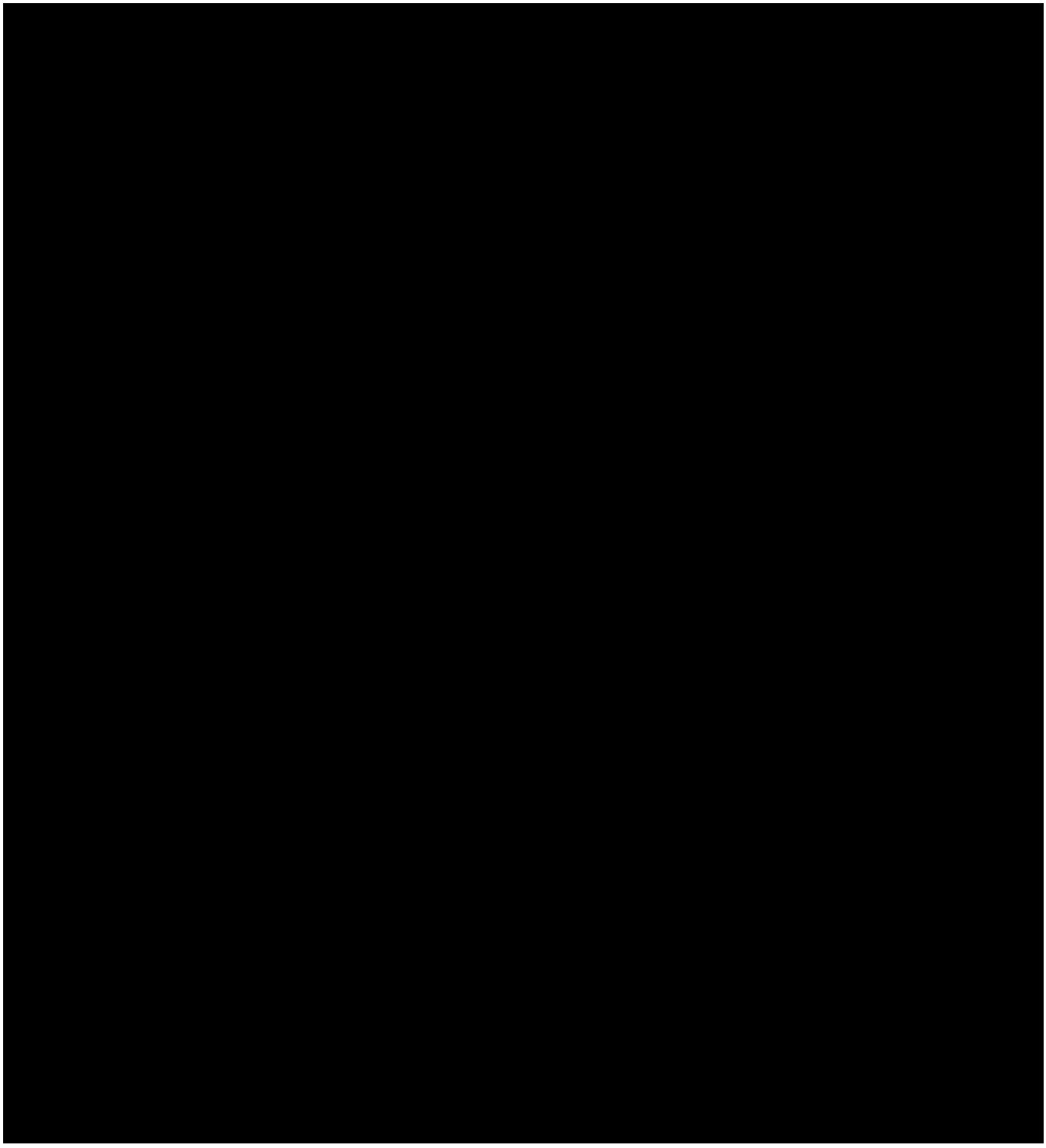
domains that are not normally juxtaposed. Immunogenicity of the CAR can lead to the rejection of the adoptively transferred T cells. The basis for this supposition is that human retrovirally-modified CTLs expressing a fusion protein consisting of hygromycin:HSV thymidine kinase were eliminated by host CTLs in patients with advanced HIV infection[86]; importantly, this immune mediated elimination was not accompanied by adverse effects and required 6 to 8 weeks to occur. There is one report where CAR containing a scFv with mouse sequences have been given to cancer patients. Following a single dose of CAR T cells (0.6 to 4 x 10^9 T cells), the CIR T cells were detected in circulation from 23, 32, and 53 days after infusion in three patients with renal cell carcinoma[87]. All three patients developed low levels of anti-scFv antibodies between 37 and 100 days after the CIR T-cell infusion. It is important to note that it is possible the CART-19 T cells will be rejected in our patients. We expect the cells to persist for a sufficient period of time to determine safety, T cell subset specific persistence, and effects on tumor burden and tumor specific immunity at 4 weeks following the first infusion.

1.3 Preclinical Data

An extensive literature supports the use of engineered T cells for tumor immunotherapy in rodent tumor models, reviewed[40, 41, 88-90]. 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[34, 35, 80, 91, 92]. The incorporation of signaling modules such as CD28 and 4-1BB in T body constructs increases potency of the engineered T cells in pre-clinical studies[59, 79, 93-97].

Over the past decade we have developed improved T cell culture systems and T cell transduction conditions. The T cell culture systems have been tested in phase I/II trials in patients with HIV infection and hematologic malignancies. The culture systems use anti-CD3 and CD28 costimulation and have proven to be efficient and feasible for large scale manufacturing, thereby overcoming a major barrier to adoptive immunotherapy. No significant safety concerns have emerged with more than 200 patients treated to date with CD4 and CD8 T cells, with and without genetic engineering with retroviral vectors[70, 71, 84, 98-103]. Our collaborators have used replication defective retroviruses for our initial studies to test chimeric receptors in patients with HIV infection[84, 85, 98]. However, HIV-based lentiviral vectors have the potential to be substantially more efficient and to be safer from the perspective of insertional mutagenesis for adoptive immunotherapy[75, 76]. Since 2001, we have collaborated with Boro Dropulic at Lentigen Corp. to develop lentiviral engineered T cells for adoptive immunotherapy[104, 105]; the data from our first pilot trial to test lentiviral vectors is described in section 1.4 below.

We have made research use only grade lentiviral vectors to test CART-19 T cells in preclinical models. We have found that the various constructs can be efficiently and stably expressed in primary human T cells (data not shown).



[redacted]

[redacted]

1.4 Initial Clinical Data

CART-19 cells with a 4-1BB:TCRζ have not been previously tested in humans; however a recent report testing CART-20 cells with a TCRζ reported safety in patients with mantle cell lymphoma [106]. Results from related approaches testing T bodies / CARs in humans are available from

patients with HIV infection and cancer. Human data relevant to CART-19 cells is summarized below:

CD4:TCRζ experience in HIV infection. CD4ζ is a genetically engineered, MHC–unrestricted receptor composed of the ζ subunit of the CD3 T cell receptor, the cytoplasmic domain involved in signal transduction, fused to the transmembrane and extracellular domains of human CD4, which targets HIV *env* expressed on the surface of infected cells [107]. Upon binding to HIV envelope, $CD8^+$ T cells engineered to express the CD4ζ fusion protein proliferate and initiate effector functions such as cytokine secretion and HIV-specific cytolytic activity [108, 109]. In collaboration with K. Hege at Cell Genesys Inc. and others, we assessed the survival of co-stimulated gene–marked T cells in 3 published studies [84, 85, 98]. CD4ζ modified T cells were detected by DNA PCR in the peripheral blood of all patients following infusion, and sustained mean levels of 1 to 3% of T cells were detected at all time points after infusion. In extended follow-up, CD4ζ was detected in the blood of 17 of 18 patients 1 year following infusion. In the only published phase II HIV gene therapy trial, 40 patients were randomized to receive an infusion of CD4ζ–modified T cells or non-transduced costimulated T cells; the treatment was found to be safe and associated with treatment-related increases in CD4 T cell counts[98]. Together these clinical results indicate that CD4ζ CAR anti-HIV vector is safe and has stable engraftment and persistence in lymphopenic patients with HIV infection.

G250:TCRζ experience in renal cell carcinoma. Lamers and colleagues recently reported the interim results of a trial testing T bodies in 3 patients with metastatic renal cell carcinoma[87, 110]. The T bodies were targeted with an scFv specific for carboxy-anhydrase-IX (the G250 antigen) that is overexpressed on clear cell RCC and in the biliary tract. The T body was expressed in autologous T cells using retroviral transduction. The subjects were treated with a dose-escalation scheme of intravenous doses of 2×10^7 T cells at day 1; 2×10^8 T cells at day 2; 2×10^9 T cells at days 3 through 5 (treatment cycle 1); and 2×10^9 cells at days 17 to 19, in combination with human recombinant IL-2, given subcutaneously at 5×10^5 U/m2 twice daily administered at days 1 to 10 and days 17 to 26. Infusions of these G250 T bodies were initially well-tolerated. However, after four to five infusions, liver enzyme disturbances reaching NCI CTC grades 2 to 4 developed. These toxicities necessitated the cessation of treatment in subjects 1 and 3, corticosteroid treatment in patient 1, and reduction of the maximal T-cell dose in subjects 2 and 3. All three patients developed low levels of anti-scFv (G250) antibodies between 37 and 100 days after the start of T-cell therapy. The liver enzyme elevations resolved. The T bodies circulated from 32 to 53 days after infusion, and the authors could demonstrate anti-tumor activity of the G2550 T body cells in the peripheral blood of the patients after infusion. The authors concluded that the liver toxicity was most likely due to the reactivity of G250 T bodies against the target antigen expressed on normal tissue, that is, the epithelial cells lining the bile ducts. Thus, this is interpreted as a form of "on target, off organ" toxicity.

Anti-CD20 expressing CD8 cells for follicular lymphoma. Jensen, Press and colleagues reported a clinical trial evaluating escalating doses of CD8 T cells modified by electroporation to express a chimeric immune receptor targeting the CD20 B cell marker and linked to the CD3zeta activation domain for the treatment of refractory follicular lymphoma and mantle cell lymphomas [106 106]. A total of 7 subjects were enrolled in the trial, and each received 3 infusions of escalating doses of CD20 specific CARTs of 10^8 cell/m^2, 10^9 cell/m^2, and 3.3×10^9 cell/m^2 spaced 2 to 5 days apart. The first three patients had poor persistence of the cells post infusion, which was improved in the last 4 patients who received subcutaneous low dose (500,000 IU/m^2)

IL-2 twice daily for 14 days. No subjects developed an immune response to the infused cells, which was evaluated by chromium release assay against targets expressing the scFv and neomycin resistance gene products (for cellular immunity), and by ELISA for antibody responses to the anti-CD20 antibody. Two patients became positive for HAMA 3 and 6 months after infusion. Since the chimeric immune receptor does not contain the murine Fc region, the conversion to HAMA positivity may reflect immune reconstitution and response to prior therapy. Clinical responses were modest, with no grade 3 or 4 toxicities. Toxicities were restricted to the four patients receiving IL-2, and were attributed to an IL-2 induced flu like syndrome.

Folate Receptor-Fcγ expressing T cells for ovarian cancer. Kershaw, Hwu and colleagues carried out a 14 patient trial with two cohorts evaluating a CIR targeting the folate receptor and linked to the Fcγ signaling chain[109b]. This trial consisted of two cohorts, the first of which received three cycles of escalating doses of T bodies expanded with antiCD3 Ab and IL-2 for doses of 3 x 10^9, 1 x 10^{10} and 3-5 x 10^{10} respectively, combined with systemic IL-2. The second cohort received 1-2 cycles (depending upon the number of cells available) of cells expanded with allogeneic PBMCs and IL-2, followed by allogeneic stimulation in vivo with live unirradiated PBMCs. Doses ranged from 4-169 x 10^9 cells, and two of six patients received 2 cycles. Cells were modified using a clinical grade retroviral vector containing the neomycin resistance gene, and were selected for using G418 after transduction. Grade 3 and 4 adverse events were observed in cohort 1, but were attributed to IL-2 administration. No serious adverse events were attributed to CIR administration. Persistence of modified cells was poor and did not exceed 3 weeks. This is likely a result of generation of antibodies to the infused T cells, as serum inhibitory factor that could be removed by incubation with protein G was found in patient serum post but not pre infusion. No effect on tumor, as measured by imaging and CA-125 antigen was observed in patients.

αCD19 redirected T cells with CD28-ζ signaling chain for CLL. Recently Brentjens, Sadelain, Riviere and colleagues initiated a clinical trial evaluating a chimeric immune receptor targeting CD19 and linked to the CD28 and CD3ζ signaling chains, for treatment of CLL. This is a two cohort study, with the first cohort receiving a low dose of cells to evaluate safety. The second cohort is designed to better evaluate potential efficacy, and the patients receive cytoreductive chemotherapy and a higher dose of cells. Cells are modified using a retroviral vector, and undergo rapid expansion using ClinExVivo beads from Invitrogen. To date, the treatment in cohort 1 has been well tolerated and resulted in transient anti-tumor effects in some patients. Results from this ongoing trial have not been published.

Lentiviral vector clinical experience. Considerable data shows that retroviral vectors are safe when expressed in human T cells; however, retroviral vectors appear less safe when used in human stem cells[111, 112]. Lentiviral vectors have the potential to be safer from the perspective of insertional mutagenesis, and they have substantially higher efficiency for genetically engineering human T cells[76, 113, 114]. Therefore, we propose to use lentiviral vector technology to introduce the CART-19 T body constructs into the T cells. [redacted]

[redacted]

CD19 immunotoxin experience. Ideally, the target antigens for T body/CIR anti-tumor protocols should be carefully chosen, so as to be expressed only by malignant cells and not by normal cells. CD19 expression has been reported to be restricted to the B cell lineage, and thus the expectation is that toxicity from CART-19 T cells should be confined to the hematopoietic compartment[13, 18, 19]. Most importantly, CD19 is not expressed on hematopoietic stem cells. Empiric clinical data with CD19 antibodies, radiolabeled CD19 antibodies and anti-CD19 immunotoxins supports this contention[28, 115-119], as the infusions have not caused aplasia. Furthermore, the widespread clinical use of anti-CD20 (Rituximab), targeting CD20, an antigen with a distribution of expression similar to CD19, has shown an acceptable clinical toxicity profile that would be expected from B cell depletion[120]. Recent clinical trials using chimeric immune receptors targeting CD20 and CD19 have also shown that redirected T cell immunotherapy appears safe for these targets.

Pheresis experience in young children. We have extensive experience over the past 15 years pheresing young children for therapeutic cells on our high-risk neuroblastoma trials (median age 3 years; studies including CHP-594, CHP-667, CCG A3973, COG ANBL00P1 and ANBL0532, various NANT studies), as well as a protocol for multiple cycle stem cell transplant in young patients with medulloblastoma (COG 99703). The effective lower limit for pheresis is 6 kg, and the effective lower limit for Medcomp catheter placement (as opposed to a femoral line) is 12 kg. Young children will be evaluated for suitability as a pheresis subject using our standard apheresis SOPs as approved by FACT.

1.5 Initial experience with CD19 redirected T cells and the CART-19 trial in adults at UPenn. [redacted]

[redacted]

[redacted]

UPN 03 received a low dose of CART19 cells ($1.5x10^5$ CAR+ T cells/kg divided over 3 days). Again, there were no acute infusional toxicities. However, 14 days after his first infusion, he began having rigors, fevers, nausea and diarrhea. By day 22 after infusion, tumor lysis syndrome was diagnosed requiring hospitalization for treatment. The patient was given rasburicase and IV fluids with resolution of biochemical abnormalities. He had resolution of constitutional symptoms, and within 1 month of CART19 infusions, he had clearance of circulating CLL from the blood and bone marrow by morphology, flow cytometry, cytogenetic, and FISH analysis. CT scans showed resolution of abnormal adenopathy. His complete remission has been sustained beyond 6 months from the initial CART19 cell infusion (Porter, et al. NEJM 2011).

[redacted]

[redacted]

GVHD risk. The patients in the allo cohort will be pheresed after allo SCT and will have some degree of residual donor engraftment, which will include T cells of donor origin. Most children who relapse after allo SCT will not have reverted to full recipient chimerism, and would be enrolled in the allo cohort. The collection of cells from the patients (as opposed to the donors) has 3 potential advantages: 1) many pediatric SCTs use umbilical cord cells as a donor source, in which case the donor would not be available; 2) in matched sibling donor SCT, the donor may be a minor and pheresing that donor would introduce further complications and potential risks; 3) most importantly, the allo cells collected from a subject without GVHD are tolerized and less likely to cause GVHD after infusion. There is limited experience with using activated allogeneic

cells in this setting. A prior UPENN/CHOP study of "activated DLI" (ex vivo activated cells collected from the donor and grown in the same fashion as CART19 but without the CAR introduction) did not show high rates of GVHD (2/18 pts with grade 3 GVHD and none with grade 4; Porter et al. Blood 2006). A patient recently treated at the NIH with CAR-modified "allo" T cells collected from the patient did not experience GVHD (A. Wayne, personal communication). While the risk of GVHD still exists, we feel it is low in this setting and the risk-benefit favors treating this incurable group of ALL patients with CART19 cells. At the same time, it must be acknowledged that treatments for GVHD would necessarily limit or destroy CART19 cells, so GVHD remains an important risk for these patients. The allo cohort will allow us to test CART19 therapy in this group of patients with significant unmet medical need and assess the GVHD risk. In 10 post-allo patients treated to date, there has been no GVHD.

Risk of neuropathy. In addition to the toxicities described as part of the CRS, we have also seen transient encephalopathy. Several of our patients have experienced confusion and/or aphasia lasting 2-3 days, and then recovering over another 2-3 days. For some patients, we have seen some confusion or delirium during periods of high fever while on opiates. These patients are very difficult to assess as to mental status. However, in 3/14 patients treated to date, we have seen this confusion occur at a point when the inflammatory phase was subsiding and the fevers were improved or gone. [redacted]

Although the etiology of this is unclear, it may well be related to the CART19 cells or CRS/MAS/HLH in some way. None of these patients have required any treatment, and all of them recovered fully in a few days.

1.6 Inclusion of children in the research.

Broadly speaking, we feel inclusion of pediatric patients in phase 1 or pilot protocols at the point where we have initial feasibility data from adult trials is appropriate. This is the approach that is generally pursued in early phase cancer trials for three reasons: i) to get clinical experience in pediatric populations, ii) to learn about issues with dosing or administration that might be specific to younger patients, and iii) allow pediatric access to novel therapies earlier in the development process. The population of pediatric relapsed/refractory leukemia and lymphoma patients targeted for enrollment in this study have no curative options for treatment. There is very little available on the horizon, especially for ALL patients, in terms of novel therapies at this stage of disease. New and highly innovative approaches are desperately needed for this group of pediatric patients. Our initial adult experience suggests a possible signal for efficacy. **With this, we believe there exists both compelling reasons to include children and the prospect of benefit in patients with no curative options.**

1.7 Dose Rationale and Risk/Benefits

The primary objective of this protocol is to test the safety and feasibility of administering CART-19 cells to patients with advanced refractory hematologic malignancies. A secondary objective of this protocol is to determine which cytosolic signaling domain is superior for permitting CIR T cell engraftment and persistence. In this "competitive repopulation" scenario, we must administer a dose of cells that is 1) safe and 2) feasible to produce and can be tracked in the subject after infusion in order to determine if one T body construct is superior to the other. The basis for setting the starting dose was based on 1) Guidance for Industry: S6 Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals; 2) Joint taskforce report from the BioIndustry Association (BIA) and the Association of the British Pharmaceutical Industry (ABPI) and 3) the available literature on phase I trials to date conducted with CIR T cells. The FDA guidance on safe starting dose (FDA Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers) does not apply well for cell and gene therapy, and as such was not used to guide the selection of the starting dose for this study.

We believe that an initial i.v. dose of $1.5x10^7$-$5x10^9$ ($0.3x10^6$-$1.0x10^8$/kg) CART-19 cells is optimal for this protocol. Because there are about 1 x 10^{12} T cells in a healthy adult (equivalent to $2x10^{10}$ T cells/kg), the proposed total (100%) dose is equivalent to about 0.5% of the total body mass of T cells[122, 123]. Therefore, we expect the initial frequency of cells to be present at about 0.5% at baseline following infusion. In addition, in our animal models, we find that a dose of 5 million cells per animal causes a robust antitumor response. The dose given to animals, when scaled, is similar to $5x10^9$ cells in humans. As an additional safety feature [124], the cells will be administered using an intrapatient dose escalation approach (see section 6.6). The proposed dose in children is also consistent with or less than our prior T cell infusion trial in young children (CHP-667).

Safety. As described above in section 1.2, we propose to insert the scFv CIR constructs into T cells using lentiviral vector technology. At the University of Pennsylvania we have treated [redacted] patients using a related approach for patients with HIV infection with autologous T cells that contained a 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. We have given doses of up to $1x10^{10}$ autologous *ex vivo* expanded T cells in several trials to patients with hematologic malignancies, HIV, and neuroblastoma and have found this to be well tolerated[70, 71, 99, 100]. The CART-19 cells may eventually be rejected by an immune response directed against the mouse scFv sequences. In summary, this dose was chosen because it is anticipated to be safe based on previous studies, and because it will permit sufficient engraftment in order to evaluate the primary and secondary endpoints of this study.

Risk of tumor lysis related to cytoreductive chemotherapy. The risk of tumor lysis syndrome (TLS) is dependent on the disease and burden of disease, but in most cases, this risk will be very low. Patients at highest risk for TLS have aggressive lymphomas (Burkitt's or other 'high grade" NHL) and ALL. The risk of TLS in CLL, follicular lymphoma, mantle cell lymphoma and diffuse large cell lymphoma is thought to be low, but may be higher with highly effective therapy (Porter et al., NEJM 2011). Patients will be closely monitored both before and after chemotherapy and infusions including blood tests for potassium and uric acid. Patient-

subjects will receive hydration and allopurinol to minimize any toxicity should significant acute tumor lysis occur.

Feasibility. The manufacturing feasibility is not expected to be an issue in this protocol, as given the combination of ex vivo cell culture and lentiviral vector technologies shown in figure 3, we expect to readily exceed the target dose of CART-19 cells that we have chosen.

Risk/benefits. Participation in this study will expose the patient to genetically engineered autologous T cells. The risk of the cells alone is low based on clinical experience. The unknown risk is that of the signaling domains in the CAR. T cell proliferation could be uncontrolled, however we have not observed this in our pre-clinical models. In this case, corticosterioids and chemotherapy would be given in an attempt to eradicate the CIR cells; this has worked in previous cases[87]. 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 subjects developed HAMA and loss of T cell engraftment in the Lamers study, but zero of 7 patients in the Press study developed immune responses to their T bodies. It is possible that the CART-19 T cells will exert an anti-tumor effect; in a preliminary study, two patients treated with T bodies encoding an scFv specific for CD20 had a response that has lasted for a year [106].

Transient host B cell depletion is also a likely risk with CART-19 cells, since normal B cells express CD19. This is expected to resolve when the CART-19 cells are cleared.

2 STUDY OBJECTIVES

Primary objectives:

1. Determine the safety and feasibility of administration of chimeric antigen receptor T cells transduced with the anti-CD19 lentiviral vector (referred to as "CART-19" cells), in patients who either:
    a. have not received a prior allogeneic SCT or have 0% residual donor engraftment ("no allo" cohort), or
    b. have relapsed after prior allogeneic SCT with any degree of residual donor engraftment ("allo" cohort).

   In each case, the cells used will be collected from the subject, not from an allogeneic donor.
2. Determine duration of *in vivo* survival of CART-19 cells. RT-PCR analysis of whole blood will be used to detect and quantify survival of CART-19 TCRζ:4-1BB and TCRζ cells over time.

Secondary objectives:

1. For patients with detectable disease, measure anti-tumor response due to CART-19 cell infusions.
2. To determine if the 4-1BB transgene is superior to the TCRζ only transgene as measured by the relative engraftment levels of CART-19 TCRζ:4-1BB and TCRζ cells over time.
3. For patients with stored or accessible tumor cells (such as patients with active CLL, ALL, etc) determine tumor cell killing by CART-19 cells in vitro.

4. Determine if cellular or humoral host immunity develops against the murine anti-CD19, and assess correlation with loss of detectable CART-19 (loss of engraftment).
5. Determine the relative subsets of CART-19 T cells (Tcm, Tem, and Treg)
6. Determine safety and efficacy in a test cohort of patients with CNS3 disease.

Exploratory objectives:

1. Determine if insufficient in vitro T-cell test expansion in response to CD3/CD28 costimulation is predictive of manufacturing or clinical outcome.

3 STUDY DESIGN

3.1 General Design

We have designed a single-arm open-label phase I/IIa study to determine the safety, tolerability and engraftment potential of CART-19 T cells in patients with CD19+ hematologic malignancies. The general protocol schema is shown above in **figure 1** in section 1. All subjects will be dosed with CART-19 T cells. In all subjects treated to date, the CART-19 T cells contained only the TCRζ:4-1BB transgene. A total of 72 subjects will be infused across "no allo", "allo", and CNS-3 cohorts. In order to infuse 72 patients, assuming a 20% drop out rate, we would enroll up to 86 patients.

A secondary objective is to determine if the 4-1BB transgene is superior to the TCRζ only transgene. To test this, a cohort of 5 patients will be dosed with mixtures of TCRζ:4-1BB and TCRζ only cells, using a competitive repopulation strategy to determine the optimal signal transduction module in the chimeric receptor (**figure 13**). This cohort will proceed only after the 2 CARs to be compared are finalized. It is reasonable to assume that engineered T cells containing signaling domains that mimic physiologic signaling will function best in vivo, and therefore, we think that CART-19:TCRζ:4-1BB will be superior to CART-19:TCRζ only. **The null hypothesis is that they are equivalent.**

At entry, subjects will be staged and the suitability of their T cells for CART-19 manufacturing will be determined. Subjects in both the no allo and allo cohorts who have adequate T cells will be leukapheresed to obtain large numbers of peripheral blood mononuclear cells (PBMC) for CART-19 manufacturing. In each cohort, the subject is pheresed; however, in the allo cohort, T cells derived from donor engrafted cells may be collected from the subject. The T cells will be purified from the PBMC, transduced with CART-19 lentiviral vector, expanded in vitro and then frozen for future administration. Chemotherapy will then be given. Following tumor burden reassessment, CART-19 cells will be thawed and infused.

Subjects will have blood tests to assess safety, and engraftment and persistence of the CART-19 cells at regular intervals through week 4 of the study (see table in Appendix 2). The subsets of circulating T-cells that contain the CART-19:TCRζ:4-1BB and CART-19:TCRζ only lentiviral vector will be assessed at various times after infusion and compared to the baseline sample. Following the 6 months of intensive follow-up, subjects will be evaluated quarterly for two years with a medical history, a physical examination, and blood tests. Following completion of the primary follow-up phase, subjects will continue to be followed for disease-free survival (as applicable) and overall survival every 3 months until they withdraw consent or until two years post the last subject infusion, as part of Secondary Follow-up. Subjects will also be asked

to enroll in a 15 year long-term follow-up protocol to evaluate specific long-term adverse events related to the study product.

3.2 Primary Study Endpoints

This phase I/IIa study is designed to test the safety and relative engraftment of the autologous T cells transduced with the anti-CD19 lentiviral vector in patients with relapsed, refractory and incurable CD19+ B cell malignancies.

Primary safety, feasibility and engraftment endpoints include:

1. Occurrence of study related adverse events, defined as NCI CTC $\geq$ grade 3 signs/symptoms, laboratory toxicities and clinical events that are possible, likely or definitely related to study treatment at any time from the infusion until week 24. This will include infusional toxicity and any toxicity possibly related to the CART-19 cells including but not limited to:
    a. Fevers
    b. Capillary leak
    c. Rash
    d. Neutropenia, thrombocytopenia, anemia, marrow aplasia
    e. Hepatic dysfunction
    f. Pulmonary infiltrates or other pulmonary toxicity
    g. Hypotension
    h. In the allo cohort only, GVHD
2. Feasibility to manufacture CART-19 cells from patient apheresis products. The number of manufactured products that do not meet release criteria for vector transduction efficiency,

T cell purity, viability, sterility and tumor contamination will be determined (defined as "manufacturing failures").

3. Duration of *in vivo* survival of CART-19 cells is defined as "engraftment". The primary engraftment endpoint is the # DNA vector copies per ml blood of CART-19 cells on week 4 after the first infusion. Q-PCR for CART-19 vector sequences will also be performed after each infusion, weekly x 4, monthly x 6, and every 3 months thereafter until any 2 sequential tests are negative documenting loss of CART-19 cells.
4. New onset secondary malignancies.

3.3 Secondary Study Endpoints

Secondary endpoints include:

1. Describe anti-tumor responses to CART-19 cell infusions.
    a. For subjects with active disease, typical response criteria for partial response (PR) or complete response (CR) will be determined. Response determination will be only "descriptive" given the small number of subjects to be treated. The main endpoint for efficacy is Day 28 overall response rate. Other endpoints, including duration of remission, best overall response, CR/CRi with MRD negative bone marrow, RFS and EFS may also be summarized.
    b. For subjects treated with MRD (identified by PCR analysis of blood or marrow), determine elimination of MRD scored as yes/no.
    c. For subjects treated in remission, describe progression-free survival (PFS).
2. Describe overall survival and cause of death
3. For patients with stored or accessible tumor cells (such as patients with active CLL or ALL) determine if in vitro cell killing assays of CART-19 cells against patient tumor correlates with clinical response.
4. Determine if host immunity develops against the murine anti-CD19 or other elements of the transgene or vector such as VSV-G, and assess correlation with loss of detectable CART-19 (loss of engraftment).
5. In subjects infused with mixtures of CART-19 TCRζ and TCRζ:4-1BB cells, RT-PCR analysis of whole blood will be used to detect and quantify relative survival of CART-19 TCRζ and TCRζ:4-1BB cells over time.
6. Determine the relative subsets of CART-19 T cells (Tcm, Tem, and Treg)

3.4 Exploratory Study Endpoints

Exploratory endpoints include:

1. Describe the correlation of test expansion results with manufacturing feasibility and clinical outcome.

4 SUBJECT SELECTION AND WITHDRAWAL

4.1 Inclusion Criteria

Inclusion criteria are designed to include a broad spectrum of subjects with documented CD19+ hematologic malignancies. Enrollment at CHOP will largely be limited to ALL and high-grade B cell NHL. Male and female subjects with CD19+ B cell malignancies in patients with no available curative treatment options (such as autologous or allogeneic SCT) who have limited

prognosis (several months to <2 year survival) with currently available therapies will be enrolled:

1. Eligible diseases: CD19+ leukemia or lymphoma
    a. ALL without curative options for therapy, including those not eligible for allogeneic SCT because of:
        - age
        - comorbid disease
        - other contraindications to TBI-based conditioning (required for ALL SCT)
        - lack of suitable donor
        - prior SCT
        - Declines allo SCT (in CR3) as a therapeutic option after documented discussion about the role of SCT with a BMT physician not part of the study team (see note below).

    Patient may be in any complete response, or patient may have active disease but responding or stable after most recent therapy (see 5.2 Patient Eligibility to Receive CART-19 Transduced T Cells, below). The intent is not to enroll patients with no degree of disease control, or rapidly increasing disease burden between enrollment and cell infusion.
    b. Follicular lymphoma, previously identified as CD19+
        - At least 2 prior combination chemotherapy regimens (not including single agent monoclonal antibody (Rituxan) therapy.
        - Stage III-IV disease.
        - Less than 1 year between last chemotherapy and progression (i.e. most recent progression free interval <1 year).
        - Disease responding or stable after most recent therapy (chemotherapy, MoAb).
    c. CLL
        - At least 2 prior chemotherapy regimens (not including single agent monoclonal antibody (Rituxan) therapy.
        - Less than 1 year between last chemotherapy and progression (i.e. most recent progression free interval <1 year).
        - Not eligible or appropriate for conventional allogeneic SCT.
        - Disease responding or stable after most recent therapy (chemotherapy, MoAb)
    d. Mantle cell lymphoma
        - Beyond 1st CR with relapsed or persistent disease and not eligible or appropriate for conventional allogeneic or autologous SCT.
        - Disease responding or stable after most recent therapy (chemotherapy, MoAb).
        - Relapsed after prior autologous SCT.
    e. B-cell prolymphocytic leukemia (PLL) with relapsed or residual disease after at least 1 prior therapy and not eligible for allogeneic SCT.
    f. Diffuse large cell lymphoma or other high-grade NHL, previously identified as CD19+
        - Residual disease after primary therapy and not eligible for autologous SCT.
        - Relapsed after prior autologous SCT.
        - Beyond 1st CR with relapsed or persistent disease and not eligible or appropriate for conventional allogeneic or autologous SCT.
2. Age 1 to 24 years. Patients ages 22-24 will only be enrolled if they are currently being treated at CHOP or another pediatric facility/oncologist.

3. Expected survival > 12 weeks
4. Creatinine < 2.5 mg/dl and less than 2.5x normal for age
5. ALT <= 5x normal
6. Bilirubin <2.0 mg/dl
7. Any relapse after prior SCT will make patient eligible regardless of other prior therapy.
8. Patients with relapsed disease after prior allogeneic SCT (myeloablative or non-myeloablative) will be eligible if they meet all other inclusion criteria and
   a. Have no active GVHD and require no immunosuppression
   b. Are more than 4 months from transplant (6 months at infusion; see Section 5.2)
9. For those patients who require leukapheresis for T cell collection (i.e. no previously collected product exists), adequate venous access for apheresis or eligible for appropriate catheter placement, and no other contraindications for leukapheresis.
10. Voluntary informed consent is given.
11. Patients with CNS3 disease will be eligible if CNS disease is responsive to therapy (at infusion, must meet criteria in Section 5.2) (see section 15.5 for definitions of CNS1, CNS2 and CNS3 disease)

NOTE on 1.a.vi – declines SCT: SCT is performed in patients with ALL in CR3, but the outcomes are problematic. Most data suggest about a 30% OS in the highly selected group of patients. A report which places this in context was published in JCO by Saarinen-Pihkala et al., looking at the overall (population-based) experience in CR3 ALL. Of 71 pts with ALL in CR3, OS after SCT was 30% and EFS 15%. Overall EFS of the 71 patients in CR3 was 7%. These numbers indicate that a decision to pursue a therapy other than SCT in CR3 ALL is rational, and that CART-19 therapy may offer a benefit to patients in this group.

4.2 Exclusion Criteria

1. Pregnant or lactating women. The safety of this therapy on unborn children is not known. Female study participants of reproductive potential must have a negative serum or urine pregnancy test performed within 48 hours before infusion.
2. Uncontrolled active infection.
3. Active hepatitis B or hepatitis C infection.
4. Concurrent use of systemic steroids at the time of cell infusion or cell collection, or a condition, in the treating physician's opinion, that is likely to require steroid therapy during collection or after infusion. Steroids for disease treatment at times other than cell collection or at the time of infusion are permitted. Use of inhaled steroids, or hydrocortisone for physiological replacement in patients with adrenal insufficiency are permitted as well.
5. ~~Presence of grade 2-4 acute or extensive chronic GVHD. See section 15.2 for definition of chronic GVHD.~~ (NO LONGER APPLICABLE- Retired from V14)
6. ~~Under treatment for GVHD~~. (NO LONGER APPLICABLE- Retired from V14)
7. Previous treatment with any gene therapy products.
8. ~~Feasibility assessment during screening shows insufficient expansion in response to CD3/CD28 costimulation.~~(NO LONGER APPLICABLE – Retired from V11)
9. Any uncontrolled active medical disorder that would preclude participation as outlined.
10. HIV infection.

11. [redacted]
12. CNS3 disease that is progressive on therapy, or with CNS parenchymal lesions that might increase the risk of CNS toxicity
13. Acute or chronic GVHD requiring systemic therapy (topical allowed).

4.3 Subject Recruitment and Screening

Subjects 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. The investigators will identify patients potentially meeting the study eligibility criteria through medical record reviews and the results of tests performed as part of routine clinical care. Subjects who are determined to be potentially eligible will be informed about the study. If the subject indicates willingness to participate in the study, the consent process will begin. Although many screening/eligibility tests are done routinely as part of standard oncology care, some are not, and written informed consent will be obtained before any non-SOC screening tests are conducted for research purposes. After informed consent has been obtained, the subjects will be evaluated for eligibility to ensure all inclusion and exclusion criteria are met. Eligible subjects will then be assigned a subject ID and considered enrolled by the sponsor. The test expansion (performed for exploratory purposes) will also occur in this phase. If and when the patient is eligible and enrolled with the sponsor, pheresis will occur if needed, and cell manufacturing begun.

The test expansion has been used to determine whether the subjects have an adequate number of T cells that can be successfully expanded with the anti-CD19 lentivirus vector, as determined from a sample of PBMC obtained by phlebotomy or prior apheresis at the first screening visit (~week -8).

Staggered enrollment on the CNS3 cohort: infusion of any subsequent patient on the CNS3 cohort will be delayed until 21 days after the prior CNS3 patient's infusion to allow for toxicity monitoring.

Female subjects 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], or are not <11 years of age or who are not Tanner 1,) must have a negative serum or urine pregnancy test performed within 48 hours prior to entry.

Due to the high risk level of this study, while enrolled, all subjects 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 subject must agree to use reliable and double barrier methods of contraception during the follow-up period of the protocol.

Acceptable birth control includes a combination of two 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

Subjects who are not of reproductive potential (girls<11 years of age or who are Tanner 1, women who have been post menopausal for at least 24 consecutive months or have undergone hysterectomy, salpingotomy, and/or bilateral oophorectomy or men who have documented azoospermia) are eligible without requiring the use of contraception. Acceptable documentation includes written or oral documentation communicated by clinician or clinician's staff of one of the following:

- Demographics show age<11
- PE indicates Tanner 1 female
- Physician report/letter
- Operative report or other source documentation in the subject record (a laboratory report of azoospermia is required to document successful vasectomy)
- Discharge summary
- Laboratory report of azoospermia
- Follicle stimulating hormone measurement elevated into the menopausal range

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subjects 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 subject is lost to follow-up.
2. The judgment of the principal investigator that the patient is too ill to continue.
3. Pregnancy
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.
6. Grade 3 or 4 toxicity, which is unmanageable, unexpected, unrelated to chemotherapy, and attributable to protocol therapy, or a serious adverse event that requires the subject's being withdrawn from the trial.
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 criteria outlined in Section 15.3.
8. Termination of the study by the principal investigator, the sponsor, the study funder, the IRB, or the Food and Drug Administration.

4.4.2 Data Collection and Follow-up

Follow-up data collection after 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. Many patients who respond to cell therapy may also have prolonged DFS but are also at risk for late relapse. Therefore,

subjects will continue to be followed for 1) engraftment as long as patients are at risk (until evidence of loss of detectable transduced T cells), 2) DFS until there is disease progression or they begin a new cancer therapy (except for HSCT); 3) survival until the time of death; or until the patient withdraws consent for clinical data collection or until two years after the last subject's infusion.

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 may be asked to provide information from the subject's medical record to the study team at protocol defined timepoints (including the results of any routine care examinations and/or laboratory assessments), and assist in the collection of protocol required blood samples which will be sent to the University of Pennsylvania for protocol required analysis. The local provider will be contacted by a member of the study team to assess any potential toxicity. In numerous previous cell therapy trials at the CHOP and UPENN, loss of follow-up is estimated to occur in less than 5% of cases. Every effort will be made to contact subjects who appear to be lost to follow-up in order to at least obtain survival data. In the event a subject fails to complete the follow-up requirements, documentation of all attempts to contact the subject 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 this study, subjects will also be asked to participate in a separate 15 year long-term follow-up destination protocol to further evaluate long term adverse events related to the study product.

5 STUDY DRUG

5.1 Description

CART-19 cells are autologous T cells (collected from the patient) that have been engineered to express an extracellular single chain antibody (scFv) with specificity for CD19 linked to an intracellular signaling molecule comprised of a tandem signaling domain of the 4-1BB and TCRζ signaling modules. The CART-19 cells are cryopreserved in infusible cryomedia and will be administered in either 1 or 2 bags. Each bag will contain an aliquot (volume dependent upon dose) of cryomedia containing the following infusible grade reagents: plasmalyte-A, dextrose, NaCl, up to 7.5% DMSO, dextran 40, human serum albumin with up to $5x10^9$ autologous T cells per bag.

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. Later toxicities (7-21 days post infusion) are likely to be related to cytokine release syndrome (CRS), or possibly tumor lysis. Toxicities that could potentially occur but are unprecedented are primarily related to the gene transfer and are described in Section 8.4.2. 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

- Disease response:

- No evidence of high disease burden, that would, in the opinion of the treating physician, put the patient at significant potential risk for tumor lysis syndrome.
- Failure to maintain performance status as indicated in initial eligibility criteria
- If s/p allogeneic transplant, 6 months from transplant.
- Patients experiencing toxicities from their preceding cytoreductive chemotherapy will have their infusion schedule delayed until these toxicities have resolved. The specific toxicities warranting delay of T cell infusions include:
  - Pulmonary: Requirement for supplemental oxygen to keep saturation greater than 95% or presence of radiographic abnormalities on chest x-ray that are progressive
  - Cardiac: New cardiac arrhythmia not controlled with medical management
  - Hypotension requiring pressor support
  - Active Infection: Positive blood cultures for bacteria, fungus, or virus within 48 hours.

CNS3 cohort infusion criteria:

1) Disease status:
   a. If CNS3 by spinal fluid involvement, stable/responding disease as indicated by:
      i. stable or decreasing CSF WBC, and
      ii. total CSF WBC < 100 in a sample obtained within 72 hours of CART-19 infusion.
   b. If CNS3 by MRI findings, there must be interval stability or improvement on MRI within 2 weeks of infusion
   c. If CNS3 by cranial nerve findings, there must be stability or improvement of these cranial nerve findings on exam post intervention
2) At least 21 days since any prior CNS3 patient was infused.
3) Patients with CNS3 disease requiring radiation therapy must be at least 8 weeks post radiation at CART-19 infusion
4) Patients must have no acute/ongoing neurologic toxicity > Grade 1 with the exception of a history of controlled seizures or fixed neurologic deficits that have been stable/improving over the past 3 months

5.2.1 Release criteria for the manufactured T cell product:

[redacted]

5.3 Treatment Regimen

5.3.1 Patients 1-72 (infused), including CNS3 cohort

CART-19 transduced T cells will be given, up to a total dose of $1.5x10^7$-$5x10^9$ ($0.3x10^6$-$1.0x10^8$/kg)- total cells transduced with the 41BB-z vector. We will use the following schedule, starting with a 10x dose reduction but allowing for intra-patient dose escalation (see section 6.6):

- Day 0: 10% of total dose
- Day 1: 30% of total dose if patient is clinically stable from prior day's infusion

See section 6.6 for details on timing and doses of subsequent CART-19 infusions. The toxicities that would preclude the next dose of T cells are DLTs or GVHD attributable to the cell infusion, and not toxicities attributable to the prior chemotherapy, such as cytopenias. A DLT will prevent the infusion of the next dose, even if fully resolved by that time.

5.3.2 Comparison Cohort

NOTE: This cohort is on hold pending redesign of the comparison strategy (i.e., which CAR the 41-BB CAR will be compared to). Both the protocol and the consent form will require modification and IRB approval prior to this cohort being treated.

CART-19 transduced T cells will be given consisting of a mixture of $1.5x10^7$-$5x10^9$ ($0.3x10^6$-$1.0x10^8$/kg) total cells transduced with either the 41BB-zeta or zeta only vector. The first patient to receive the combined T cell infusion will be an adult, either at PENN on their CART-19 protocol, or an adult at CHOP. This dose will include 50% of cells of each CART-19-T product, which is separately manufactured and released, and then mixed prior to infusion. Currently, the plan is to give the CART-19 cells using the original "split dose" regimen on day 0, 1 and 2, but this will be reassessed based on the response in the next patients we treat with the single T cell infusions. If we use this schedule, a second dose of CART-19 cells will be planned on day 11 to patients, providing they had adequate tolerance of the first dose and sufficient CART-19 cells were manufactured. If we proceed with the modified dosing schedule, this day 11 dose will not occur. We will clarify this prior to enrolling to this phase of the study.

5.4 Preparation and Administration of Study Drug

Preparation

The CART-19 T cells are prepared in the [redacted] as described in Section 1.2, and are not released from the [redacted] until release criteria for the infused cells (e.g., cell purity, sterility, average copy number of vectors/cell, etc.) [redacted]. Upon release, the cells are taken to the CHOP [redacted].

Cell thawing:

After logging the cells in the [redacted], frozen cells will be thawed and transported to the subject's bedside for infusion.

Premedication

Side effects following T cell infusions include transient fever, chills, and/or nausea. It is recommended that the subject be pre-medicated with acetaminophen and Benadryl prior to the infusion of CART-19 cells. These medications may be repeated every 4-6 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 systemic corticosteroids such as hydrocortisone, prednisone, prednisolone or dexamethasone at any time, except in the case of a life-threatening emergency, since this may have an adverse effect on T cells.

Febrile reaction

In the unlikely event that the subject develops sepsis or systemic bacteremia following 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 [redacted].

Administration

The transduced T cells will be administered by slow IV push or IV infusion -targeting an infusion time of 10-15 minutes, adjusted as appropriate for smaller children and smaller volumes. No more than 10cc/kg of total volume will be delivered to the patient. One or two bags of CART-19 modified cells will be delivered. Each infusion bag will have affixed to it a label containing the following: "FOR AUTOLOGOUS USE ONLY." In addition the label will have at least two unique identifiers such as the subject's initials, birth date, and study number. Prior to the infusion, two individuals will independently verify all this information and confirm identity using the subject's wristband, and so confirm that the information is correctly matched to the participant.

Emergency medical equipment will be available during the infusion in case the subject has an allergic response, or severe hypotensive crisis, or any other reaction to the infusion. Vital signs (temperature, respiration rate, pulse, oxygen saturation, and blood pressure) will be taken before and after infusion, then every 15 minutes for at least one hour and until these signs are satisfactory and stable.

5.5 Subject Compliance Monitoring

Investigator-initiated phase I/II trials at CHOP are subject to routine auditing in accordance with the high risk policy of the CRQA. Ongoing monitoring of each patient-subject is performed by the monitor designated by the Sponsor.

5.6 Prior and Concomitant Therapy

All prescription and nonprescription medication, vitamins, herbal and nutritional supplements, taken by the subject during the 30 days prior to screening will be recorded at the screening visit.

Concomitant medications will be recorded in the medical record and on the appropriate CRF at every visit following the Day -1 Pre-Infusion Visit through the End of Study Visit. Any additions, deletions, or changes of these medications will be documented.

5.6.1 Anti-neoplastic Therapies

All prior antineoplastic therapy for the study indication received prior to the start of study treatment will be documented and recorded on the appropriate CRF as required per the CRF Completion Guidelines.

All antineoplastic therapies for subjects in remission post CART19 T-cell infusion (HSCT and non-HSCT therapy for their study disease) will be collected and recorded on the appropriate CRF, until the time of disease relapse.

5.7 Packaging

Each infusion bag/syringe will contain 10-50 ml of cells containing up to $5x10^9$ total cells.

5.8 Receiving, Storage, Dispensing and Return

5.8.1 Receipt of Drug Supplies

The CART-19 T cells are prepared in the [redacted] as described in Section 1.2. Upon release, the cells are taken to the CHOP [redacted]. Upon receipt of the investigational product by the CHOP [redacted], an inventory must be performed and an Investigational Product Accountability log completed. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The investigator must notify study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.

5.8.2 Storage

Bags (10 to 100 ml capacity) containing CART-19-transduced T cells will be stored under blood bank conditions in a monitored -135^{0}C freezer at the University of Pennsylvania [redacted] until dispensed to the clinical site. Infusion bags will be stored in the freezer until needed.

5.8.3 Dispensing of Study Drug

After logging the cells in the CHOP [redacted], frozen CART-19 cells will be thawed and transported for infusion to the subject's bedside. If the CART-19 T 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 [redacted] as specified below.

5.8.4 Return or Destruction of Study Drug

CART-19 T cells may require return to the [redacted] for a variety of reasons, including but not limited to: 1) Mislabeled product; 2) Condition of patient prohibits infusion/injection, and 3) Subject refuses infusion/injection; any unused product will be returned to the [redacted] for disposal as per [redacted].

There is an ongoing reconciliation of drug shipped, drug consumed, and drug remaining, performed by the [redacted]. Final disposition of the investigational product will also be documented in the site Investigational Product Accountability logs appropriately. This information is submitted on an annual basis to the FDA in annual reports.

6 STUDY PROCEDURES

Overview

The study consists of 1) a screening phase, 2) followed by an intervention/treatment phase consisting of apheresis, chemotherapy (to be determined according to disease as per section 6.5), 3) infusions of CART-19 cells, and 4) follow-up. Schedule of evaluations and infusion are included in Attachment 2.

6.1 Pre-Entry Evaluations

Subjects will be tested for the Human Immunodeficiency Virus (HIV). If this was obtained as part of the pheresis process, this test result can be used. The HIV test must be completed no more than 8 weeks before the infusion date as per the Schedule of Study Procedures (Section 15.1).

6.2 Enrollment and Baseline Assessment.

Subjects who have signed an informed consent and have an adequate pre-entry evaluation will undergo a routine lymphoma/leukemia staging workup including:

- History and Physical Examination (including height, weight, body surface area)
- CT scans of the Chest, Abdomen, and Pelvis done within 21 days of study entry if required for disease evaluation.
- Complete Blood Count, Differential.
- Chemistry Panel (Glucose, BUN, Creatinine, Sodium, Potassium, Calcium, Total Protein, Albumin, Total Bilirubin, Alk Phos, AST, ALT, Mg, Phos, LDH, Uric Acid)
- Serum pregnancy test for females of child bearing potential
- B2 Microglobulin Level if used in disease evaluation
- Serum immunoglobulin levels
- Performance Status Assessment
- GVHD assessment (allo cohort)
- Viral serologies (may use Miller-Keystone Autologous Panel)
- Autoantibody screen (ANA, ESR)
- Bone marrow aspirate, spinal tap (for any patient where spinal fluid sampling is part of disease surveillance, such as ALL) and lymph node biopsy (if accessible). Samples are sent to hematopathology for MRD assessment and CD19 expression. These tests will often already be available from prior treatment, in which case they will not be repeated at this point unless clinically indicated.
- For patients with ALL, CLL, PLL or other malignancy with circulating disease, bone marrow assessments will be done if clinically indicated to identify disease burden. If there is circulating disease, blood will be sent for flow cytometry to confirm CD19 expression if this has not been done in the past 6 months.
- An ECHO/MUGA should be performed within 6 weeks of the first infusion.

6.3 Enrollment

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

[redacted]

Documents required:

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

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

6.4 Apheresis

A single large volume apheresis procedure is carried out at the apheresis center. PBMC are obtained for CART-19 manufacture during this procedure. From a single leukapheresis, the intention is to harvest at up to 50 x 10^9 (10^9/kg) white blood cells to manufacture CART-19 T cells. Baseline blood leukocytes for FDA look-back requirements and for research are also obtained and cryopreserved. The cell product is expected to be ready for release approximately 4 weeks after beginning manufacturing.

Timing of leukapheresis. Leukapheresis may occur after enrollment or may also occur prior to enrollment, in the case of cells collected for another clinical purpose or in anticipation of possible immunotherapy, using the cell collection protocol CHP-784, prior to determining eligibility for this study. In these cases, cells which were collected under appropriate conditions and with adequate number of PBMC available may be used for manufacturing, obviating the need for another collection procedure after enrollment.

6.4.1 Cytoreductive chemotherapy

It is anticipated that many patients will have been receiving chemotherapy for relapse or resistant disease. For inclusion, they will have responding or stable disease to the most recent therapy. Prior to CART-19 cell infusion and after apheresis, an additional chemotherapy cycle is planned. Patients referred with stable disease on no recent therapy will be eligible as well. The use of additional chemotherapy prior to the recommended preinfusion chemotherapy will be at the discretion of the investigator and dependent on the patient's disease burden.

When given, chemotherapy may be started approximately 8-13 days before infusion so that CART-19 cells may be given 1-5 days after completion of the chemotherapy. The timing of

chemotherapy initiation therefore depends on the length of the regimen. The purpose of the chemotherapy is to induce lymphopenia in order to facilitate engraftment and homeostatic expansion of CART-19 cells. The chemotherapy may be chosen also to reduce disease tumor burden. The cytoreductive chemotherapy is not part of this investigational protocol and may be chosen and administered by the primary/referring oncologists. The choice of chemotherapy will depend on the patient's underlying disease and prior therapies. Fludarabine (30 mg/m2/day x 4 days) and cyclophosphamide (500 mg/m2/day x 2 days) are the agents of choice, as there is the most experience with the use of these agents in facilitating adoptive immunotherapy.

6.5 Restaging assessment

A limited restaging is done at the completion of chemotherapy in order to provide baseline tumor burden measurements. This may include imaging, physical examination, and MRD assessments. Subjects will undergo the following for pre-infusion testing: physical exam, documentation of adverse events and blood draws for hematology, chemistry and pregnancy testing (if applicable).

6.6 CART-19 Infusion #1 With Intra-Patient Dose Escalation

Infusions may begin a minimum of 1 to 5 days after completion of chemotherapy.

The day of (but prior to) the first 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.

All patients must undergo a rapid influenza diagnostic test during the flu season months of October through May and within 10 days prior to the planned CART-19 infusion. If the patient is positive for influenza, oseltamivir phosphate or zanamivir should administered for 10 days as preventative treatment. The patient must complete their 10 day preventative treatment course prior to receiving CART-19. The test does not need to be repeated prior to CART-19 infusion however if flu-like or respiratory signs and symptoms are present, CART-19 infusion should be delayed until the patient is asymptomatic.

CART-19 transduced T cells will be given, up to a total dose of $1.5x10^7$-$5x10^9$ ($0.3x10^6$-$1.0x10^8$/kg) total cells transduced with the 41BB-z vector. We will use the following schedule, starting with a 10x dose reduction but allowing for intra-patient dose escalation:

- Day 0: 10% of total dose
- Day 1: 30% of total dose if patient is stable from prior dose (no significant toxicity such as fever from 10% dose). This dose may be given between day 1 and 4 if the patient develops transient fever and is subsequently afebrile and clinically stable. If not given on day 1-4, this dose will then become available for infusion at day 14 or later (see next paragraph regarding timing and subsequent infusions).

Timing and doses of subsequent CART-19 infusions. For patients who have had i) evidence of brief B cell aplasia with subsequent B cell recovery (suggesting rapid CAR clearance), or ii) fever and other reversible toxicities without evidence of CAR expansion/LGLs or response, or iii) no response, or a partial or temporary response to the initial infusion, it may be that the initial dose of cells was not adequate to produce a full therapeutic effect, or the cells may not have persisted long enough to produce longer-term disease control. In these cases, it may be

appropriate to give more CART-19 cells (subsequent infusions). In a recent case, a CLL patient at HUP experienced fevers but not CAR expansion after his first (100%) dose of CART-19 cells. He then was redosed 4 months later, and subsequently experienced a delayed marrow CR 45 days after this second infusion (D Porter, personal communication). Patients may receive doses of up to 60%, no less than 14 days from any prior infusion (including the first infusion).

It is possible that a subject's cumulative dose could exceed 100% of the dose specified above. More than 100% of the cell dose may be given in aliquots to sustain an initial response or address rapid CAR clearance (as evidenced by e.g. B cell recovery) if the cells grew well and a sufficient number are available. In this scenario, additional doses of 30% (if available) would be given at 2 week+ intervals. The rationale for this dosing regimen is that there does not appear to be a significant dose-response relationship with the initial dose. We have observed varying and significant degrees of cell expansion post infusion, making the amount of the infused cells less relevant. Thus, a multiple doses given over time may be more efficacious in sustaining a response. In terms of safety, the worst toxicities have been observed with the first infusion. There has been minimal toxicity with subsequent infusions in the few patients that have received them. Therefore, we consider the potential benefits of administering a greater cell dose over time to outweigh the potential risks. However, the cumulative dose will not exceed 1.5 x 10^8/kg administered over time in several doses.

The goal is to give 30% aliquots for these subsequent infusions, unless only a 60% aliquot exists to be infused. Prior to amendment 10, the [redacted] has preparing the product in 10%, 30% and 60% aliquots, thus, it was not possible to administer less than 60% if that was the only aliquot remaining. Going forward, the manufacturing facility will prepare the product in several 30% aliquots (if sufficient cells exist), making subsequent administration of 30% doses possible. We will not administer several 60% doses over time (i.e. only one 60% dose would be given after the initial 10% and 30% doses).

Regardless of timing, toxicities that would preclude a subsequent dose of T cells are DLTs (defined in section 8.1) or GVHD attributable to the cell infusion, and not non-DLT toxicities or toxicities attributable to the prior chemotherapy, such as cytopenias. A DLT will prevent the infusion of the next dose, even if fully resolved by that time.

The cells are thawed by the [redacted]. The thawed cells will be given as a slow IV push or infusion rate as quickly as tolerated so that the duration of the infusion will be approximately 10-15 minutes, adjusted for size of patient and volume of infusion. In subjects 15-20 who receive mixtures of CART-19 cells, in order to facilitate mixing, the cells may be administered simultaneously using a Y-adapter. Subjects will be infused and premedicated as described in section 5.3. Subjects' vital signs will be assessed and pulse oximetry will be done prior to dosing, at the end of the infusion and every 15 minutes thereafter for 1 hour and until these are stable and satisfactory. A blood sample for determination of baseline CART-19 level is obtained before infusion.

Patients experiencing toxicities from their preceding cytoreductive chemotherapy will have their infusion schedule delayed until these toxicities have resolved. The specific toxicities warranting delay of T cell infusions include: 1) Pulmonary: Requirement for supplemental oxygen to keep

saturation greater than 95% 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. These delay criteria apply to subsequent doses of CART-19 cells as well.

A serum sample for potassium and uric acid will be collected before each infusion.

6.7 Post infusion assessments of clinical status, engraftment and persistence

Subjects will return to CHOP twice weekly for 2 weeks after each CART-19 cell infusion to 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.

Two weeks after all infusions are complete, subjects will then return once a week for three weeks to undergo some or all of the following: physical exam, documentation of adverse events and blood draws for hematology, chemistry, engraftment and persistence of CART-19 cells and research labs. Subjects will be instructed to call if there are any fevers or other side effects such as rigors experienced. These instructions, part of routine Oncology AVS instructions, will be reinforced by the study team.

6.8 CART-19 Subsequent Infusions

Subsequent CART-19 infusions are considered in circumstances such as: 1) after disease relapse or 2) No response or 3) CART loss/B cell recovery while still in CR. Please refer to Attachment 15.1 for further details.

6.9 Day 28 Evaluation

PBMC will be obtained by a peripheral blood draw. Subjects 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 disease type and may include imaging, MRD assessments, lumbar puncture, bone marrow aspirate and biopsy and/or lymph node biopsy – these biopsies will be performed if necessary for determination of disease status and not for research. The day 28 evaluation may be repeated 28 days after the last infusion if the patient receives subsequent infusions.

6.10 Monthly evaluations 2 to 6 months post infusion

Subjects will be monitored on a monthly basis during months 2 to 6 post CART-19 cell infusion. At these study visits, subjects will undergo the following: review of concomitant medications and post treatment antineoplastic therapy, physical exam with GVHD assessment if applicable, documentation of adverse events and blood draws for hematology, chemistry, engraftment and persistence of CART-19 cells and research labs.

An appropriate RCL test (i.e. HIV-gag or VSV-G or similar) will be performed at 3 and 6 months post the initial CART-19 cell infusion to exclude the presence of detectable RCL.

6.11 Quarterly evaluations for up to 2 Years Post Infusion

Subjects will be evaluated on a quarterly basis until 2 years post infusion. At these study visits, subjects will undergo the following: review of concomitant medication and post treatment

antineoplastic therapy, physical exam, documentation of adverse events and blood draws for hematology, chemistry, engraftment and persistence of CART-19 cells and research labs.

6.12 Secondary Follow-up Phase

For subjects who complete or prematurely discontinue from the primary follow-up phase while in remission, follow-up attempts will be made to assess the subject's relapse, post treatment antineoplastic therapy, and survival status every 3 months post CART-19 infusion until two years post the last subject infusion. Once subjects' relapse or they begin a new cancer therapy, additional follow-up for relapse will not be required, and subjects will be followed for survival only.

6.13 Long-term Follow-up Protocol

After subjects' complete or prematurely discontinue participation in this study, subjects will be asked to participate in a separate 15 year long-term follow-up destination protocol. This long-term follow-up protocol includes evaluations that will be performed for up to 15 years on all subjects as recommended by the FDA for protocols utilizing integrating viral vectors. Evaluations include: physical exam and medical history (including concomitant medications and adverse events) with careful attention to features possibly related to oncoretroviral diseases such as cancer, neurologic disorders or other hematologic disorders. In addition, labs will be drawn to evaluate engraftment, T body PCR and research labs. Blood for an appropriate RCL test (i.e. HIV-gag or VSV-G or similar) will be drawn yearly in order to detect RCL. If all RCL tests are negative during the study period to the first annual evaluation, then plasma may be archived for analysis on an as needed basis.

6.14 Tumor Response Assessments

Tumor response assessments will be done according to standard of care and practices at baseline (~21 days prior to CART 19 infusion), a limited reassessment after chemotherapy (see section 6.5), at the 4 week response evaluation and day 28 after last infusion if the patient receives subsequent CART-19 cells, and then every 3 months for 1-2 years after CART-19 cell infusions or until the patient requires alternative therapy for their disease. Tumor assessments will depend on the patients underlying disease as follows:

Disease	**Physical Exam**	**CBC with differential**	**Bone marrow Aspirate +/- bx with MRD**	**Lymph node biopsy**	**Lumbar puncture**	**CT scans**
ALL	+	+	+		+	
CLL/PLL	+	+	+/- (If clinically indicated)	If clinically indicated		+/-
NHL/ Mantle Cell	+	+	+/- (If clinically indicated)	If clinically indicated	If clinically indicated	+

7 STATISTICAL PLAN

7.1 General Design Issues

This is an open label, phase I/IIa study to evaluate the safety and tolerability, and persistence and engraftment of autologous T cells engineered to express a chimeric antigen receptor targeting CD19, that is linked to the CD3z-4-1BB signaling chains in the setting of a competitive repopulation in patients with chemotherapy resistant or refractory CD19+ leukemia or lymphoma. Patients at CHOP will be enrolled on this protocol (including some adults up to age 24), while patients at UPENN will be enrolled on the UPENN protocol. Inclusion of patients ages 22-24 will be limited to patients already being treated at CHOP or another pediatric center. The eligibility criteria are similar. The accrual goals at CHOP and UPENN will be separate. SAEs will be reported to both IRBs, with CHOP SAEs being reported according to the timing below and UPENN SAEs being provided according to the "outside center" reporting rules. Events on both the CHOP and UPENN protocols will be evaluated by the same DSMB. Possible triggering of pausing and stopping rules on this protocol will reflect events at CHOP only unless recommended by the DSMB.

Failure to complete the study due to the stopping rules being invoked will be the main basis for determining safety and feasibility of this study. This study is primarily intended to provide data that might allow the investigators to conduct a preliminary assessment of safety and feasibility.

Upon enrollment, patients will undergo leukapheresis (if needed) and an optional bone marrow aspirate approximately four weeks prior to dosing. Between leukapheresis and treatment, patients may undergo an additional chemotherapy treatment depending upon their disease. At dosing, patients will receive redirected autologous T cells against CD19 (CART-19 cells), containing a CD3z-4-1BB intracellular signaling chain. Patients will be monitored weekly for four weeks. At the end of four weeks, patients will undergo a second evaluation.

Observation and monitoring of patients will continue on a monthly basis until 24 weeks after a cell infusion is given. In subjects given a mixture of CART-19 cells, the change in the ratio of the vector transduced cells to each other between baseline and week four will be evaluated. After two years, annual follow-up for lentiviral vector safety will be carried out under a separate destination protocol for 15 years post infusion in accordance with FDA guidelines for retroviral vectors.

72 subjects will be targeted for infusion in this study. We have observed a drop out rate of less than 20% or less due to disease progression or clinical deterioration between enrollment and initial infusion (this will take about 6-8 weeks for screening-apheresis to dosing). However, as we are eliminating the test expansion, we may increase this dropout rate by 5-15%. Thus, up to 86 subjects will be initially enrolled to allow our goal of 72 infusions.

All safety data after initial CART-19 infusion will be summarized regardless of whether patient receives subsequent CART-19 infusions or not (Section 6.6). Efficacy data will be summarized from initial CART-19 infusion to first documented relapse or end of follow-up, whichever occurs first. Additional analyses may be planned as needed.

7.2 Endpoints

7.2.1 Primary Endpoints

- Monitor the occurrence of study related adverse events (defined as ≥ Grade 3 signs/symptoms, laboratory toxicities, and clinical events) that are "possibly",

"likely", or "definitely" related to study treatment any time from the first day of study treatment until week 24.

- Feasibility to manufacture CART-19 cells from patient apheresis products. The number of manufactured products that do not meet release criteria for vector transduction efficiency, T cell purity, viability, sterility and tumor contamination will be determined (defined as "manufacturing failures").
- Duration of *in vivo* survival of CART-19 cells is defined as "engraftment". PCR for CART-19 vector sequences will also be performed after infusion at 24 hours, weekly x 4, monthly x 6, and every 3 months thereafter until any 2 sequential tests are negative documenting loss of CART-19 cells.
- New onset secondary malignancies.

7.2.2 Secondary Endpoints

- Describe anti-tumor responses to CART-19 cell infusions.
  i. For subjects with active disease, typical response criteria for partial response (PR) or complete response (CR) will be determined. Response determination will be only "descriptive" given the small number of subjects to be treated.
  ii. For subjects treated with MRD (identified by PCR analysis of blood or marrow), determine elimination of MRD scored as yes/no.
  iii. For subjects treated in remission, describe progression-free survival (PFS).
- Describe overall survival and cause of death
- Rates of grade 2, 3 and 4 GVHD
- For patients with banked or accessible tumor cells (such as patients with active CLL, ALL, etc) determine if in vitro cell killing assays of CART-19 cells against patient tumor correlates with clinical response.
- Determine if host immunity develops against the murine anti-CD19, and assess correlation with loss of detectable CART-19 (loss of engraftment).
- For patients with stored or accessible tumor cells, evaluate the post infusion tumor response in comparison to baseline responses prior to cytoreductive chemotherapy (week -4), to evaluate the effect on general anti-tumor immunity.
- In subjects infused with mixtures of CART-19 TCRζ and TCRζ:4-1BB cells, RT-PCR analysis of whole blood will be used to detect and quantify relative survival of CART-19 TCRζ and TCRζ:4-1BB cells over time.
- Determine the relative subsets of CART-19 T cells (Tcm, Tem, and Treg)

7.2.3 Exploratory Endpoints

- Describe the correlation of test expansion results with manufacturing feasibility and clinical outcome.

7.3 Sample Size

This is a 86 patient phase I/IIa study with an anticipated dropout rate of 20%. If this exploratory study suggests that one vector persists and engrafts better than the other vector, then a larger follow-on trial will be designed that has the statistical power to assess the potential efficacy of that vector.

7.4 Safety

This trial will infuse up to 72 patients. This may require enrollment of more subjects because of the dropout rate. In order to assess the confidence with which we can obtain an initial estimate on the toxicity rate in each cohort, the table below presents the exact 95% confidence intervals for the toxicity rate based on the first 7 or 10 subjects

.

n=7 Evaluable Subjects		n=10 Evaluable Subjects	
Observed Toxicity Rate	**95% Exact Confidence Interval**	**Observed Toxicity Rate**	**95% Exact Confidence Interval**
0% (0/7)	(0%, 41%)	0% (0/10)	(0%, 31%)
14% (1/7)	(0%, 58%)	10% (1/10)	(0%, 45%)
29% (2/7)	(4%, 71%)	20% (2/10)	(3%, 56%)
43% (3/7)	(10%, 82%)	30% (3/10)	(7%, 65%)

With 10 subjects and a true underlying toxicity rate of 20%, the probability of failing to observe an adverse event is 11%. The probability decreases to 3% if the true toxicity rate is 30%. Taking into account potential drop out due to disease progression either prior to infusion or before the planned response assessment is possible, with seven subjects the probability of failing to observe an adverse event is 21%, if the true toxicity rate is 20% and decreases to 8% if the true toxicity rate is 30%.

7.5 Efficacy

Anti-tumor activity will be assessed as a secondary trial endpoint. Anti-tumor efficacy of CART-19 cells will be determined in a follow-on study.

Another exploratory endpoint is to test the relative engraftment of CART-19 cells expressing TCRζ or TCRζ:4-1BB domains. It is recognized that there will be statistical power to detect only very large change in the ratio of CART-19 cells. The results of this study will provide information on the expected change in ratios of CART-19 cells from baseline to four weeks post dosing to provide guidance on which vector should be considered for a future larger study, as well as pilot data to allow determination of required samples sizes for that study.

With a total of 20 evaluable subjects in each cohort, there would be 80% power to detect a 0.76 standard deviation difference in the ratio between the two different CART-19 cell between just post dosing baseline values and values obtained from the week four apheresis, using a paired t-test at a 0.05 two-sided level of significance. With a total of seven evaluable subjects in each cohort, there would be 80% power to detect a 1.27 standard deviation difference in the CART-19 ratio under the same conditions.

7.6 Subject Population(s) for Analysis

The subject population to be analyzed for primary and secondary endpoints will include all patients infused with CART-19 cells.

A second population of patients will include all patients enrolled on study but who do not receive CART-19 cells. Reasons patients do not receive cell infusions are likely to include 1) ineffective

transduction of autologous T cells; 2) rapid progression, clinical deterioration, and/or death between the time of enrollment and infusion; 3) subject withdrawal.

The number of patients enrolled versus the number of patients infused will be described and is a measure of the feasibility of this therapy for patients with various malignancies to be treated.

7.7 Statistical Analysis

The statistical analysis will be primarily descriptive in keeping with the exploratory nature of the study. All adverse events will be described and exact 95% confidence intervals will be produced for adverse event rates, both overall and within major categories. The change in the ratio of CART-19 cells over time will be compared using a Wilcoxon signed-rank test for paired data. This nonparametric test is very efficient (>95%) compared to the t-test if the underlying data are normally distributed. Analysis of other secondary endpoints such as anti-tumor activity will also be primarily descriptive and may include summary statistics such as means and standard deviations or Kaplan-Meier curves for survival information. Descriptive statistics may also be summarized for the exploratory endpoint.

8 SAFETY AND ADVERSE EVENTS

Safety will be assessed by monitoring and recording potential adverse effects of the treatment using the Common Toxicity Criteria version 3.0 at each study visit. Subjects will be monitored by medical histories, physical examinations, and blood studies to detect potential toxicities from the treatment.

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others

Any incident, experience, or outcome that meets <u>all</u> of the following criteria:

- <u>Unexpected in nature, severity, or frequency</u> (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- <u>Related or possibly related to participation in the research</u> (i.e. possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research)
- <u>Suggests that the research places subjects or others at greater risk of harm</u> (including physical, psychological, economic, or social harm).

Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

1. results in study withdrawal
2. is associated with a serious adverse event
3. is associated with clinical signs or symptoms
4. leads to additional treatment or to further diagnostic tests
5. is considered by the investigator to be of clinical significance

Serious Adverse Event

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

1. fatal
2. life-threatening
3. requires or prolongs hospital stay
4. results in persistent or significant disability or incapacity
5. a congenital anomaly or birth defect
6. an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, 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 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***.

Suspected Adverse Reaction (21 CFR 312.32(a))

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

Unexpected Suspected Adverse Reaction (21 CFR 312.32(a))

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

"Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Dose Limiting Toxicity (DLT)

Dose limiting toxicity is defined as a grade 3 or 4 toxicity as determined by CTC 3.0, which is unmanageable, unexpected and unrelated to chemotherapy and attributable to protocol therapy. For instance, grade 4 neutropenia or thrombocytopenia related to pre-infusion chemotherapy would not be related to study drug and would not be considered as a

DLT. In addition, if the toxicity is autoimmune or allergic in nature and occurs after repeat dosing, a grade 2 toxicity will be considered a DLT.

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 0 until the subject is off study or until year 2 end of study visit. The only exception is for patients that are discontinued due to an adverse event after enrollment and prior to infusion. This AE or SAE leading to study discontinuation will be recorded in the clinical database. Events occurring during chemotherapy, and occurring as a direct result of the cytotoxic chemotherapy will not be reported as an adverse event but should be listed under medical history.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

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:

1. The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
2. The abnormality suggests a disease and/or organ toxicity
3. 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.

Medical conditions and laboratory abnormalities due to underlying disease and chemotherapy regimen are **expected** and will not be reported as adverse events (e.g. abnormal hematology values, mucositis, etc.).

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event. As was noted above, events related to the cytotoxic chemotherapy will be excluded from adverse event reporting.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the adverse event reporting period (defined in Section 8.1 above) must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

8.3.1 Study Sponsor Notification by Investigator

All serious adverse events must be reported to the study sponsor by email within 24 hours of knowledge of the event. Report serious adverse events by email to:

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

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

Within the following 3 business days, the investigator must provide further information on the serious adverse event in the form of a written report. This should include a copy of the completed SAE Form, and 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. Significant new information on ongoing serious adverse events should be provided promptly to the study sponsor.

Follow-up information on SAEs should be reported when received, on a copy of the original SAE form, and should include both the follow-up number and report date. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or withdrew from study participation.

8.3.2 Investigator reporting: notifying 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, or where required by the Sponsor. 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 event was more likely than not to be caused by the research procedures.)

Reporting Process
Unanticipated problems posing risks to subjects or others as noted above will be reported to the IRB as a written or online 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 IRB for specific situations:

1. Report the event within 24 hours when the death is unforeseen (unexpected) and indicates participants or others are at increased risk of harm.
2. Report the event within 72 hours, for all other deaths, regardless of whether the death is related to study participation.

For reportable deaths, the initial submission to the IRB may be made through the eIRB system. The AE/Unanticipated Problem Form is required as a follow up to the initial submission.

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

1. 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 agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
2. 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 subjects.
3. 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.
4. Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
5. Breach of confidentiality

6. Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
7. 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 subject to remain on the study.
8. Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
9. 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 subjects.

8.3.3 Reporting obligations to the ACC DSMC

The Abramson Cancer Center has developed a detailed data safety and monitoring plan. The Clinical Trials Scientific Review and Monitoring Committee (CTSRMC) serves as a rigorous scientific peer review mechanism for all cancer protocols conducted within the University of Pennsylvania. The CTSRMC's focus is the scientific merit, priorities, and progress of Cancer Center clinical research while the DSMC's focus is data quality and subject safety monitoring and auditing. The guidelines governing both committees have been adapted from the NCI/NIH policies.

The DSMC requires AE/SAE submission through Velos as follows

- All grade 3 or higher events, including GVHD, within 5 business days of knowledge of the adverse event. 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, do not require DSMC reporting. It is important to note that these events must be reported in the absence of a diagnosis of a specific toxicity.
- All unexpected deaths within 24 hours of knowledge
- All other deaths within 30 days of knowledge (including death of subjects off study)
- In the event of a grade 4 or 5 unexpected event regardless of attribution, the investigators and the study team must meet or have a teleconference within 24 business hours 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.

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.

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

Within 15 calendar days

Any study event that is:

- Serious and unexpected
- Suspected adverse reaction 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 other studies, findings from animal or in vitro testing:

- that suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, 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 identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

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

Reporting Process

Adverse events may be submitted on FDA Form 3500A or in a narrative format.

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 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. The review of these adverse events, and any decision to prematurely stop subject enrollment, will be determined by the DSMB and reviewed by the IRB.

Premature termination of the clinical trial may occur because of a regulatory authority decision, change in opinion of the IRB, the DSMB, or determination that there are problems in the cell product generation or safety at the discretion of the 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 subject develops uncontrolled T cell proliferation that does not respond to management.
- The Investigator, Study Funder, Sponsor, DSMB, ACC DSMC, or any independent review board or regulatory body decides for any reason that subject safety may be compromised by continuing the study.
- The Sponsor or Study Funder decides to discontinue the development of the intervention to be used in this study.

The protocol will be paused pending submission of protocol pause to the FDA and review by the IRB and DSMB if any patient experiences any of the following events within two weeks of the CART-19 infusion:

- Life-threatening (grade 4) toxicity attributable to protocol therapy that is unmanageable, unexpected, unrelated to chemotherapy, and attributable to protocol therapy. This includes grade 4 GVHD. In terms of expected toxicity, we expect high fevers, hypotension, possible ICU admission and even mechanical ventilation. CRS and MAS can result in grade 4 liver toxicity, nephrotoxicity and other organ involvement. In addition, we have seen reversible confusion in 3 patients which fully resolved spontaneously over 2-4 days without treatment or sequelae.
- Death.
- If the Vβ analysis 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 subject in consultation with gene therapy experts, study investigators, DSMB, FDA and NIH.

8.4.2 Stopping rules related to the CNS3 cohort

Accrual to the CNS3 cohort will be stopped for review if:

1) Any subject develops a grade 5 neurological toxicity attributable to CART-19 infusion.
2) Any subject develops grade 3/4 neurotoxicity or agitation, confusion, delirium, or psychosis (psychiatric) toxicity that has not resolved to </= Grade 2 within 4 weeks with or without medical intervention (ie: anti-epileptics).
3) Any non-resolving grade 3 or 4 neuro toxicity or death due to neuro/psychiatric toxicity clearly attributable to the CART cells will lead to a hold on any further CNS3 patients enrolled in this high risk stratum pending subsequent review.

Note on attribution: Toxicity is attributable to CART-19 infusion if, in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the CART-19 cells. In making this determination, the investigator will assess:

- Timing (most other toxicities, other than cytopenias and hypogammaglobulinema are resolved within 28 days of infusion).
- Predisposing structural CNS lesions, prior strokes, or prior seizure history
- Neurologic baseline at study entry
- Events during CRS that may have been predisposing to CNS toxicity (such as significant coagulopathy)
- Infections are most likely attributable to cytopenias due to disease and chemotherapy, and are therefore less likely to be due to CART-19 infusion

8.4.3 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 subject. Nevertheless, generation of an RCL following infusion of the vector product remains a theoretical possibility. The consequences of such recombination events in subjects without a known lentiviral infection are unknown, and therefore subjects 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 subject 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 subject. However, because the probability and characteristics of an RCL are unknown, no guidelines have been put in place. Nevertheless, all agree that the subject must be isolated until an understanding of how to manage the subject becomes clear. Some considerations are

- Intensive follow-up of subject 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 Testing: If a positive RCL assay result is obtained from a subject blood specimen, the PI will be informed and the subject rescheduled for a retest for the DNA test. If the second RCL 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 subjects can

resume, and the patient will be referred to treatment for HIV. If an RCL is confirmed, or the virus cannot be isolated from the blood draw, the patient will be scheduled for apheresis and will undergo a full biological RCL testing for detection and/or characterization of the RCL.

- Clonality and insertional oncogenesis: The occurrence of adverse events caused by insertional mutagenesis in three 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[112]. 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[113]. Monitoring for T cell clonal outgrowth will be performed by flow cytometric analysis for T-body-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 subject's Vβ repertoire is found to be monoclonal or oligoclonal, the subject'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 subject in consultation with gene therapy experts, study investigators, DSMB, FDA and NIH. Further evaluation of the subject will comprise confirmation of the persistence of the clonality within a 3 month period, and monitoring of the subject 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 T 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[69, 132]. If any subject develops excessive CART-19 T cell accumulation, corticosteroids will be administered to eradicate the infused cells.

8.4.4 Management of toxicity

Uncontrolled T cell proliferation. 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[87]. If uncontrolled T cell proliferation occurs, subjects will be treated with corticosteroids. Subjects will be treated with pulse methylprednisolone (2 mg/kg i.v. divided q6-8 hr x 2 days), followed by a rapid taper.

Recommended treatment of cytokine release/ possible macrophage activation syndromes. Given the dramatic clinical improvement of several of the patients treated on this protocol after treatment with anti-cytokine therapy, we will prospectively manage potential cytokine toxicities

with tocilizumab (anti-IL-6), with or without other agents such as steroids and etanercept (anti-TNFα). This management approach is designed to avoid life-threatening toxicities, while attempting to allow the CART-19 cells to establish the proliferative phase that appears to correlate with anti-tumor efficacy. Thus, the timing of the interventions below will be individualized in close consultation with the admitting service.

- Subjects will be treated with tocilizumab (CHOP Formulary dose 8 mg/kg, or 12mg/kg for patients<30kg, as per the package insert) with or without corticosteroids if grade 4 toxicity related to CART-19 cells is observed, such as hypoxia, respiratory distress, or hypotension. If possible and when used, steroids will be rapidly tapered.
- Subjects who have not responded to tocilizumab may be treated with a single dose of etanercept (CHOP Formulary dose 0.8mg/kg) if they have developed significant respiratory distress including intubation.
- Subjects will be treated with a single dose of tocilizumab (as above) if they develop hypotension requiring more than low-dose pressor support.

Events of Cytokine Release Syndrome (CRS) will be reported and graded based on the below criteria for all patients that experience CRS moving forward. Events prior to this amendment will be reviewed and retrospectively be graded and reported by the primary investigator per source documentation. The Penn Grading Scale for Cytokine Release Syndrome (PGS-CRS) will be used for grading of CRS. Please see below for details.

CTL019-Therapy-Associated Grading for Cytokine Release Syndrome: The Penn Grading Scale for Cytokine Release Syndrome (PGS-CRS)

- Marked elevations in IL-6, interferon gamma and less intensely TNF
- Symptoms occur 1 to 14 days after cell infusion in ALL
- Symptoms may include: High fevers, rigors, myalgia, arthralgia, nausea, vomiting, anorexia, fatigue, headache, hypotension, encephalopathy, dyspnea, tachypnea, and hypoxia
- 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.

Table 8-1

1	2	3	4	5
Mild reaction: Treated with supportive care such as anti-pyretics and anti-emetics.	Moderate reaction: Requiring intravenous therapies 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 intravenous fluids* 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
*Defined as: multiple fluid boluses for blood pressure support.				

Table 8-2 High Dose Vasopressor Use

Definition of "High-Dose" Vasopressors	
Vasopressor	Dose for ≥ 3 hours
Norepinephrine monotherapy	≥ 0.2 mcg/kg/min
Dopamine monotherapy	≥ 10 mcg/kg/min
Phenylephrine monotherapy	≥ 200mcg/min
Epinephrine monotherapy	≥ 0.1 mcg/kg/min
If on vasopressin	High-dose if vaso + Norepinephrine Equivalent (NE) of ≥0.1 mcg/min (using Vasopressin and Septic Shock Trial (VASST) formula)
If on combination vasopressors (not vasopressin)	Norepinephrine equivalent of ≥ 20 mcg/min (using VASST formula)
Vasopressin and Septic Shock Trial (VASST) Equivalent Equation: Norepinephrine equivalent dose = [norepinephrine (mcg/min)] + [dopamine (mcg/kg/min) ÷ 2] + [epinephrine (mcg/min)] + [phenylephrine (mcg/min) ÷10] Criteria from Russell et al 2008. **Note:** Pediatric weight adjustments should be taken into consideration.	

B cell depletion. It is likely that B cell depletion and hypogammaglobulinemia will occur in CART-19 patients who have a disease response. This is common with anti-CD20 directed therapies[120]. In the event of clinically significant hypogammaglobulinemia (i.e. systemic infections), subjects will be given intravenous immunoglobulin (IVIG) by established clinical dosing guidelines to restore normal levels of serum immunoglobulin levels, as has been done with Rituximab.

8.4.5 Criteria for discontinuing a subject's participation in the study:

- If a subject develops a condition that precludes CART-19 infusion after enrollment but before infusion, the subject 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.
- For other considerations for premature discontinuation from the study, see Section 4.4.1.

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 approval from the Regulatory Sponsor, and local regulatory review committees per institutional guidelines, is required.

No exception will 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.

Deviation:
A one time, **unintentional** action or process that departs from the approved study protocol, involving one incident and **identified retrospectively**, after the event occurred. If the impact on the protocol disrupts the study design, may affect the outcome (endpoints) or compromises the safety and welfare of the subjects, the deviation must be reported to the Regulatory Sponsor within 5 business days and local regulatory review committees per institutional guidelines.

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

- May have affected subject safety.
- Impacts the integrity of the study design or outcome
- Violates eligibility
- Dose adjustment
- Stopping criteria
- Affect sample size (adding more subjects, decreasing number of subjects, changing the number of subject in a specific arm/cohort)

Other deviations should be explained in a memo to file (such as a subject 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: Study identifier, subject identifier, description of deviation from protocol or exception request, and necessity for exception/deviation. Ensure all completed exception/deviation forms are signed by the Principal Investigator (or co-investigator) and submitted to the Sponsor Project Manager.

The Sponsor Project Manager will submit the exception request or deviation notification to the Regulatory Sponsor for review and approval. Once approval of the exception request or acknowledgement of the deviation has been received from the Regulatory Sponsor, the event will be submitted by the Investigator to the IRB and other local regulatory review committees as required 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 9 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.6.1 Independent Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) comprised of 4-6 individuals including physicians with experience in oncology and/or gene transfer therapy will be assembled and will work under a charter specifically developed for safety oversight of this study. The DSMB will provide advice to the investigators, and consult with Sponsor as necessary. The DSMB will evaluate patient-subject safety as specified in the DSMB Charter.

If necessary, additional meetings of the DSMB may be held if safety issues arise in between scheduled meetings.

8.6.2 Clinical Monitors

Representatives of the Sponsor will conduct a site initiation visit and periodically audit, at mutually convenient times during and after the study, all CRFs and corresponding source documents for each subject. At the site initiation visit, monitors will assure that proper study-related documentation exists, provide training to investigators and other site personnel in study procedures and GCP guidelines, and assure that acceptable facilities and adequately trained staff are available to conduct the study.

Periodic monitoring visits throughout the study provide the Sponsor with the opportunity to evaluate the progress of the study and inform the Sponsor of potential problems. The monitors will assure that submitted data are accurate and in agreement with source documentation; verify that investigational products are properly stored and accounted for, verify that subjects' consent for study participation has been properly obtained and documented, confirm that research subjects entered into the study meet inclusion and exclusion criteria, and assure that all essential documentation required by Good Clinical Practices (GCP) guidelines are appropriately filed.

At the end of the study, monitors will conduct a close-out visit and will advise on storage of study records and disposition of unused investigational products.

9 DATA HANDLING AND RECORD KEEPING

9.1 Confidentiality

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

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

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

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' 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, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

Electronic case report forms will be used.

9.4 Records Retention

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

10 STUDY MONITORING, AUDITING, AND INSPECTING

10.1 Study Monitoring Plan

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

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups 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.

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. The investigator should provide a list of IRB members and their affiliate to the sponsor.

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

12 STUDY FINANCES

12.1 Funding Source

This study is funded by a Pennsylvania Department of Health CURE grant. The cell manufacturing is supported by Novartis Pharmaceuticals.

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All University of Pennsylvania investigators will follow the University conflict of interest policy.

12.3 Subject Stipends or Payments

There is no subject 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 ACGT, the FDA, or other government agencies as part of their duties to ensure that research subjects are protected.

13 PUBLICATION PLAN

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

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15 ATTACHMENTS

15.1 Schedule of Study Procedures

	~Week (-) 8	~ Week (-) 6 to 8	~ Week (-) 4	~ Week (-) 1	~ Day (-) 1	Day 0	Day + 1	Day +5 (+/- 3D)	D 10 §(+/-1D)	D 14(or later)	D17(+/-3D)[26]	D21(+/-4D)	D 28(+/-4D)	D 35 (+/-4D)	**D42 (+/-4D)**	**Monthly to 6 mo. (+/-2 weeks)**	**Quarterly to Year 2[8](+/-1 month)**	**Secondary Follow-up[40]**
	Pre-entry eval/ Screening	Enrollment	Apheresis	Chemotherapy[1]	Pre Infusion	*Infusion*	*Infusion[24]*	Follow-up	Follow-up	*Possible Infusion[23]*	Follow-up (if dose #3 given)	Follow-up	1º endpt.	Follow-up	**Follow-up**	**Follow – up**		**Secondary Follow-up**
Consent	**X**																	
Recent Med. History and PE, PS, GVHD[12]		**X**			**X**	**X[11]**	**X[11]**	**X**	**X**	**X[11]**	**X**	**X**	**X**			**X**	**X**	
Concomitant Meds.		**X**			**X**	**X[11]**	**X[11]**	**X**	**X**	**X[11]**	**X**	**X**	**X**			**X**	**X**	
Adverse Events						**X[11]**	**X[11]**	**X**	**X**	**X[11]**	**X**	**X**	**X**			X	**X**	
Antineoplastic Therapies[33]			**X**							**X**						**X**	**X**	**X**
Influenza A and B				**Within 10 days of infusion**														
HIV test (1 ml SST)	**X**																	
Leukapheresis screening		**X**																
CBC, differential (includes atypical lymphocytes) (5 ml lavender)		**X**			**X**	**X[11]**	**X[11]**	**X**	**X**	**X[11]**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Chemistry[13] (3 ml SST)		**X**			**X**	**X[11]**	**X[11]**	**X**	**X**	**X[11]**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Serum pregnancy test[2] (1 ml SST)		**X**																
Urine pregnancy test[2]					**X**													
Autoantibody Panel[3] (4 ml SST and 3ml EDTA)		**X**																
CMV, EBV, Hepatitis B/C (5ml)[18]		**X**																

	~Week (-) 8	~ Week (-) 6 to 8	~ Week (-) 4	~ Week (-) 1	~ Day (-) 1	Day 0	Day + 1	Day +5 (+/- 3D)	D 10 §(+/-1D)	D 14(or later)	D17(+/-3D)[26]	D21(+/-4D)	D 28(+/-4D)	D 35 (+/-4D)	**D42 (+/-4D)**	**Monthly to 6 mo. (+/-2 weeks)**	**Quarterly to Year 2[8](+/-1 month)**	**Secondary Follow-up[40]**
	Pre-entry eval/ Screening	Enrollment	Apheresis	Chemotherapy[1]	Pre Infusion	*Infusion*	*Infusion[24]*	Follow-up	Follow-up	*Possible Infusion[23]*	Follow-up (if dose #3 given)	Follow-up	1º endpt.	Follow-up	**Follow-up**	**Follow – up**		**Secondary Follow-up**
CD3/CD4/CD8 [17]		**X**				**X**							**X**			X[9]		
β2 Microglobulin[15]		**X**																
Serum Ig levels[16]		**X**																
Chemotherapy[1]				**X**														
CART-19 cell infusion						**X**	**X**			**X**								
Large volume Leukapheresis			**X**[22,25]															
Bone marrow[5]		**X**			**X**								**X**				**X**[27]	
CSF (assessment by lumbar puncture)[5]		**X**			**X**								**X**				**X**[10]	
Lymph Node and other tissue Aspirate/Biopsy		**As clinically indicated**																
Other tumor assessments (i.e. CBC, physical exam)[7]		**X**											**X**			**X**	**X**	
CT Scan/MRI[4, 7]		**As clinically indicated[28,29]**																
ECHO/MUGA[14]			**X**															
Research chimerism (allo cohort only)	**X**[20]				**X**[21]													
Relapse and Survival Follow-up																		**X**[30]
DNA 5cc (Lavender)			**X**		**X**	**X**	**X**	**X**	**X**	**X**		**X**	**X**			**X**	**X**	
QPCR			X		X	X	X	X	X	X		X	X			X	X	

	~Week (-) 8	~ Week (-) 6 to 8	~ Week (-) 4	~ Week (-) 1	~ Day (-) 1	Day 0	Day + 1	Day +5 (+/- 3D)	D 10 §(+/-1D)	D 14(or later)	D17(+/-3D)[26]	D21(+/-4D)	D 28(+/-4D)	D 35 (+/-4D)	**D42 (+/-4D)**	**Monthly to 6 mo. (+/-2 weeks)**	**Quarterly to Year 2[8](+/-1 month)**	**Secondary Follow-up[40]**
	Pre-entry eval/ Screening	Enrollment	Apheresis	Chemotherapy[1]	Pre Infusion	*Infusion*	*Infusion[24]*	Follow-up	Follow-up	*Possible Infusion[23]*	Follow-up (if dose #3 given)	Follow-up	1º endpt.	Follow-up	**Follow-up**	**Follow – up**		**Secondary Follow-up**
DNA RCL (VSV-G Q-PCR)					x[6]											x[6]	x[6]	
Serum 2cc (Red)[31]			**X**		**X**	**X**	**X**	**X**	**x**	**X**		**X**	**X**			X	**X**	
HAMA			X										X			X	X	
HACA									X[32]									
cytokines					X	X	X	X	X	X		X	X			-	-	
PBMC 25cc (Lavender)[31]			**X**		**X**		**X**	**X**	**X**			**X**	**X**			**X[9]**	**X**	
Flow			X		X		X	X	X			X	X			X	X	
Spectra-typing			X		X		X	X	X			X	X			X	X	
Total Research Needs			32		32	7	32	32	32	7		32	32			32	32	
Total Blood Volume (ml)**	21	20	40	20	9	13	13		18	13		38	44			38-44	42	
Total Blood Volume (tbsp)	1.4	1.4	1.4	1.4	0.6	0.9	0.9		1.2	0.9		2.6	3.0			2.6-3.0	2.8	

NOTE: study days may be adjusted as indicated above, and also to avoid weekends. Based on patient status, research blood may be obtained on days other than those indicated above.

**** limit blood volume removed to 3cc/kg at any sampling point. All research blood volumes subject to adjustment for size and patient condition.**

1. Chemotherapy. Fludarabine and cyclophosphamide recommended. Timing and regimen as appropriate for disease type as determined by treating physician.
2. Pregnancy test. Females of reproductive potential only (Tanner I and/or <11 years of age excluded)
3. Autoimmune screen (ANA, ESR)
4. Disease monitoring: staging scans if appropriate to disease. Should be done within 21 days of study entry if required.

5. Bone marrow aspirate and LP (for ALL patients) should be sent as part of standard disease assessment and for research assessments including flow/QPCR for CART-19 cell detection. The enrollment marrow and LP can be (and usually are) data from a prior procedure.
6. The RCL (see protocol for details) test is performed at months 3 and 6 and annually. If all DNA tests during the first year (including the first annual visit) are negative, then samples for future annual tests may be archived.
7. Tumor response assessments will be done according to standard care and practices every 3 months for 2 years after infusion. Assessments are disease specific and are described in Section 6.11.
8. After year 2 or at the time of premature protocol discontinuation, patients will be asked to participate in a destination protocol for evaluation for up to 15 years post T cell infusion, to monitor for delayed adverse events associated with the lentiviral vector genetic modification.
9. Research blood taken at months 3 and 6 only. CD3/4/8 counts will be collected at month 3 only.
10. CSF assessments by LP are performed on Day -1, Day 28, and every 3 months up to 1 year after infusion.
11. Hx, PE, concomitant medications are to be done prior to infusion. Lab results are to be obtained prior to infusion for days 0 & 1 and if applicable day 14 based on the intra-patient dose escalation.
12. GVHD assessment for allo cohort only
13. Chemistry – Glucose, BUN, Creatinine, Sodium, Potassium, Calcium, Total Protein, Albumin, Total Bilirubin, Alk Phos, AST, ALT, Mg, Phos, LDH, Uric Acid
14. Should be performed within 6 weeks of the 1st infusion
15. If used for disease evaluation
16. IgG, IgA, IgM
17. CD3, CD4, CD8 (absolute and %)
18. May use Miller-Keystone autologous panel results.
19. Visit only required if subject received cells on day 28
20. Preferably at time of pheresis. CD3+ lineage-specific & B cell chimerism preferred.
21. Send from manufactured product
22. If a product is not already available
23. Adjust post infusion follow-up days if third infusion is given at a time other than D14. Recommended twice weekly follow-up visits x 2-3 weeks after each infusion.
24. May delay second dose up to 3d for scheduling reasons or to allow a 72h rule out if cultures drawn.
25. Test expansion for exploratory purposes is often sent from pheresis product.
26. Twice weekly follow-ups after each subsequent dose, which may be on D14 or later. The two initial infusion visits will count as the two visit days.
27. Bone marrow assessment (morphology) and MRD assessment on bone marrow are performed on Day -1, Day 28, and every 3 months up to 1 year after infusion.
28. For CNS3 cohort only: Brain MRI/CT (MRI preferred) within 2 weeks of enrollment.
29. For CNS3 cohort only: Brain MRI (same modality as above) within 2 weeks of infusion if known chloromatous disease: otherwise an MRI within 6 weeks of infusion

30. For subjects who complete or prematurely discontinue from the primary follow-up phase while in remission, follow-up attempts will be made to assess the subject's relapse, post treatment antineoplastic therapy, and survival status every 3 months post CART-19 infusion until two years post the last subject infusion. Once subjects' relapse or they begin a new cancer therapy, additional follow-up for relapse will not be required, and subjects will be followed for survival only.
31. In the event that something unexpected occurs, additional research sample collection may be done as necessary.
32. Serum samples may also be used for HACA testing when sufficient samples are available.
33. All prior antineoplastic therapy for the study indication received prior to the start of study treatment will be documented and recorded on the appropriate CRF as required per the CRF Completion Guidelines. All antineoplastic therapies for subjects in remission post CART19 T-cell infusion (HSCT and non-HSCT therapy for their study disease) will be collected and recorded on the appropriate CRF, until the time of disease relapse.

Recommended additional labs after subsequent infusions:

/

Recommended disease assessment and visit schedules vary for these additional infusions based on the scenario:

- **After disease relapse from primary infusion:**
  - Study labs are performed at Day 3, 7, 10, 14, 28, 42, and then monthly.
  - Bone marrow disease assessments are performed from the point of this additional infusion at Day 28, month 3, month 6, month 9, and month 12.
- **No response from primary infusion:**
  - Study labs are performed at Day 3, 7, 10, 14, 28, 42, and then monthly.
  - Bone marrow disease assessments are performed from the point of this additional infusion at Day 28, and then continued per the original schedule from the primary infusions.
- **CART loss/B cell recovery from primary infusion:**
  - Study labs are performed at Day 3, 7, 10, 14, 28, 42, and then monthly.
  - Bone marrow disease assessments are continued per the original schedule from the primary infusions and no additional assessments will be added.

15.2 Suggested Labs

Suggested Supportive Care Labs	
Lab test	Suggested Time Points
Ferritin, D-dimer	3x weekly if admitted
LDH/CRP	Daily if admitted
CD19	Visits D +28 and following
IgG	Visits D +28 and following

15.3 Monitoring Plan

Please refer to the study Monitoring Plan for details.

15.4 Definition of CNS leukemia:

- CNS-1 (no detectable blast cells in a sample of cerebrospinal fluid)
- CNS-2 (blast cells detected in a sample with <5 WBC/mm^3 per cubic millimeter and <10 RBC/mm3)
- CNS-3 (blast cells detected in a sample with ≥5 WBC/mm^3 and <10 RBC/mm3)