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Seattle Children's Hospital and Research Institute

Clinical Research Protocol

Pediatric and Young Adult **L**eukemia **A**doptive **T**herapy (**PLAT**)-**02**: A Phase 1/2 Feasibility and Safety Study of CD19-CAR T Cell Immunotherapy for $CD19^{+}$ Leukemia

Protocol Number:	PLAT-02
Investigational Product:	Defined Composition CD4 and CD8 T Cells Lentivirally Transduced to Express a Second Generation 4-1BB:zeta CD19 CAR and EGFRt
IND Number:	BB IND# 15829
NCT Number	NCT02028455
Development Phase:	Phase 1/2
Study Chair:	Rebecca Gardner, MD Seattle Children's Hospital Seattle, WA 98105 Telephone: 206-469-3300

This study is to be performed in compliance with the protocol, Good Clinical Practice (GCP) and applicable regulatory requirements.

This confidential information about an investigational product is provided for the exclusive use of investigators of this product and is subject to recall at any time. Subject to the foregoing, this information may be disclosed only to those persons involved in the study who have a need to know, with the obligation not to further disseminate this information.

SIGNATURE PAGE

Protocol Title: **P**ediatric and Young Adult **L**eukemia **A**doptive **T**herapy (**PLAT**)-**02**: A Phase 1/2 Feasibility and Safety Study of CD19-CAR T Cell Immunotherapy for $CD19^{+}$ Leukemia

Protocol Number: PLAT-02

Sponsor Acknowledgement:

As the Sponsor representative, I confirm that SCRI will comply with all Sponsor obligations as detailed in all applicable regulations and guidelines. I will ensure that the investigator is informed of all relevant information that becomes available during the conduct of this study.

Rebecca Gardner, MD — Date

Associate Medical Director, Immunotherapy Coordinating Center
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Principal Investigator:

I have read Protocol, including all appendices, and I agree to conduct the study as detailed in this protocol and in compliance with the Declaration of Helsinki, Good Clinical Practices (GCP) and all applicable regulatory requirements and guidelines.

Principal Investigator — Date

LIST OF ABBREVIATIONS AND GLOSSARY OF TERMS

Abbreviation	Term
AE	Adverse Event
Allo-HCT	Allogeneic hematopoietic cell transplantation
ALP	Alkaline Phosphatase
ALT	Alanine transaminase
ANC	Absolute Neutrophil Count
ALL	Acute Lymphoblastic Leukemia
AST	Aspartate transaminase
BUN	Blood Urea Nitrogen
CAR	Chimeric antigen receptor
CBC	Complete Blood Count
CLL	Chronic lymphocytic leukemia
CMV	Cytomegalovirus
CR	Complete remission
CRA	Clinical research associate
CRF	Case report form
CRS	Cytokine release syndrome
CXR	Chest x-ray
CSF	Cerebrospinal fluid
CTCAE	Common Terminology Criteria for Adverse Events
CSL	Correlative Studies Laboratory
CTL	Cytotoxic T lymphocytes
DLI	Donor lymphocyte infusion
DLT	Dose-Limiting Toxicity
DMC	Data Monitoring Committee
EBV	Epstein Barr virus
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EFS	Event-Free Survival
FDA	Food and Drug Administration
g	Gram
GCP	Good Clinical Practice
GVHD	Graft-versus-host disease

h	Hour
HCT	Hematopoietic Cell Transplant
HCT	Hematopoietic Cell Transplant
IBC	Institutional Biosafety Committee
ICF	Informed Consent Form
IRB	Institutional Review Board
ITAMs	Immunoreceptor tyrosine activation motifs
IVIG	Intravenous immunoglobulin
L	Liter
LTFU	Long term follow-up
m^2	Meters squared
mg	Milligram
MHC	Major Histocompatibility Complex
MRD	Minimal residual disease
mL	Milliliter
mm^3	Millimeter cubed
MPF	Multiparameter flow cytometry
MRD	Minimal residual disease
MRD-CR	Minimal residual disease negative Complete Remission
MRD+CD	Minimal residual disease positive Complete Remission
MRP-CRp	Minimal residual disease negative Complete Remission without platelet recovery
MRP+CRp	Minimal residual disease positive Complete Remission without platelet recovery
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NIH	National Institutes of Health
NHL	Non-Hodgkin lymphoma
OS	Overall survival
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase chain reaction
PD	Progressive disease
PR	Partial Remission
RCL	Replication Competent Lentivirus

rhuIL-2	Recombinant Human Interleukin-2
SAE	Serious Adverse Event
SCH	Seattle Children's Hospital
SD	Stable disease
SIN	Self-inactivating
SITE	Study Implementation Team
SPRT	Statistical Probability Ratio Test
T_{CM}	Central Memory T Cell
T_E	Effector T Cell
Treg	Regulatory T cell
TIL	Tumor infiltrating lymphocytes
WBC	White blood cells

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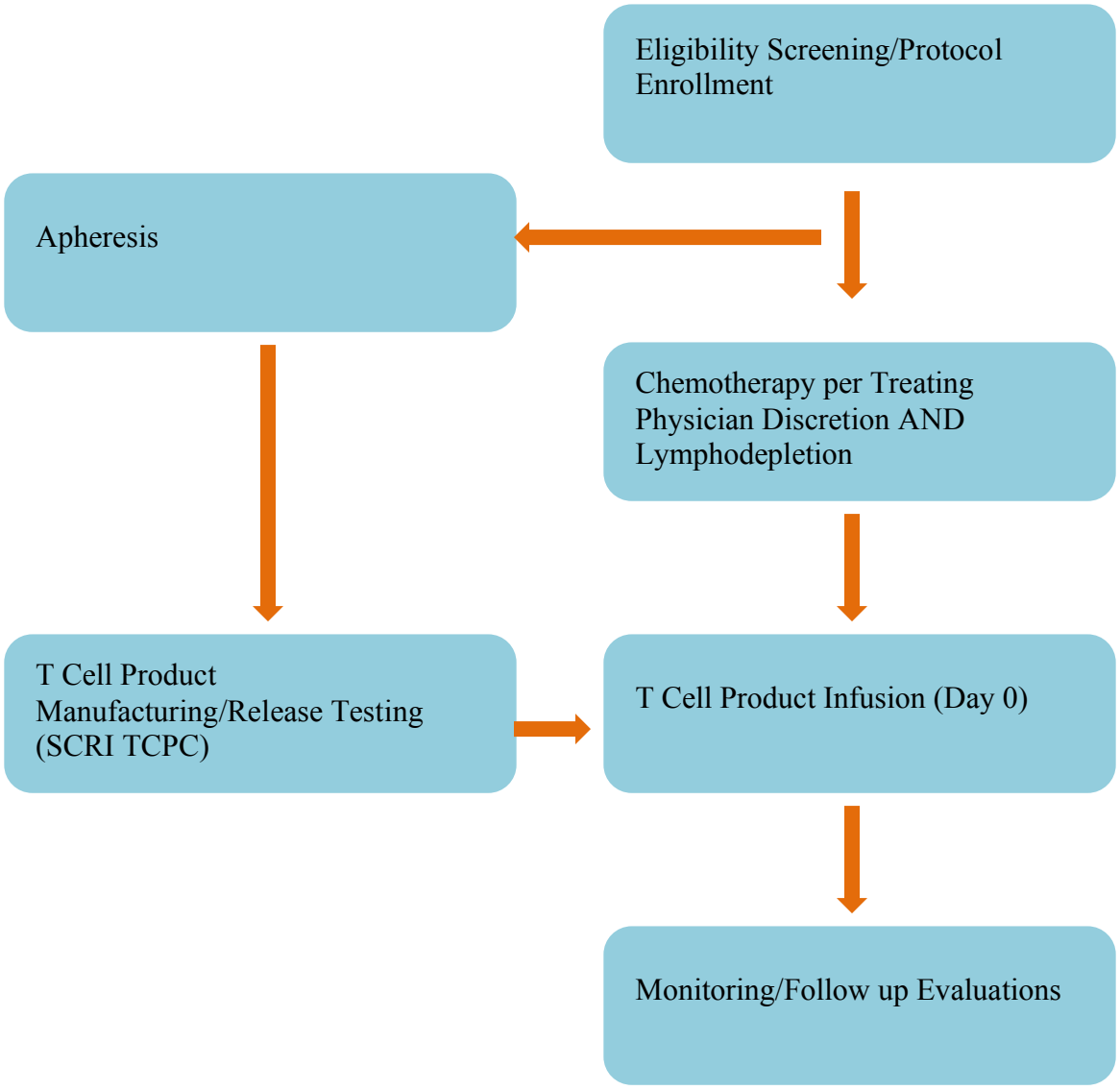
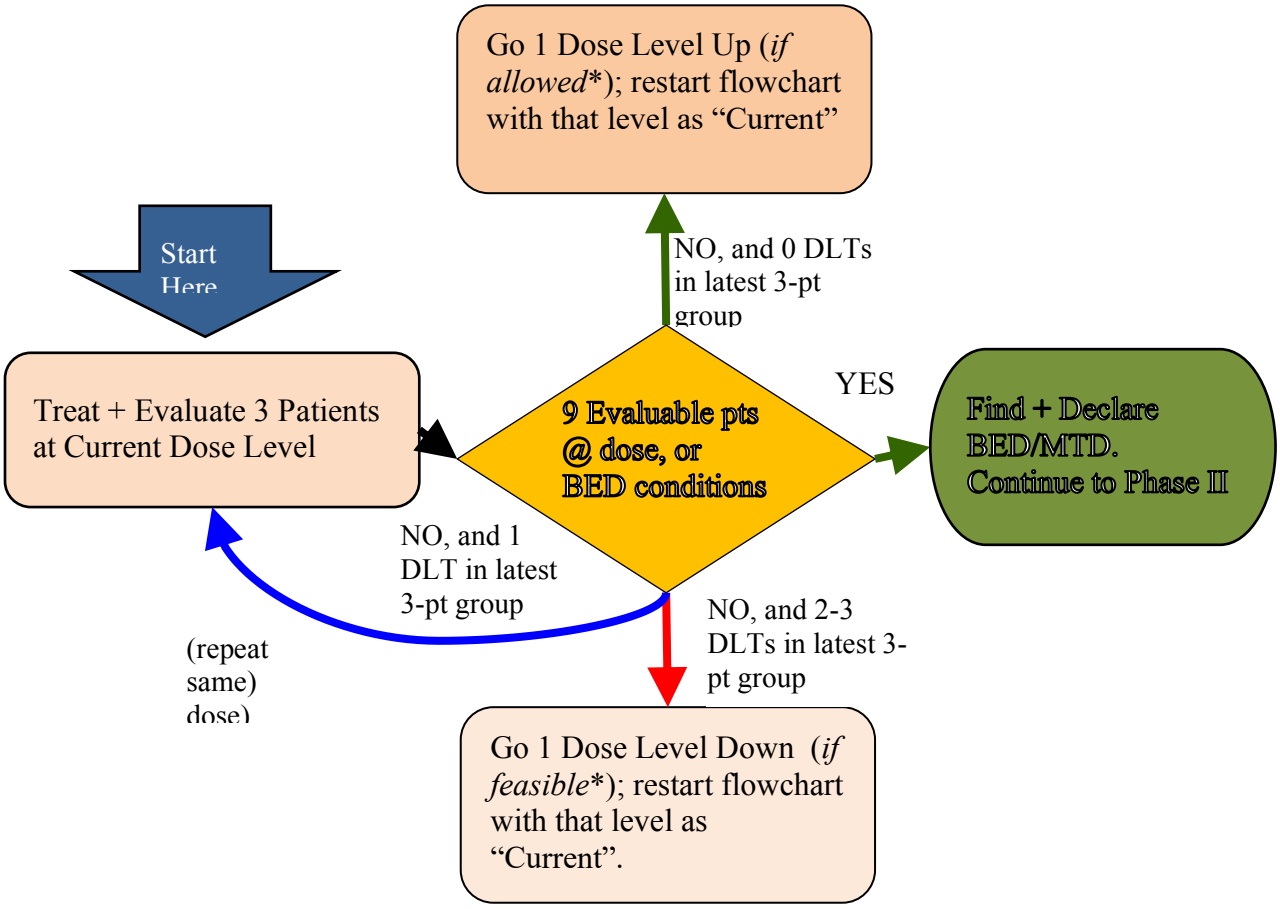
ABSTRACT

This phase 1/2, open-label, non-randomized study will enroll pediatric and young adult research participants with relapsed or refractory $CD19^+$ leukemia with and without prior history of allogeneic stem cell transplant, to examine the safety, feasibility, and efficacy of administering T cell products derived from patient peripheral blood mononuclear cells (PBMC) that have been genetically modified using a self-inactivating SIN lentiviral vector to express a CD19-specific chimeric antigen receptor (CAR) and the selection-suicide marker EGFRt. The primary objectives of the phase 1 portion of the study will be to determine the feasibility of manufacturing the cell product, the safety of the T cell product infusion, and to describe the full toxicity profile, including development of clinically significant GVHD. In the phase 1 cohorts, subjects will receive a single dose of T cells within the framework of a 3+3 dose escalation trial design. Subjects in the phase 2 portion of the study will receive 1 dose of T cells (the dose that has been determined as the maximum tolerated dose during phase 1) and have the additional primary objective of evaluating the efficacy of the T cell product on $CD19^+$ disease as measured by rates of minimal residual disease complete remissions. Two cohorts of participants will enroll in the phase 2, those receiving fludarabine/cyclophosphamide lymphodepletion and those receiving alternative lymphodepletion. During the phase 2 portion, CD19+ Non-Hodgkin lymphoma patients will also be eligible to enroll in the cohorts but they will be analyzed separately for both toxicity and efficacy. CD4 and CD8 T cell subsets will be isolated from apheresis products obtained from the research participant. The CD4 and CD8 CD19 CAR modified T cells will be administered as two separate infusions. Once the patient has recovered from any acute toxicity from re-induction/salvage chemotherapy, the T cell product will be infused via an indwelling catheter or peripheral IV. The secondary objectives of this protocol are to study the engraftment and *in vivo* persistence of transferred cells by quantitative polymerase chain reaction (PCR) for lentiviral vector-specific sequence, to assess T-cell accumulation in the bone marrow and effect on tumor/$CD19^+$ B cell numbers, describe efficacy and toxicity in the MRD positive CR patients and in the frank relapse/refractory disease patients separately, and to assess the incidence of acute GVHD exacerbation and/or T cell engraftment syndrome. Patients who experience significant and potentially life-threatening toxicities (other than clinically manageable cytokine storm/engraftment syndrome) will receive infusions of cetuximab to assess the ability of the EGFRt transgene to be an effective suicide mechanism for the ablation of transferred T cell products.

EXPERIMENTAL DESIGN SCHEMA



PLAT-02 Phase I: Dose Escalation Rules, Visualized



Flow-chart depiction of the main dose transition rules of Sections 10.3.2 - 10.3.3. These rules apply separately to the transition between levels 1Z-1D in Phase I Escalation, and between levels 1E1-1E2 in Phase 1 Extension. Moving one dose level up or down might be infeasible or disallowed, either due to the lack of such a level, or due to additional safety rules described in detail in Section 10.3.2. Rules for whether Phase 1 Escalation continues to its Extension or directly to Phase II are found in Section 10.3.3

1 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Objectives

- To assess the safety and toxicity of cellular immunotherapy utilizing ex-vivo expanded patient-derived CD4+/CD8+ T cells genetically modified to express a CD19-specific scFvIgG4hinge: CD28tm/4-1BBζ chimeric antigen receptor (CAR) and EGFRt in children and young adults who relapse with CD19+ leukemia.
- To describe the full toxicity profile.
- To assess the feasibility of manufacturing and releasing T cell products from pediatric and young adult patients who relapse with $CD19^+$ leukemia after both before and after allo-HCT.
- To estimate the efficacy, defined as a complete remission (CR), minimal residual disease (MRD) negative response to *ex-vivo* expanded patient-derived $CD4^+/CD8^+$ T cells genetically modified to express a CD19-specific scFvIgG4hinge: CD28tm/4-1BBζ CAR and EGFRt in patients with relapsed or refractory $CD19^+$ leukemia.
- To estimate the rate of persistence of functional CAR^+ T cells past day +63.

1.2 Secondary Objectives

- To determine the duration and magnitude of *in vivo* persistence of adoptively transferred T cells in the peripheral blood.
- To assess the accumulation of transferred T-cells in the bone marrow and cerebrospinal fluid (CSF).
- To quantitate anti-leukemic responses by measuring changes in leukemia burden using multi-parameter flow cytometry (MPF), IgH deep sequencing and/or induction of $CD19^+$ B-cell aplasia.
- To determine the incidence of recrudescence or development of acute graft-versus-host-disease (GVHD) in treated patients and its association with the engraftment of transferred T cells for the post-allogenic HCT cohort.
- To assess the efficacy of infusional cetuximab in ablating transferred T cells and ameliorating acute toxicities and/or facilitating B cell recovery in treated patients.
- To separately determine response rates and toxicity rates in the CR, MRD+ group and the refractory group.
- To explore relationships of CD19 antigen load/disease burden and conditioning regimen on persistence of CAR^+ T cells.

2 BACKGROUND

2.1 Introduction/Rationale for Development

Acute Lymphoblastic Leukemia (ALL) is the single most common type of cancer afflicting children with a high overall cure rate of >80%. Nevertheless, recurrent disease is a significant contributor to childhood cancer morbidity and mortality, with overall survival rates of only 40-50% [1-3]. The upfront treatment for childhood ALL involves a prolonged course of multidrug chemotherapy that is dose and time intensive and is augmented for those patients who are identified as high risk [1, 4]. As event free survival (EFS) and overall survival (OS) have greatly improved for newly diagnosed patients with ALL, those who relapse after intensive upfront therapy are not experiencing improved salvage rates and often have chemo-resistant disease[5]. At present, the best therapeutic option capable of achieving disease eradication for children with relapsed ALL almost invariably includes an allogeneic hematopoietic cell transplantation (HCT), provided a suitable donor is available and the disease is controlled prior to transplant [6-8]. Post relapse survival (PRS) is affected by timing of relapse, site of relapse, and age at diagnosis, with increasing age portending a worse prognosis. For those who relapse early (< 36 months from diagnosis), the 3-yr PRS is 30%, whereas those who relapse late (≥ 36 months from diagnosis) have a 3-yr PRS of 57.8% [9]. Additionally, patients who have a marrow relapse, with or without extramedullary disease, have the worst outcomes. Additionally, patients who relapse a second or greater time, have decreasing rates of responsiveness to chemotherapy, with declining rates of CR (44% with 3rd treatment, and 27% with 4th treatment) and OS of <20% [3].

Current data indicate that the presence of minimal residual disease (MRD), as defined by greater than 0.01% leukemic cell burden in bone marrow by multiparameter flow (MPF), at the time of allo-HCT is a significant predictor of post-HCT relapse and poor overall survival. The 2-year disease free survival (DFS) for children with relapsed ALL who achieve a complete remission with no detectable disease prior to transplant is 70%, whereas those who have minimal residual disease at the time of transplant do much worse, with a 2-year DFS of about 20% [10]. In analyzing the patients on the COG relapsed ALL study AALL01P2, MRD was determined at the end of each block of chemotherapy [2]. Of the patients who were in CR2 at the end of block 1 (81%), 62% remained MRD positive, indicative of a worse prognosis. If MRD negative CR is the desired response, only 30% of patients achieved that endpoint. The 3-year EFS rate for all patients achieving CR at the end of the first month of reinduction who were MRD positive at the end of block 1 is roughly 20%. For patients who were MRD positive, there was a conversion to MRD negative after 2 additional cycles of chemotherapy in approximately 20% of the patients.

What this means is that for relapsed ALL patients who remain MRD positive after re-induction chemotherapy, their overall outcome with standard of care, including HCT is substantially inferior with an EFS of only 20% or less. Many transplant centers are now deferring HCT in recipients who are MRD positive due to their poor outcomes. Thus, the development of therapeutic strategies with augmented anti-ALL activity is warranted to increase the percentage of patients who achieve an MRD negative remission prior to allo-HCT, which in turn would be expected to increase the proportion of patients who are ultimately cured.

Relapse following HCT is often not responsive to further chemotherapy and survival is dismal. Strategies for the treatment of relapse following HCT includes weaning of immunosuppression to invoke a graft versus leukemia (GVL) response, and if feasible, the use of donor lymphocyte

infusion (DLI). Unfortunately, ALL is amongst the least responsive hematologic malignancies to a GVL effect, and this is demonstrated by the frequent occurrence of relapse despite graft versus host (GVH) disease and low response rate to DLI for relapse post HCT [11, 12]. Therefore alternative strategies are called for in the treatment of relapsed ALL post HCT.

$CD19^+$ Non Hodgkin Lymphoma (NHL) occurs in pediatrics, most commonly as Burkitt, Diffuse Large B cell, and lymphoblastic lymphoma. In general, B cells NHL are highly curable with chemotherapy, however with recurrence, they are difficult to salvage and EFS is similar to OS. Due to the lack of effective therapies in this group, they will be eligible for this trial as an exploratory aim.

2.2 Development of Adoptive Therapy for B-Lineage Malignancies using Chimeric Antigen Receptor Re-directed CD19-Specific T Cells

2.2.1 Adoptive Therapy for Viral Infections and Malignant Disease

The feasibility of isolating and expanding antigen-specific T cells for adoptive therapy (AT) of human disease has been validated in numerous clinical trials [13-17]. Dr. Riddell initially studied adoptive T cell therapy with donor-derived $CD8^+$ cytotoxic T-lymphocyte (CTL) clones specific for cytomegalovirus (CMV) as a means of reconstituting deficient viral immunity in allogeneic HCT recipients; and defined the principles and methodologies for T cell isolation, cloning, expansion, and re-infusion[18-20]. A similar approach pioneered by Heslop et al using polyclonal EBV-specific T cells has been effective for preventing or treating post-transplant EBV-associated lymphoproliferative disease after allogeneic HCT [21, 22]. These studies and others have demonstrated that virus-specific T cells derived from donors with established viral immunity and expanded *in vitro* can be transferred with minimal toxicity, mediate virus specific effector functions, and control viral replication as well as eradicate virally (EBV) transformed B-cell immunoblastic lymphoma [23-29].

While the adoptive transfer of tumor-specific T cells can result in tumor eradication in a variety of animal models [30-41], adoptive T cell therapy for human malignancy has been significantly more challenging and less effective than for viral diseases. Perhaps the most clinically robust AT data comes from efforts of the Surgery Branch at the National Cancer Institute (NCI) to treat melanoma [42-52]. Their studies demonstrated that administration of lymphodepleting chemotherapy followed by the adoptive transfer of polyclonal tumor infiltrating lymphocytes (TIL's) specific for melanocyte lineage antigens/tumor-testis antigens and high-dose recombinant human interluckin-2 (rhuIL-2), results in demonstrable tumor regressions in nearly one half of treated patients. Of note, the response rate in patients that exhibited high-level persistent engraftment of transferred T cells was nearly 90%, while no responders are seen in the approximately 50% of patients without T cell engraftment. Interestingly, persisting TILs represented only a small subset of the infused product repertoire, suggesting that the capacity for engraftment and persistence may be linked to the intrinsic programming of a rare population of T cells derived from TIL [51, 52]. The generalization of the NCI group's AT results for melanoma to other tumor types, including hematological malignancies, is the subject of intense ongoing investigation.

2.2.2 Barriers to the Clinical Success of AT

Tumor antigens presented by HLA molecules and recognized by T cells have been identified and can originate from processing of normal or over-expressed tissue-specific proteins, aberrantly expressed onco-fetal antigens and mutated proteins, or minor histocompatibility (H) antigens in the setting of allogeneic HCT [53-58]. However, isolating and expanding high-affinity major histocompatibility complex (MHC)-restricted tumor-reactive T cells from tumor-bearing patients is technically difficult and even when successful, these T cells often fail to eliminate tumors after adoptive transfer [59-61]. Our evolving understandings of the counter-regulatory mechanisms that impede successful tumor therapy have been derived largely from murine models [59-61]. Because many tumor antigens are self-proteins that are expressed in some normal tissues, tolerance mechanisms shape the frequency, avidity, and function of tumor-reactive T cells. Additionally, tumors evade immune recognition through a variety of mechanisms including local recruitment of regulatory T cells (T_{REG}) and other suppressor cells, loss of antigen expression, down-regulation of MHC and co stimulatory molecules, and expression or secretion of inhibitory molecules or cytokines [62-74]. Finally, cultured tumor-reactive T cells often exhibit limited persistence *in vivo* after adoptive transfer, even if high doses of rhuIL-2 are administered to support their survival [44, 75, 76].

2.2.3 Redirected Specificity of T Cells Engineered to Express scFvFc:ζ Chimeric Antigen Receptors

In order to overcome some of these obstacles, we have focused on T cell genetic engineering strategies that serve to equip T cells with tumor specificity through the expression of CARs [77]. CARs are engineered to consist of an extracellular single chain antibody fused to the intracellular signaling domain of the T cell antigen receptor complex zeta chain, and when expressed in T cells, are able to redirect antigen recognition based on the monoclonal antibody's specificity [78]. These chimeric cell-surface molecules have the ability to bind antigen and transduce activation signals via immunoreceptor tyrosine activation motifs (ITAMs) present in the CD3-ζ cytoplasmic tail [79], or both activation and co-stimulatory signals when CD28 and/or 4-1BB/OX40 domains are incorporated in series with CD3-ζ [38, 80].

The design of scFvFc:ζ receptors with target specificities for tumor cell-surface epitopes is a conceptually attractive strategy to generate antitumor immune effector cells for adoptive therapy as it does not rely on pre-existing anti-tumor immunity. These receptors are "universal" in that they bind antigen in a MHC independent fashion, thus, one receptor construct can be used to treat a population of patients with antigen positive tumors. Several constructs for targeting human tumors have been described in the literature including receptors with specificities for Her2/Neu, CEA, ERRB-2, CD44v6, and epitopes selectively expressed on renal cell carcinoma [29, 81-84]. These epitopes all share the common characteristic of being cell-surface moieties accessible to scFv binding by the chimeric T cell receptor. The function of primary human T cells expressing tumor-specific scFvFc:ζ receptors have been evaluated *in vitro*; these cells specifically lyse tumor targets and secrete an array of pro-inflammatory cytokines including IL-2, TNF-α, IFN-γ, and GM-CSF [85].

Adoptively transferred CAR-modified T cells can eradicate established tumors in a variety of animal models [33, 35, 37, 86-89]. Moreover, T cells expressing second or third generation

CARs consisting of co-stimulatory signaling domains appear to be more resistant to activation induced cell death, exhaustion, and T_{REG} mediated functional energy [33, 38, 80, 90-95].

2.2.3.1 CD19 as a target epitope for re-directed T cells

Efforts to target hematologic malignancies with CAR-modified T cells have focused predominantly on cell lineage specific molecules. Normal and malignant B cells express surface molecules that define their lineage commitment and maturation stage, and several are being investigated as targets for antibody or cellular therapy. These include CD20, which is present on B cell lymphomas and CLL, and has been targeted effectively with rituximab [96-98]. However, CD20 is expressed late in B cell development and is often not present on ALL [99]. By contrast, CD19 is expressed on all human B cells beginning from the initial lineage commitment until terminal differentiation into plasma cells, and is present on the majority of adult and pediatric ALL and B cell lymphomas. Genetic studies, in vitro assays, and engraftment assays in NOD/SCID mice have indicated that the leukemia repopulating cells in the common forms of ALL are $CD34^+$ and $CD19^+$, although a subset of Ph^+ ALL may contain $CD19^-$ progenitors [100-103]. Thus, for therapy of ALL and B cell lymphomas, we have constructed a CD19-specific CAR.

2.2.3.2 Construction of the FMC63scFvIgG4hinge: CD28tm/4-1BBζ-T2A-EGFRt CAR cDNA and third generation SIN lentiviral vector.

We have developed a CAR consisting of tumor targeting single-chain variable fragments (scFv's) specific for CD19 [38, 104-110]. Based on the V_H and V_L sequences of the CD19-specific murine IgG1 monoclonal antibody FMC63 published by Nicholson et al, a scFv sequence was constructed *de novo* utilizing PCR [111]. A full length CD19scFvIgG4hinge:CD28tm/41BBζ-T2A-EGFRt cDNA designated ZRX014 and property of ZetaRx Biosciences, Inc, encodes for a 2nd generation CD19scFv CAR and was constructed by PCR splice overlap extension and consists of the human GM-CSF receptor alpha chain leader peptide, FMC63 V_L, Gly-Ser linker, FMC63 V_H, human IgG_4 hinge, human CD28 transmembrane domain, 4-1BB intracellular cytoplasmic domain (residues 191-232) and CD3ζ (residues 31-142). Further, a truncated human EGFR selection/tracking/suicide construct is co-expressed with the CAR in constructs designed with T2A cleavable linkers [112]. When incorporated into third generation SIN lentiviral vectors, transduced T cells exhibit CD19-specific tumor cell recognition, lysis, cytokine secretion and proliferation [113].

The surface expression of EGFRt in conjunction with the CAR provides for a second cell surface marker that allows easy examination of transduction efficiency [112]. Biotinylated Erbitux binds to the EGFRt expressed on the cell surface and can be labeled with fluorochrome for analysis with flow cytometry. Additionally it can be used for enrichment through selection with anti-biotin beads to increase the frequency of transduced cells and thus creating a purified population of CAR expressing T cells. Because the EGRFt lacks an intracellular domain, it does not have any signaling capacity, thus binding of Erbitux to the EGFRt does not affect the cell. Lastly, it has the potential to be used as a suicide gene in the clinical setting with the treatment of Erbitux, a clinically available antibody with a low toxicity profile [112, 114]. After binding of Erbitux, cells undergo antibody-dependent cellular cytotoxicity in vivo. Ongoing work is currently taking place in a primate model to further evaluate the use of Erbitux as a treatment for ablation of

transferred T cells and with its success, we anticipate the use of Erbitux as a strategy in which cells can be ablated if they are no longer required for efficacy or alternatively, could be used in conjunction with glucocorticoids for ablation of T cells due to toxicity.

2.2.4 CD4$^+$ T cell help enhances the function and persistence of CD8+ T cells

CD4$^+$ T cells enhance CD8$^+$ effector T cell persistence, enhance memory formation, and trafficking to antigen rich tissues. Without CD4$^+$ help, CD8$^+$ memory cells undergo a transient partial exhaustion [115]. Additional work has shown that CD4$^+$ help is required to allow CD8$^+$ effector cells entry into infected tissue [116]. Activated CD8$^+$ T cells have shown poor survival when CD4 help is absent and CD4 help is required during recall expansion of memory CD8 cells [117]. Likewise, CAR$^+$ CD4$^+$ T cells enhance CAR$^+$ CD8$^+$ cytolytic effector function both in vitro and in vivo [118]. For these reasons, this protocol will evaluate the use of cell products formulated to comprise a 1:1 mix of CD4$^+$ and CD8$^+$ CAR expressing T cells.

2.2.5 Enhancing Persistence of Transferred Cells

While T cell genetic modification can address some of the issues relevant to tumor AT, our animal studies, as well as data from initial clinical trials demonstrate that the capacity of gene-modified CAR$^+$ effector T cells to persist following adoptive transfer is a major obstacle to achieving therapeutic responses. However, patient preparation for AT by the induction of lymphopenia can enhance the engraftment of adoptively transferred T cells as described below.

2.2.5.1 Lymphodepletion prior to T cell transfer

The size of the T cell pool is subject to homeostatic regulation, and the induction of lymphopenia results in the proliferation of residual T cells to restore T cell numbers [119-121]. The lymphopenic environment may be more favorable for T cell transfer because of less competition for homeostatic cytokines such as IL-15 and IL-7 that promote lymphocyte proliferation and survival [122-124], and the elimination of CD4$^+$ CD25$^+$ regulatory T cells [125, 126]. Direct evidence that the induction of lymphopenia improves the persistence of transferred tumor-reactive T cells was provided by studies at the NCI in which melanoma patients were treated with fludarabine and cyclophosphamide to induce lymphopenia prior to the transfer of 10^{10}-10^{11} polyclonal melanoma-specific T cells. A subset of these patients achieved prolonged high-level engraftment of a few clonotypes in the transferred T cell population, and this correlated with antitumor efficacy [45, 127, 128]. Studies in murine models subsequently confirmed the human data that lymphodepletion can be exploited to improve the antitumor efficacy of transferred T-effector (T_E)cells, and provided evidence that hematopoietic stem cell (HSC) infusion further promoted the antitumor activity of T cell transfer [129, 130]. No systematic analysis has been done to directly compare various lymphodepletion regimens.

2.2.5.2 Lymphopenia post hematopoietic cell transplant

Prolonged lymphopenia is common following allo-HCT. If often takes a year or more to obtain a full repertoire of lymphocytes assuming that there are no major episodes of chronic GVH requiring longer duration of immunosuppression or recurrent infections, particularly CMV reactivation. The CD3$^+$ subset of lymphocytes reaches the 5th percentile of reference range on average 2 months post allo-HCT [131]. Helper phenotype (CD3$^+$CD4$^+$) T cells are delayed until

9 months post-transplant to reach the 5th percentile of reference range, and cytotoxic $CD8^+$ T cells recover more quickly and are within normal range by day+60. Naïve T cells begin their recovery 6 months following allo-HCT, but typically do not reach normal numbers until 2 years post allo-HCT. We anticipate this patient population will be highly lymphodepleted at the time of T cell transfer based on prior allo-HCT and the re-induction/salvage chemotherapy patients will receive.

2.3 Clinical trials using CAR modified T cells

Several clinical trials using CAR modified T cells have been published, most recently using CD19 CAR specific autologous T cells, and at least a dozen are currently accruing patients.

2.3.1 1st Generation CARs

A study of CD19 and CD20 specific CAR modified T cells in adult patients with recurrent non-Hodgkin lymphoma (NHL) was recently published from City of Hope [132]. Autologous $CD8^+$ T cells were isolated from a leukapheresis product. A total of 15 infusions (dose range of 10^8 to $2x10^9$ cells/m^2) were given to four patients with no overt toxicities attributable to the T cell product. Noted toxicities include hepatic toxicity, lymphopenia, and eosinophilia. Also of interest, two patients were noted to have a cellular anti-transgene immune rejection response. The persistence of the transferred T cells was short lived, lasting between 24 hours and 7 days. A similar study was conducted in Seattle using CD20 specific CAR modified T cells for patients with relapsed or refractory indolent B-cell lymphoma or mantle cell lymphoma [133]. Of the nine patients enrolled, products were generated from seven. The seven patients received a total of 20 T-cell infusions with minimal toxicities (dose range of 10^8 to $3.3x10^9$ cells/m^2). In this group, two patients maintained a previous complete response (CR), while one achieved a partial response (PR), and 4 had stable disease (SD). In this report, no anti-transgene immune rejection responses were identified. Of the adverse events (AEs) reported, all grade 2 or lower, they were attributed to the concurrent IL-2 treatment rather than the cell product. In both of these clinical trials, there was only a minimal persistence of the transferred T cells, indicating that additional modifications needed to be made in order to improve persistence.

2.3.2 Enhanced persistence and clinical results with second generation CARs

Both of the above mentioned trials used first generation CARs, which included CD3ζ as the only intracellular signaling domain. By combining a co-stimulatory signal, from either CD28 or 4-1BB, enhanced persistence and clinical responses can be demonstrated. A recent clinical trial performed by the Baylor group examined the persistence of transferred T cells expressing either a first or second generation CD19 specific CAR in the same patient and found that the second generation CAR, which contained the co-stimulatory signal from CD28, had better in vivo expansion and persistence [134]. Six adult patients with NHL were treated on this trial and had two separate T cell products generated over a two-week period with an intra-patient dose escalation, resulting in individual doses between $2x10^7/m^2$ to $2x10^8/m^2$. The infusions were well tolerated, without SAE. The first generation CAR^+ T cells failed to expand after transfer and were undetectable by 6 weeks, whereas the second generation CAR^+ T cells expanded 6.82 fold two weeks after transfer and were detectable up to 6 weeks post infusion. These results suggest a confirmation of animal studies suggesting that 2nd generation CAR^+ T cells have enhanced in-

vivo expansion and persistence. It should be noted that there was no sustained clinical benefit noted in any of the six patients.

The NCI recently reported their findings in a single adult patient with NHL, using autologous CD19- specific second generation (CD28 signaling domain) CAR modified autologous T cells [135, 136]. The first reported patient received $4X10^8$ total cells divided over 2 days following lymphodepletion with cyclophosphamide and fludarabine. The patient also received IL-2 for eight doses following T cell infusion. Acute toxicities included fever for two days, which self-resolved and the patient demonstrated an impressive partial response to treatment that lasted for 32 weeks before progression was noted with increased lymphadenopathy. It should be noted that the patient experienced prolonged B cell aplasia with resulting decreased serum immunoglobulin levels that was clinically not significant and the patient did receive IVIG. In the follow up report, 6/8 patients with either NHL or CLL have had clinical responses.

Table 2-1 Published Trial Results with CD19 CAR modified T cells

NCT	Site	Co—stimu-lation	Disease Treated	Pediatric patients	Lympho-depletion	Dose Range	Clinical Response	Reference
01029366	U Penn	41BB	CLL	N	Optional	145 x 10^6 transduced T cells	6/9	139, 140, 142
01626495	CHOP	41BB	ALL	Y	Optional	1.4-12 x10^6 transduced T cell/kg	2/2	143, 144
00924326	NCI	CD28	NHL, CLL	N	Y	3-30 x10^6 transduced T cell/kg	6/8	136
01593696	NCI	CD28	NHL, ALL	Y	Y	1 x10^6 transduced T cell/kg	3/4	144
00466531	MSKCC	CD28	CLL	N	Y	4-30 x10^6 transduced T cell/kg	3/10	136, Park, JH et al. ASH abstract 2012
01044069	MSKCC	CD28	ALL	N	Y	1.5-3 x10^6 transduced T cells/kg	5/5	137
00586391	Baylor	CD28	NHL/CLL	N	N	2-20 x 10^7 transduced cells/m^2	0/6	134

CHOP Children's Hospital of Philadelphia; NCI National Cancer Institute; MSKCC Memorial Sloan Kettering Cancer Center; Baylor

Preliminary results from two trials conducted at Memorial Sloan Kettering have also been reported using a CD19-specific CAR with a CD28 costimulatory domain [137, 138]. The ex-vivo culture time was short; between 11-19 days. This was a dose escalation trial, with starting doses of 1.2-3.0x10^7 CAR^+ T-cells/kg initially without lymphodepletion, followed by cohorts

that were treated with increasing cyclophosphamide doses, first with 1.5gm/m^2 followed by 3gm/m^2. For the patients who did not receive lymphodepletion prior to T cell transfer, there were no detectable T cells at one month. However, there was persistence of the transferred T cells for 6 weeks noted in some of the patients who received cyclophosphamide conditioning prior to T-cell transfer. There have been 13 reported patients from these trials, 1 of whom was enrolled in cohort 2 and had a SAE with subsequent death that has been reported separately and discussed below as a potential toxicity of CAR therapy [139]. Following the patient death, the next 3 patients received dose level -1 without lymphodepletion, with doses of 0.4-1x10^7 CAR$^+$ T cells/kg. Among the eight patients who received the T cells, the most common side effects included rigors, chills and transient fevers, all occurring within 24 hours of receiving the modified T cells infusions. Most recently, there were five adult patients reported in the trial for ALL[138]. Two had frank relapse at the time of enrolling, two were in CR with MRD$^+$ disease, and one was MRD $^-$. All five patients were MRD negative by day 60 following the T cell infusion. One patient relapsed following loss of the modified T cells in vivo after treatment with steroids. Four of the patients went on to an allo-HCT, 3 of whom are alive today with no evidence of disease and one died from transplant complications with no disease detectable at the time of death.

Researchers from the University of Pennsylvania have an ongoing trial using modified autologous T cells which express a CD19 CAR that has a 4-1BB co-stimulation domain rather than CD28 [140, 141]. Polyclonal T cell preparations were generated ex-vivo by PBMC stimulation with anti-CD3/CD28 beads over a period of approximately 10-12 days. The first three reported patients had CLL and received between 1.46x10^5 to 1.6x10^7 cells/kg. Infused T cells expanded in vivo up to 1000 fold, induced B cell aplasia and in 2 of the 3 patients have induced tumor remissions. A third patient received corticosteroids due to toxicities, which blunted the engraftment and anti-tumor response that did not achieve a full regression of CLL [140, 141]. This trial was updated at ASH 2012 and at that time a total of 10 patients (9 adults with CLL and 1 child with ALL) had been treated, of whom six have had a clinical response, including 4 CRs that have been durable. Maximal T cell response was seen by day+30 following T cell infusion in all cases. See Section 2.4 for full details of toxicities described with these trials.

2.3.3 CD19 CAR T cell Therapy Experience for Pediatric CD19$^+$ Malignancies

Two groups, CHOP and NCI have now reported experience treating relapse CD19$^+$ acute lymphoblastic leukemia and lymphoma in children both before and following allo-HCT. Both groups reported at the ASH conference 2012. At that time CHOP had treated two pediatric relapsed ALL patients, 1 with no prior allo-HCT, and 1 with a prior allo-HCT [142-144]. Both obtained a CR, with the later patient going on to recur with a CD19 negative ALL. Both subjects experienced a cytokine storm, and the first patient required tocilizumab to target elevated IL-6 levels [145] with more details provided below (Section 2.4). NCI reported on the treatment of 3 patients post-allo-HCT with patient-derived CAR modified T cells of donor origin [146]. The first patient obtained a CR following T cell infusion with evidence of mild cytokine elevation not necessitating intervention. The second two patients had detectable modified T cells (1 in peripheral blood and 1 in a malignant pleural effusion) without signs of cytokine elevation and did not have a clinical response. See Section 2.4 for full details of toxicities described with these trials.

2.4 Potential Toxicity Associated with the Infusion of CD19 Specific CAR+ T Cell Lines

Cytokine Release Syndrome: It is commonly observed that patients receiving therapeutic T cells have a mild cytokine release syndrome (CRS) upon infusion of the product, with symptoms of fever, chills, rigor, mild hypotension and mild respiratory distress. These toxicities are typically self-resolving within 48 hours of onset and have been reported in the most recent results from CD19 CAR specific clinical trials [135, 137]. From the U Penn group, they reported no toxicity greater than grade 2 associated with infusion, and NCI and MSKCC reported no infusional toxicities in pediatric patients [143, 146]. Unlike these transient and mild infusional side effects, emerging clinical data with second generation CAR AT for patients with bulky B-cell malignancies has revealed a new toxicity syndrome that occurs in patients who have a robust engraftment of T cells (usually occurring two weeks after infusion) and who experience a concomitant therapeutic response. The most robust data is from the University of Philadelphia in both pediatrics and adults and is described in detail [140, 141, 145]. Several patients required corticosteroids for ablation of the cells in addition to tumor lysis therapy (IV fluids and allopurinol). All patients had serum cytokine levels followed and frequently IL-6, INFγ, IL-8, IL2Rα and MIP1b were elevated. Four patients experiencing severe cytokine storm and high levels of serum IL-6 received tocilizumab and subsequently experienced rapid resolution of their symptoms. One patient required tocilizumab on day D3, and subsequently had no T cell proliferation and no disease response, suggesting the early modulation of the immune response tempers that efficacy. It should be noted that the 3 patients who were non responders did not have elevated cytokines. Memorial Sloan Kettering and CHOP both also reported acute mental status changes during the cytokine surge that are fully reversible [138, 144].

Deaths on study: Two deaths following CAR therapy have been reported in the literature. The first was a patient with metastatic breast cancer receiving a high dose of third generation CAR targeting ErbB2 who had pulmonary toxicity and cytokine release syndrome immediately following the infusion of T cells [147]. The patient received a total dose of 10^{10} cells, and on autopsy, was found to have large numbers of transferred T cells present in lung tissue that also was heavily seeded with metastatic breast cancer nodules. It has been hypothesized that this was due to expression of ErbB2 at low levels on lung epithelial however the impact of tumor targeting in the lung cannot be excluded. The second event occurred in a patient with CLL who developed a sepsis like picture following infusion of $1.2\text{-}3x10^7$ T cells/kg containing a second generation CD19 CAR [139]. Autopsy did not reveal any overt cause of death and the clinical investigators have hypothesized that the patient had low-grade sepsis prior to the infusion of T cells that was magnified in the context of lymphodepletion and transferred T cell expansion.

Alloreactive T cell activation: The use of expanded T cells of donor origin carries the potential risk that a portion of the cell product will consist of T cells that have an endogenous TcR that is alloreactive and capable of mediating a graft versus host response. In this study, for the post allo-HCT cohorts, the donor derived T cells are being obtained from the peripheral blood of the patient following a prior allo-HCT at a time when GVHD is quiescent, off all immunosuppression. Evidence to date that GVHD recrudescence is not a common consequence of CAR T cells infusion when the product is derived from post-transplant patient PBMC comes from the reports of several patients treated with CD19 directed donor derived T cells that were in this setting. Four total pediatric patients have been reported to date, all of who did not

experience GVH following in fusion of the cells. One child treated at CHOP did have an in vivo expansion of cells of donor origin CAR$^+$ T cells, accompanied by CRS and a complete remission [142, 143, 145]. Likewise, of the reported NCI patients, there has not been GVH reported [146]. Additional evidence for GVH immune tolerance that is not reactivated upon T cell activation in vivo comes from reports on the use of blinatumomab (a bispecific antibody binding CD3 and CD19) in the post allo-HCT setting, including after haploidentical transplantation [148]. In these patients there is substantial expansion of CD3 T cells and no GVHD has been observed, suggesting that the activation of donor derived T cells, possibly even including T cells with alloreactive potential does not necessarily lead to clinical manifestations of GVHD and in fact GVHD may occur infrequently or not at all in this context.

Collectively, these data suggest that the repertoire of donor T cells engrafted in patients who fail to flair with GVH following immune suppression withdrawal are not reactivated by their inclusion in CAR T cell adoptive therapy products. More experience is required to better define the true incidence of this theoretical complication and this trial will seek to carefully assess this outcome in treated patients.

Potential long-term toxicity:

B cell Aplasia: A potential long-term toxicity of CD19 directed CAR therapy is prolonged B-cell aplasia. Since non-malignant CD19$^+$ B-cells will be subject to recognition by re-directed T cells, their long-term persistence of the adoptively transferred CD19-specific CTL has the potential to cause B-cell immunodeficiency. In research participants who are predicted to succumb to recurrent/progressive ALL, the clinical sequelae of B-cell lymphopenia that can be ameliorated by intravenous immunoglobulin (IVIG) therapy is an acceptable side effect of CD19-directed immunotherapy, especially since prolonged ablation of normal CD20$^+$ B cells in patients receiving rituximab therapy does not appear to result in clinically significant complications attributable to depleted numbers of normal B cells. In fact, this side effect has been reported in the literature in adult patients who have received CD19 CAR$^+$ T cell therapy with persistence of the transferred T cells and has not been thought to be clinically relevant [135, 137, 140, 141]. Pediatric ALL patients undergoing chemotherapy are already known to be profoundly B cell depleted, so it shouldn't have a large effect on an already almost absent cell population [149]. If the hypogammaglobulinemia becomes clinically significant, then the research participant may be given IVIG as replacement therapy until B-cell function returns. This trial provides for the intervention of cetuximab infusions to potentially ablate the infused CAR T cells that co-express EGFRt and this is an explicit study objective of this protocol for patients that achieve a prolonged remission, continued CAR T cell engraftment in the face of B cell aplasia and opportunistic infection.

Secondary Malignancy/Insertional Mutagenesis: A second potential long-term toxicity relates to Dr. Alain Fischer's gene therapy trials at the Necker Children's Hospital in Paris [150, 151]. Since April 2000, Dr. Fischer's team has treated approximately 20 patients with a SCID-X condition by transducing bone marrow-derived CD34$^+$ cells with a retrovirus encoding the common cytokine-receptor γ-chain gene that forms a T cell growth-factor receptor. Following re-infusion of the transduced stem cells, there is a selective advantage for the survival of T cells that bear intact cytokine receptors that is manifested by the emergence of peripheral transduced T cells within 6 to 12 weeks of treatment. However, as of October 2010, five of the children treated on this protocol have developed a T cell leukemic disease, resulting in 1 death.

Preliminary analysis revealed that the retrovirus used for transduction was inserted into a hematopoietic precursor that was then differentiated into a T cell having a profound proliferative advantage. This adverse event has raised concern that retroviral transduction may have caused the lymphoproliferation as the consequence of an insertional mutagenesis event. However, in 19 patients with ADA deficiency treated with gene therapy, none developed leukemic disease suggesting that there may be disease modifiers which predispose to the development of leukemia.

Recently data regarding the risks of gammaretroviral vectors for T cell modification, rather than stem cell modification was published [152]. The University of Pennsylvania analyzed 221 patient samples obtained over a decade from 43 subjects who had been enrolled in T cell trials, in which CD4ζ CAR's where expressed on autologous T cells (CD4 and CD8) through genetic modification with gene integration directed by gammaretroviral vectors. There was durable engraftment of the modified T cells in 212 of the samples. Through whole genome analysis, a thorough analysis of the integration sites was evaluated in 11 individuals with high-level marking. A total of 7222 unique integration sites were detected and were enriched for sites near the 5' end of a gene. There did not appear to be any clonality in the samples, and the integration sites over time in the same patient varied. Additionally, there did not appear to be enrichment of integration sites near genes involved in clonal expansion or persistence, nor was there enrichment of integration sites near the 5' ends of cancer-related genes. Overall, this report had 540 patient-years without integration-mediated toxicity, placing the true adverse event rate at less than 0.0068 per person-year. This data suggests that the use of gammaretroviruses for gene-integration in T cells carries a much safer toxicity profile than when used in hematopoietic stem cells and that T cells appear to have an inherent protection to insertional genotoxicity.

In addition to transducing T cells rather than hematopoietic stem cells, we are using a third generation SIN lentiviral vector rather than a gammaretroviral vector. Additional safety data has been published regarding the use of lentiviral vectors in clinical trials. This report detailed 65 subjects who received transduced CD4+ cells. The mean copy number was 2.3 with a range of 0.2-5.4. Additionally, all tests for replication competent lentivirus were negative. Previously some of these subjects had integration site analysis performed, which showed that there were >7000 unique insertion sites without evidence of enrichment near proto-oncogene 5' ends or within tumor suppressor genes[153, 154], suggesting that lentiviral vectors may be safer than gammaretroviral vectors in regards to malignant transformation potential.

In summary, we believe that the potential toxicities associated with the use of CD19-specific T cell therapy are outweighed by the need to develop novel treatment for patients with life-threatening ALL.

2.5 T Cell Dosing Rationale

Phase 1 *(enrollment onto Phase 1 completed on 11 May 2016)*:

As noted in the above referenced trials using autologous CAR modified T cells, there is significant inter-study variability in dosing of T cells. Doses were determined either on a per kg, or a per m^2 dose without normalizing to the percentage of T cells in the product that are CAR expressing. The successful study from the University of Pennsylvania had an average dose of 1.45 (range 0.14-5.9) x 10^8 total transduced cells[143] and in the first 3 reported patients, all of whom responded, they received 1.6x10^7/kg, 1.0 x 10^7/kg and 1.46 x 10^5/kg. Notably, the 1st and

3rd patients each obtained a CR despite having a 2 log difference in dose, while the 2nd went into a PR after requiring glucocorticoids. A pediatric CHOP patient received 1.2 x 10^7 transduced T cells/kg with a second receiving 1.4x10^6 transduced T cells/kg [144]. The three NCI pediatric patients received 1x10^6/kg transduced T cells.

There have been no serious infusional toxicities related to CD19 CAR modified T cells and the in vivo expansion with resultant cytokine storm appears to not be related to the initial dose of T cells but rather the magnitude of the in vivo expansion and size of the antigen load. These toxicities are not predicted based on the infusion dose but rather they are more likely due to both culture properties and patient properties. In the U Penn study, all of the patients who obtained a CR had between 100-1000 transduced T cells/µl of blood at the time of maximal expansion, PR patients had between 10-100 transduced T cells/µl of blood, and NR had <10 transduced T cells/µl of blood, stressing the importance of engraftment of the transferred T cells upon response [143]. For those patients who were NR, they did not develop toxicities, suggesting that only patients who have potential clinical benefit from the product are likely to develop toxicities.

Therefore, it is difficult to talk about maximum tolerated doses outside of infusional toxicities. This study started with a dose of 5x10^5 CAR^+EGFRt^+ T cells/kg as there have not been any serious adverse events or dose limiting toxicities noted with this dose in prior CD19 CAR T cell trials.

After treating the first six subjects on this study, variability has been seen in the magnitude of expansion, as well as ongoing level of T cell persistence accompanied by ongoing B cell aplasia. This variability could be attributed to several possible factors. The modified T cells undergo an impressive in-vivo expansion, which may lead to exhaustion and poor persistence.

Table 2-2 Expansion of persistence of T cells of the subjects treated at dose level 1

Subject ID	Peak % Peripheral Blood Expansion (day)	Peak Absolute/µl (day)	Longest time-point of detectable $10^5CAR^+EGFRt^+$ T cells	Length of B cell aplasia
14602-S01	16.6 (d14)	118 (d14)	5 months	5 months
14602-S02	93.6 (d10)	1288 (d10)	d +35	d +35
14602-S03	78.1 (d14)	353 (d14)	Ongoing at 7 months	Ongoing at 7 months
14602-S04	13.4 (d10)	77 (d10)	d+35	d+35
14602-S05	25.2 (d8)	63 (d10)	d+42	d +42
14602-S06	NR	NR	NR	NR

NR = non-responder

Recent data from our group and from the Fred Hutchinson Cancer Research Center (personal communication), have shown a potential benefit to enhanced expansion and long-term engraftment with the use of fludarabine and cyclophosphamide. Additionally, the FHCRC group has shown superior ability to give repeat infusions of T cells with subsequent engraftment rather than rejection. Subjects 23 and 24 were the first two patients treated on this trial to receive this combination of lymphodepletion and both were treated at dose level 3. When compared with the other subjects treated at dose level 3 who only received cyclophosphamide, 23 and 24 had

increased expansion of the T cells in the peripheral blood and bone marrow (Figure 1) but also had increased toxicity. Both S23 and S24 had neurotoxicity that met the definition for a DLT. Early time points indicate that they may also have enhanced long term persistence.

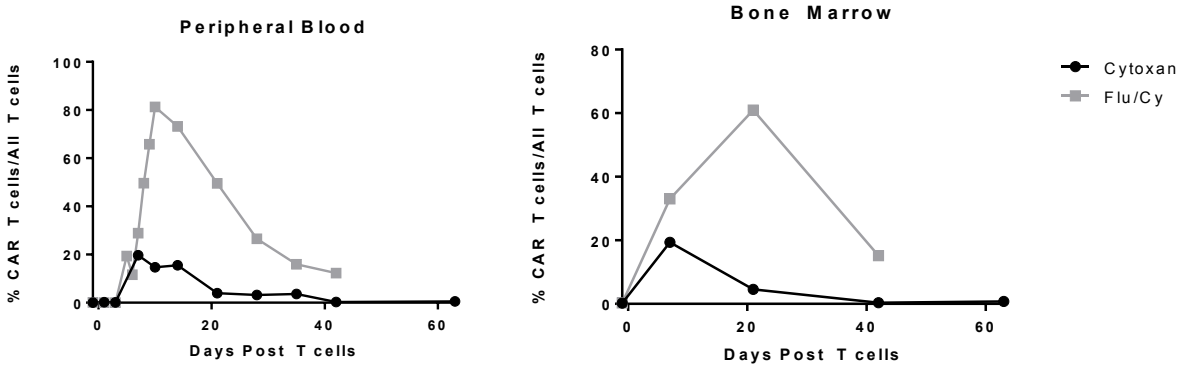


Figure 2-1 Magnitude of engraftment among the subjects treated at dose level 3 with comparison of lymphodepletion regimen.

Table 2-3 Expansion of persistence of T cells of the subjects treated at dose level 3

Subject ID	Peak % Peripheral Blood Expansion (day)	Peak % in Bone Marrow (day)	Longest time-point of detectable 10^5CAR$^+$EGFRt$^+$ T cells	Length of B cell aplasia
14602-S11	59.79 (d14)	16 (d21)	Ongoing at 15 months	Ongoing at 15 months
14602-S13	44(7)	30 (d7)	D+ 42	D+42
14602-S14	30.53 (d7)	28.9 (d7)	D+ 14	D+ 14
14602-S21	10.48 (d10)	17.94 (d7)	D+14	D+14
14602-S23	74.62 (d10)	70.82 (d21)	Ongoing at D+42	Ongoing at D+42
14602-S24	88.0 (d10)	51.09 (d21)	Ongoing at D+42, went to HSCT	Ongoing at D+42, went to HSCT

Paramount to the therapeutic efficacy of this investigational cell product is the ability to obtain reliable long-term persistence. In light of the recent data on fludarabine/cyclophosphamide use for lymphodepletion, we plan to investigate the uniform use of this specified lymphodepletion regimen. Because of the enhanced expansion of T cells with the use of fludarabine, we will begin dose escalation again, starting with dose level 1.

Phase 2 dosing

Overall, 43 subjects have been treated on the phase 1 portion of this study to date, and it has been well tolerated. There have been a total of 7 DLT's across all dose levels. The phase 1 dose escalation demonstrated that with flu/cy lymphodepletion, $0.5x10^6$ CAR^+ T cells/kg is a safe dose (0/6 subjects developed DLTs) while $1x10^6$ CAR^+ T cells/kg is a safe dose after alternative lymphodepletion (1/10 subjects developed DLTs). The phase 2 will open for accrual once the safety of $1x10^6$ CAR^+ T cells/kg is determined (per Section 10.3.3 if at least 6 patients are treated with a <34% DLT rate). If $1x10^6$ CAR^+ T cells/kg is found safe, this will be the RP2D.

Table 2-4 DLTs with flu/cy lymphodepletion

Group/Cohort	DLT description	Patient ID
1F1 (n= 6)	none	NA
1F2 (n=6)	Grade 4 encephalopathy	14602-S41
1C (n=2)	Grade 4 seizure, Grade 2 seizure	14602-S23, 14602-S24

For the cohort of patients receiving alternative lymphodepletion, $1x10^6$ CAR^+ T cells/kg will be the RP2D with a demonstrated DLT rate of 10% (1/10 treated patients).

Table 2-5 DLTs with non-flu/cy lymphodepletion

Group/Cohort	DLT description	Patient ID
1A (n=7)	Grade 4 encephalopathy	14602-S02
1B (n=10)	Grade 4 hydrocephalus	14602-S12
1C (n=4)	NA	NA
1D (n=5)	Grade 3 seizure, Grade 3 left ventricular systolic dysfunction	14602-S19, 14602-22

3 ENROLLMENT PROCEDURES AND ELIGIBILITY CRITERIA

3.1 Study Enrollment

3.1.1 Patient Registration

Patients may be enrolled on the study once all eligibility requirements for the study have been met. Registered patients that are eligible based on a screening questionnaire will be enrolled on a first-come first served selection process based on when the Research Nurse/Protocol Physician is first contacted by the referring physician or patient family.

3.1.2 Informed Consent/Assent

The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the patient or the patient's parents or guardian if the patient is a child, and a signed informed consent and assent will be obtained according to institutional guidelines.

3.1.3 Study Enrollment

Upon consent for participation in an SCRI immunotherapy clinical trial, subjects will be entered into the Immunotherapy Registration Portal. A unique Registration ID will be assigned to each potential study subject, and demographic and general eligibility information will be collected.

All subjects who are consented will be entered into a study-specific database following review of eligibility documentation.

3.1.4 Dose Assignment

Dose and Cohort Assignment will be made at the time of T cell infusion during the phase 1 portion of the study. Dose assignment will be made at the time of enrollment during the phase 2 portion of the study and cohort assignment at the time of T cell infusion based on lymphodepletion chemotherapy administered.

3.1.5 Timing

Eligibility studies must be performed within 7 days prior to enrollment unless otherwise specified.

The eligibility criteria listed below are interpreted literally and cannot be waived unless the protocol PI and IND sponsor petition the Institutional Review Board (IRB) for a planned protocol deviation. All clinical and laboratory data required for determining eligibility of a patient enrolled on this trial must be available in the patient's medical or research record which will serve as the source document for verification at the time of audit.

3.2 Patient Criteria

3.2.1 Inclusion Criteria

1. Male or female subjects ≥12 months of age and <27 years of age at the time of study enrollment.

2. Weight ≥10 kg

3. Diagnosis of $CD19^+$ leukemia or lymphoma:

4a. Disease Status for Phase 1 portion (leukemia only)

If post allogeneic HCT: Confirmed $CD19^+$ leukemia recurrence defined as ≥0.01% disease by SCH or UW Pathology Department following allogeneic HCT.

If Relapse/Refractory status with no prior history of allogeneic HCT (one of the following):

- 2nd or greater marrow relapse, with or without extramedullary disease
- 1st marrow relapse at end of 1st month of re-induction with marrow having ≥0.01% blasts by morphology and/or MPF, with or without extramedullary disease
- Primary Refractory as defined as having M2 or M3 marrow after 2 or more separate induction regimens
- Subject has indication for HCT but has been deemed ineligible

4b. Disease status for Phase 2 portion:

Leukemia

Subject status post allogeneic HCT:

Recurrent $CD19^+$ leukemia defined as ≥0.01% disease in the marrow or isolated extramedullary disease following allogeneic HCT.

Subject with no *prior history of allogeneic HCT* (one of the following):

- 2nd or greater relapse, with or without extramedullary disease (isolated extramedullary disease is eligible)
- 1st marrow relapse at end of 1st month of re-induction with marrow having ≥0.01% blast disease, with or without extramedullary disease
- Primary Refractory as defined as having M2 or M3 marrow after induction
- Subject has indication for HCT but has been deemed ineligible

Marrow involvement:

For those subjects with marrow involvement, the first 15 subjects enrolled after the 03.28.2017 amendment must have detectable disease at the time of enrollment *(this criterion was satisfied as of 1 September 2017)*.

Lymphoma:

CD19+ Non-Hodgkin Lymphoma (NHL) refractory or relapsed with no known curative therapies available

5. Patients with CNS involvement are eligible provided that they are asymptomatic and in the opinion of the study PI have a reasonable expectation that disease burden can be controlled in the interval between enrollment and T cell infusion. Patients that have a significant neurologic deterioration will be not be eligible for T cell infusion until alternate therapies result in neurological stabilization.

6. Lansky performance status score of ≥ 50 or a Karnofsky score of ≥ 50 for patients ≥ 16 years of age. Note: Patients who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.
7. Life expectancy of >8 weeks
8. All patients must discontinue all anti-cancer agents and radiotherapy, and, in the opinion of the study PI, have sufficiently recovered from significant acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy prior to entering this study.
   a. Chemotherapy: Must be at least 7 days since last chemotherapy was administered (this does not include intrathecal chemotherapy which can be administered at any given time pre study or upon enrollment nor does it include maintenance chemotherapy for the subset of patients who relapse during maintenance)

      For patients who were previously enrolled on the trial but were removed prior to receiving T cell therapy and are re-enrolling on the trial and already have a useable T cell product generated during previous enrollment, the duration and chemotherapy agents used is not restricted.

   b. Immunotherapy directed at leukemia: No antibodies within 3 half-lives prior to study enrollment (applicable to phase 1 only).

   c. Steroids: No systemic corticosteroids (unless physiologic replacement dosing) within 7 days of enrollment.

   d. Gene-Modified Cells: No prior genetically modified cell therapy that is still detectable or virotherapy allowed. Patients who are otherwise eligible but have detectable circulating CAR T cells of <5% will be eligible,but will be evaluated as a separate strata from CAR-naïve patients in the phase 2 portion of the study.
9. Adequate organ function as indicated by:
   a. Adequate Renal function defined as: a serum creatinine that is ≤ maximum based on age/gender

Table 3-1 Screening Serum Creatinine

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

b. Adequate liver function defined as:

- Total Bilirubin: ≤ 3X upper limit of normal (ULN) for age OR conjugated bilirubin ≤ 2mg/dl
- ALT (SGPT): ≤ 5X the ULN.

c. Adequate cardiac function defined as shortening fraction >28% by echocardiogram or Ejection Fraction of >50 % by MUGA

d. Adequate respiratory function defined as oxygen saturation ≥90% on room air without supplemental oxygen or mechanical ventilation.

10. Laboratory values meet the following criteria:

    a. Adequate Absolute Lymphocyte count defined as ALC ≥ 100 cells/µl

    b. Documented negative HIV antigen and antibody, Hepatitis B surface antigen, and Hepatitis C antibody within 3 months prior to enrollment. For patient with positive Hepatitis C Ab, negative PCR testing must be documented in order to be eligible.

    c. ONLY APPLICABLE TO THE PHASE 1 cohorts: For the post allo-HCT cohort, chimerism analysis must demonstrate >95% either donor or recipient in peripheral blood CD3 cells, must be done within 1 month of enrollment (no mixed chimerism are eligible)

11. Able to tolerate apheresis procedure, including placement of temporary apheresis line if required.
12. Willing to participate in long-term follow-up for up to 15 years if enrolled in the study and receive T-cell infusion.
13. Subjects of childbearing/fathering potential must agree to use highly effective contraception (Appendix III: Highly Effective Contraception) from the time of initial T cell infusion through 12 months following the last T cell infusion.

3.2.2 Exclusion Criteria

1. Presence of active clinically significant CNS dysfunction (including but not limited to such as uncontrolled seizure disorder, paresis, aphasia, cerebrovascular ischemia/hemorrhage, severe brain injuries, dementia, cerebellar disease, organic brain syndrome, psychosis, coordination or movement disorder)
2. Pregnant or breast-feeding
3. If status post allogeneic HCT, presence of active GVHD or receiving immunosuppressive GVHD therapy within 4 weeks prior to enrollment
4. Presence of an active malignancy other than CD19+ leukemia
5. Presence of an active severe infection defined as:

- A positive blood culture within 48 hours of study enrollment
- A fever above 38.2 C AND clinical signs of infection within 48 hours of study enrollment

6. Presence of any concurrent medical condition that, in the opinion of the PI or designee, would prevent the patient from undergoing protocol-based therapy. Patients with a primary immunodeficiency/ bone marrow failure syndrome are excluded from this trial.

3.3 Definitions

3.3.1 Definition of CNS status

CNS status is defined as:

- CNS 1: In cerebrospinal fluid (CSF), absence of blasts on cytospin preparation, regardless of the number of white blood cells (WBCs).
- CNS 2: In CSF, presence < 5/µL WBCs and cytospin positive for blasts, or >5/µL WBCs but negative by Steinherz/Bleyer algorithm:
  - CNS 2a: <10/µL RBCs; < 5/µL WBCs and cytospin positive for blasts
  - CNS 2b: ≥10/µL RBCs; < 5/µL WBCs and cytospin positive for blasts
  - CNS 2c: ≥10/µL RBCs; ≥ 5/µL WBCs and cytospin positive for blasts but negative by Steinherz/Bleyer algorithm (see below).
- CNS 3: In CSF, presence of ≥ 5/µL WBCs and cytospin positive for blasts (in the absence of traumatic lumbar puncture) and/or clinical signs of CNS leukemia.

STEINHERZ/BLEYER ALGORITHM FOR EVALUATING TRAUMATIC LUMBAR PUNCTURES:

If the patient has leukemic cells in the peripheral blood and the lumbar puncture is traumatic and the cytospin contains ≥ 5 WBC/µL with blasts, the following algorithm should be used to distinguish between CNS 2 and CNS 3 disease:

$$\frac{\text{CSF WBC}}{\text{CSF RBC}} > 2 \times \frac{\text{blood WBC}}{\text{blood RBC}}$$

Therefore, a patient with CSF WBC ≥ 5/µL blasts, whose CSF WBC/RBC is 2X greater than the blood WBC/RBC ratio, has CNS disease at diagnosis.

For example, the following patient would be classified as CNS3:

CSF WBC = 60/µL; CSF RBC = 1500/µL; blood WBC = 46000/µL; blood RBC = 3.0x106/µL

$$\frac{60\ /\mu L}{1500\ /\mu L} = \mathbf{0.4} \quad \mathbf{> 2x} \quad \frac{4600\ /\mu L}{3.0 \times 10^6\ /\mu L} = \mathbf{0.015}$$

3.3.2 Definition of Bone Marrow Status Morphology

- M1 Marrow: less than 5% blasts by morphology in bone marrow aspirate
- M2 marrow: 5-25% blasts by morphology in a bone marrow aspirate
- M3 marrow: >25% blasts by morphology in a bone marrow aspirate

Minimal Residual Disease (MRD)

- MRD negative (MRD-CR): M1 marrow with <0.01% blasts detected by MPF or PCR
- MRD positive (MRD+CR): M1 marrow with ≥0.01% blasts detected by MPF or PCR

4 TREATMENT PROGRAM

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 3 days) from protocol directed therapy and/or disease evaluations for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are (except where explicitly prohibited within the protocol) do not constitute failure to comply with the protocol prescribed study calendar.

4.1 Overview of Treatment Plan

This is a single center phase 1/ multi-institutional phase 2 study.

4.1.1 Phase 1 Dosing Regimen (enrollment onto Phase 1 was completed on 11 May 2016)

The primary goal of the phase 1 portion is to evaluate the feasibility and safety of cellular immunotherapy that utilizes patient-derived T cells that have been genetically modified to co-express a second generation CD19-specific CAR and EGFRt for patients with relapsed $CD19^+$ acute lymphoblastic leukemia following allogeneic HCT. The cohort dose levels are as follows:

Dose Escalation Cohorts:

1Z *Dose Level -1*: Target Dose $2.5x10^5$ # of $EGFRt^+$ T cells/kg (range $>1x10^5$ to $2.5x10^5$) on Day 0

1A *Dose Level 1*: Target Dose $5x10^5$ # of $EGFRt^+$ T cells/kg (range $>3x10^5$ to $5x10^5$) on Day 0

1B *Dose Level 2*: Target Dose $1x10^6$ # of $EGFRt^+$ T cells/kg (range $>7.5x10^5$ to $1x10^6$) on Day 0

1C *Dose Level 3*: Target Dose $5 x10^6$ # of $EGFRt^+$ T cells/kg (range $>3x10^6$ to $5x10^6$) on Day 0

1D *Dose Level 4*: Target Dose $1x10^7$ # of $EGFRt^+$ T cells/kg(range $>7.5 x10^6$ to $1x 10^7$) on Day 0

Dose Escalation Cohorts with prescribed Fludarabine/Cyclophosphamide Lymphodepletion:

1F1 *Dose Level 1*: Target Dose $5x10^5$ # of $EGFRt^+$ T cells/kg (range $>3x10^5$ to $5x10^5$) on Day 0

1F2 *Dose Level 2*: Target Dose $1x10^6$ # of $EGFRt^+$ T cells/kg (range $>7.5x10^5$ to $1x10^6$) on Day 0

As of 2/29/16 protocol amendment, the Phase 1 extension cohorts are no longer applicable.

Phase 1 Extension Cohorts – to open if no biologically effective dose (BED – see Section 10.3.3) has been identified in the proceeding cohorts and it will enroll a minimum of 6 patients and a maximum of 15 patients in order to collect data on long term engraftment strategies.

The extension cohorts will aim to overcome exhaustion of T cells in order to promote long term engraftment. The timing of the 2^{nd} dose will coincide with the contracting of the 1^{st} dose, as the antigen burden has been lowered. Responding patients are MRD negative by day 21 with <1% CD19+ cells in the peripheral blood or bone marrow. The 2^{nd} dose will begin as the MTD, but may escalate to 1 dose level higher, as the higher cell dose in the presence of less antigen should be better tolerated with less toxicity.

1E1 Dose 1 (day 0): target dose of MTD from Dose Escalation Cohorts

Dose 2 (day 21): target dose MTD from Dose Escalation Cohorts

1E2 Dose 1 (day 0): target dose of MTD from Dose Escalation Cohorts

Dose 2 (day 21): target dose of 1 dose level above MTD from Dose Escalation Cohorts

If a cell product does not make the target dose but otherwise is releasable, it will still be administered to the patient assuming they meet eligibility criteria to undergo T cell infusion. However, in the absence of a DLT, they will not be counted towards the evaluable cohort. During the phase 1 cohort, the first 3 patients on each dose level will have their T cell infusions no sooner than 14 days from the prior subject's T cell infusion.

If a subject has a releasable cell product but the trial is currently suspended for T cell infusions while awaiting the mandated period in between subjects or the data is under review by the DMC, and the patient is not medically stable to wait until the trial is open to cell infusion, subjects may receive their T cell product at one dose level below the currently enrolling dose, assuming that dose has previously been deemed tolerable.

4.1.2 Phase 2 Dosing and Lymphodepletion Regimen

There will be two separate cohorts of patients: Those receiving lymphodepletion with fludarabine/cyclophosphamide, and those receiving alternative lymphodepletion. The alternative lymphodepletion subjects are those with a contraindication to receiving fludarabine/cyclophosphamide combination chemotherapy. Cohort assignment will be made at the time of T cell infusion based on the lymphodepletion chemotherapy administered. The dosing in each cohort will be the highest tolerated dose from the Phase 1 portion of the trial. The dose is comprised of both $CD4^+EGFRt^+$ and $CD8^+EGFRt^+$ cells which are infused separately, with the intention of providing the total target dose as a 1:1 ratio of the two products.

Flu/Cy cohort (2A)

T cell infusion with prescribed Fludarabine/Cyclophosphamide Lymphodepletion

Target Dose $1x10^6$ # of $EGFRt^+$ T cells/kg (range $>7.5x10^5$ to $1x10^6$/kg with a maximum total dose of 1×10^8) on Day 0

Alternative lymphodepletion cohort (2B)

T cell infusion for patients with contraindications for Fludarabine/Cyclophosphamide Lymphodepletion

Target Dose $1x10^6$ # of $EGFRt^+$ T cells/kg (range $>7.5x10^5$ to $1x10^6$/kg with a maximum total dose of 1×10^8) on Day 0

4.2 Definition of Toxicity

4.2.1 Treatment related toxicity

Treatment related toxicity will be based on toxicities observed following the infusion of T cells and based on changes from the patient's baseline toxicity profile documented within 24 hours prior to the T cell infusion. Toxicities will be recorded using the NCI CTCAE 4 (see Section 12.3). The duration of study subject monitoring for treatment related toxicity is broken up into

acute (within 60 days following the first T cell infusion) versus late (up to 15 years from time of T cell infusion).

4.2.1.1 Dose Limiting Toxicity (DLT) (Applicable only to the phase 1 portion with CRS grading per the CTCv4):

Any grade 3 or higher toxicity; and designated as definitely or probably related (level of attribution) to the infusion of the T cells; and occurring within 30 days of T-cell infusion except for the following:

Non DLT Toxicities at time of T cell infusion:

- ≤ Grade 4 Fever for less than 48 hours after completion of the T cell infusion
- ≤ Grade 3 Chills lasting for less than 24 hours after completion of the T cell infusion
- ≤ Grade 3 Cough lasting for less than 24 hours after completion of the T cell infusion
- ≤ Grade 3 Transaminases lasting for less than 7 days after completion of the T cell infusion
- ≤ Grade 3 Hypotension lasting for less than 48 hours after completion of the T cell infusion
- ≤ Grade 3 Cytokine release syndrome lasting for less than 48 hours after completion of the T cell infusion

Non DLT Toxicities at the time of T cell engraftment/in vivo expansion (usually occurring up to 3 weeks after completion of the T cell infusion):

- ≤ Grade 4 Fever lasting up to 2 weeks
- ≤ Grade 3 Chills lasting up to 5 days
- ≤ Grade 3 Transaminases lasting up to 2 weeks
- ≤ Grade 4 Hypotension, limited to requiring a single pressor for support, resolving to <grade 3 within 72 hours
- ≤ Grade 4 Cytokine Release Syndrome with hypotension alone limited to requiring a single pressor for support (not requiring intubation) resolving to <grade 3 within 72 hours
- ≤ Grade 4 Lymphopenia lasting up to 4 weeks
- ≤ Grade 4 Leukopenia lasting up to 4 weeks
- ≤ Grade 3 Bone pain (the result of T cell expansion in marrow compartments) lasting up to 2 weeks
- ≤ Grade 4 Tumor Lysis Syndrome lasting up to 2 weeks
- ≤ Grade 3 Encephalopathy lasting up to 72 hours

OR

1. Any toxicity requiring the use of steroids and/or cetuximab to ameliorate side effects attributable to the infusion of the T cells and occurring within 30 days of T-cell infusion. This does not include the use of steroids to prevent toxicity.

OR

2. Any grade 3 or greater autoimmune toxicity (with the exception of B cell aplasia).

Toxicities of any grade normally expected with apheresis or lymphodepletion will not be considered a DLT with respect to protocol continuation, or dose escalation/de-escalation of T cell dose.

4.2.1.2 Apheresis related toxicities: These toxicities are not a determinant of this trial's safety analysis.

Side effects that can occur during cell collection include nausea, vomiting, fainting or dizziness, seizures, skin rash, hives, flushing (redness and warmness of the skin, usually the face), blood loss, and infection. Tingling of the lips, muscle cramping and, very rarely, changes in the heart rhythm can occur. These can be prevented or made milder by giving calcium supplements, either by mouth or IV. Very rarely, (less than 1 in 1,000 procedures), clotting may occur in the apheresis machine or in a patient and is potentially life-threatening. To reduce the risk of clotting, ACD (acid-citrate-dextrose) and heparin will be given. ACD may increase the risk of bleeding and may cause temporary tingling of the lips and limbs, muscle cramping, seizures, or changes in the heart rhythm. Heparin may also increase the risk of bleeding. Transfusions of both red blood cells and platelets may be required surrounding the procedure.

4.3 Treatment Details and Plan

The following guidelines are for all patients receiving study therapy. A treatment course will be through Day +63 (Day 0 = T cell infusion day).

4.3.1 Apheresis for T cell isolation

Eligibility Criteria at the time of apheresis:

- Patients must NOT have an active severe infection defined as:

A positive blood culture within 48 hours of blood draw OR

A fever above 38.2° C AND clinical signs of infection within 48 hours of apheresis.

- On the day of the apheresis, patients will be cared for in either outpatient or inpatient unit. Apheresis will be done according to institutional standard operating procedure. After undergoing medical clearance, patients will undergo apheresis with a target volume of whole blood processed for approximately 3-4 blood volumes and a total cell collection target of $1X10^9$ total nucleated cells.

Between the time of apheresis and T cell product infusion (approximately 28-35 days) subjects will be returned to the care of their treating physician and may undergo additional therapy aimed at controlling disease burden, as well as, providing lymphodepletion prior to receiving their T cell product. It is also acceptable to not undergo treatment during the time of cell manufacturing and this will be a decision made by the treating physician.

4.3.2 Lymphodepletion

Recommendations for lymphodepletion regimens during phase 1 cohorts 1Z-1D and for phase 2 alternative lymphodepletion cohort 2B: the following are general guidelines for lymphodepleting regimens that may be employed immediately prior to T cell therapy. Lymphodepleting regimens not included here may also be used after consulting with the study chair. If a subject is already lymphodeplete (ALC <500) they may opt to not undergo additional lymphodepletion.

- Single Agent Cyclophosphamide:
  - High Dose Cyclophosphamide (2-4g/m2) with MESNA
- Fludarabine
- Fludarabine/Cytarabine
- Cyclophosphamide/Etoposide

For subjects on the specified lymphodepletion arm (phase 1 cohorts 1F1, 1F2 and phase 2 cohort 2A), they will be required to receive fludarabine/cyclophosphamide lymphodepletion as prescribed below:

- Fludarabine 30mg/m2 IV once daily X 4 days (d1-4)
- Cyclophosphamide 500mg/m2 IV once daily X 2 doses (d3-4)

4.3.3 T cell Product Infusion

Within the two days prior to administering the T cell infusion, the patient will undergo a history and physical exam and protocol prescribed testing. Documented verification that the patient is eligible to proceed per below listed criteria is required.

4.3.3.1 Eligibility Criteria for T-Cell Product Infusion

- Research participant without clinically significant encephalopathy/new focal neurologic deficits.
- Research participant with no current evidence of active GVHD
- Subject has been without supra-physiologic dosing of systemic corticosteroids for at least 7 days prior to planned T cell infusion
- Adequate respiratory function defined as not requiring supplemental oxygen or mechanical ventilation, oxygen saturation 90% or higher on room air.
- Absolute Lymphocyte Count <500/µl or subject has received lymphodepleting chemotherapy administered at least 48 hours prior to T cell infusion.
- Adequate Renal Function defined as: a serum creatinine that is ≤ maximum based on age/gender

Table 4-1 Pre-Infusion Serum Creatinine

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

- Adequate Liver function defined as:
  - Total bilirubin: ≤3x ULN for age OR conjugated bilirubin ≤2mg/dL
  - ALT (SGPT): ≤5x ULN
- Patients must NOT have an active severe infection defined as:
  - A positive blood culture within 48 hours of scheduled T cell infusion OR
  - A fever above 38.2 C AND clinical signs of infection within 48 hours of T cell infusion
- Adequate Cardiac Function: If a patient has received anthracycline chemotherapy after enrollment, they must have an echocardiogram to demonstrate shortening fraction >28% or MUGA to demonstrate ejection fraction of >50%. The echocardiogram or MUGA can be done at any time following the anthracycline (does NOT need to be within 48 hours of the T cell infusion)

4.3.4 T cell Product Infusion Details of Care

In addition to institutional guidelines for infusion of cellular products, subjects are to receive the therapeutic T cell products according to the following guidelines:

- Prior to CAR T cell product administration (in instances of documented allergy or sensitivity to any of the below pre-medications clinical discretion may be used for appropriate substitution):
  - Required: acetaminophen
    - Recommended dosing: 12.5 mg/kg (maximum dose 650 mg), PO
  - Required: diphenhydramine
    - Recommended dosing: 1 mg/kg (maximum dose 50 mg), IV or PO
  - Optional: ondansetron
    - Recommended dosing: 0.15 mg/kg (maximum dose 8 mg), IV or PO
- Investigational product will be infused as rapidly as tolerated through a central venous catheter and should be infused by gravity or syringe push

- Microaggregate filters and leukodepletion filters must not be used for infusion of investigational product

The principal investigator or co-investigator will be present during the infusion and immediately available for 3-4 hours following the infusion.

4.3.5 Retreatment with modified T cells

For subjects who have lost persistence, they may be eligible for additional infusions of T cells if the below criteria are met. The dose of the T cell infusion may be up to the highest dose they previously received or at the recommended phase 2 dose, whichever is higher. Retreatment may not be given sooner than day +30. If a patient receives a 2nd dose, that dose is not counted towards evaluation of that particular dose level (including toxicity and efficacy).

Within the two days prior (unless otherwise indicated) to administering the T cell infusion, the patient will undergo a history and physical exam and protocol prescribed testing. Documented verification that the patient is eligible to proceed per below listed criteria is required.

Inclusion Criteria

- Subject has tolerated prior dose of modified T cell infusion without experiencing a dose limiting toxicity OR if patient did have a DLT, they have fully recovered back to baseline
- Subject has modified T cell product available for release
- Subject has <5 % detectable modified T cells in peripheral blood (can be done at any time prior)
- Subject has evidence of persistence of CD19+ malignant cells OR has CD19+ B cell recovery detected within 1 year of initial T cell infusion
- Research participant without clinically significant encephalopathy/new focal neurologic deficits.
- Oxygen saturation 90% or higher on room air.
- Adequate Renal Function defined as: a serum creatinine that is ≤ maximum based on age/gender

Table 4-2 Serum Creatinine Prior to Subsequent Infusion

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

- Adequate Liver function defined as:
  - Total bilirubin: ≤3x ULN for age OR conjugated bilirubin ≤2mg/dL
  - ALT (SGPT): ≤ 5x ULN

Exclusion Criteria

- Research participant with current evidence of GVHD
- Patient requiring supplemental oxygen or mechanical ventilation,
- Patients has an active severe infection defined as:
  - A positive blood culture within 48 hours of scheduled T cell infusion OR
  - A fever above 38.2 C AND clinical signs of infection within 48 hours of T cell infusion

4.4 General Information

See Section 8 for Supportive Care Guidelines

4.4.1 Hospitalization:

Patients will undergo infusion of the T cell product in the outpatient or inpatient setting. When clinically prudent, patients may be admitted to the hospital following the T cell infusion for careful observation and monitoring should they exhibit any constellation of minor symptoms, which, if to worsen, could place the patient at risk of a serious adverse event.

4.4.2 Central Venous Access

All patients should have central venous access line placed prior to the beginning of T cell infusion. Infusion of a DMSO containing cell product may NOT be infused through a peripheral IV.

4.5 Evaluations

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Section 7.1.

Prior to conducting any study-related activities, written informed consent must be signed and dated by the subject or subject's legal representative. If appropriate, assent must also be obtained prior to conducting any study-related activities. If during the screening period an assessment is performed more than once, the most recent assessment will be the relevant assessment used.

4.5.1 Clinical Assessments

4.5.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Baseline/Screening and at the pre-apheresis visit, then again starting from time of pre-T cell infusion through day +30. The following medications will not be recorded in the eCRF: contrast for radiologic procedures, hydration (unless given in response to an AE), lidocaine given during minor

procedures, saline and heparin used to flush ports, lines, catheters, and other attached medical devices.

4.5.1.2 Demographics

Demographic information (date of birth, gender, race) will be recorded at Screening.

4.5.1.3 Medical History

Relevant medical history, including history of current disease, information regarding other underlying medical conditions, and review of signs/symptoms of CTCAE toxicities will be recorded at designated intervals.

4.5.1.4 Physical Examination

A complete physical examination will be performed by either the investigator or a designated sub-investigator who is a physician at the screening visit and each subsequent evaluation that determines eligibility to enroll and proceed with protocol defined interventions. Qualified staff (MD, NP, RN, and PA) may complete the abbreviated physical exam at all other visits. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the time of presentation.

4.5.1.5 GVHD Assessment

A complete graft versus host review will occur at the time of regularly scheduled visits indicated in Section 7 and will include a history, physical exam and lab work if required. Please refer to APPENDIX II GVHD STAGING, GRADING, AND DIAGNOSIS INFORMATION.

4.5.1.6 Neurological Exam

A complete neurological exam will occur at the designated time points as indicated in Section 7. This should include cranial nerve exam, and should note the presence or absence of encephalopathy, confusion, dizziness, lethargy, seizures, tremors, and difficulty with speaking, purposeful movement, and concentration.

4.5.1.7 Bone Marrow Aspiration

Bone marrow aspiration and biopsy will be performed according to standards of care. Bone marrow specimens will be sent for morphologic evaluation and determination of MRD status as detected by MPF or PCR. Where indicated in the study calendar, portions of these aspirates will be shipped to the Correlative Studies Laboratory (CSL). If the primary oncology team feels so inclined, cytogenetics may also be obtained on bone marrow specimens and the results collected. For patients undergoing non-protocol mandated bone marrow evaluations done for clinical indications prior to patient undergoing HCT, an additional aliquot (up to 5mL) will be requested and if obtained, sent to the CSL.

4.5.1.8 Spinal Fluid Assessment

CSF sampling by lumbar puncture or Omaya accessing will be performed according to standards of care. Spinal fluid will be assessed for cell count, glucose, protein and cytology. If Grade 2 or higher CTCAE CNS adverse events occur, a lumbar puncture will be performed. When indicated

on the evaluations table, an additional 2-3mL aliquot will be taken for central measurement of cytokines and presence of CAR T cells in the CSF and shipped to CSL.

4.5.1.9 FDG-PET

FDG-PET scans will be performed in subjects with known active non-CNS extramedullary disease and for those with NHL

4.5.1.10 Cardiac Function Evaluations

Echocardiograms or MUGA scan to evaluate cardiac function. The same modality should be used at screening and subsequent time points if applicable.

4.5.1.11 Adverse Events

The baseline (within 24 hours prior to T cell infusion) and subsequent toxicity status based on review of all CTCAE version 4.0 elements will be documented by the research team at least as frequently as the medical evaluation schedule set forth in the evaluation table (Section 7). Information regarding occurrence of adverse events will be captured following start of the T cell infusion. Duration (start and stop dates), severity/grade, and relation to the study drug will be recorded on the case report form (CRF). This study will utilize the CTCAE of the NCI for toxicity and performance reporting except for phase 2 for CRS in which study specific grading should be used. The descriptions and grading scales found in the revised CTCAE version 4.03 will be utilized for reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0, which can be downloaded from the CTEP website (http://ctep.cancer.gov). Additionally, toxicities are to be reported on the appropriate data collection forms. Refer to Section 12 for reporting of Adverse Events.

4.5.2 Clinical Laboratory Measurements

Please refer to the table in section 7 for timing of when specific labs need to be obtained.

4.5.2.1 Hematology

Complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count).

4.5.2.2 Comprehensive Metabolic Panel

Serum sodium, potassium, chloride, bicarbonate, calcium, phosphorus, magnesium, BUN, creatinine, alanine aminotransferase (ALT/SGPT), total bilirubin, conjugated bilirubin, uric acid, and LDH.

4.5.2.3 Infectious Disease Studies

HIV antigen and antibody, hepatitis B surface antigen, Hepatitis C antibody and if applicable Hepatitis C quantitative PCR

4.5.2.4 Cytokine Release Syndrome Labs

CRP, LDH, ferritin, d-dimer, fibrinogen, and absolute lymphocyte count

4.5.2.5 Serum Immunoglobulin G

Serum for IgG level

4.5.2.6 Ferritin

Serum ferritin testing.

4.5.2.7 Pregnancy Test

A urine or serum pregnancy test will be obtained during the screening process and sent from female subjects who are of childbearing age prior to their enrollment on the study.

4.5.3 Correlative Studies

Please refer to section 7 for specifics of when these studies should be obtained

- Up to 1mL/kg (maximum of 40mL) peripheral blood samples and/or up to 5mL bone marrow aspirate samples will be sent to the Correlative Studies Laboratory (CSL). Refer to provided study specific lab manual for tube and shipping requirements. Specimens may be processed for molecular analysis of T cell persistence and flow cytometric analysis based on detection of EGFRt in conjunction with T cell surface markers. These samples may also be used to detect the in vitro anti-CD19 activity of the persistent CD19 CAR+ T cells and for serum cytokine analysis. Additionally, DNA based testing may be done to detect low levels of malignant cells.
- Up to 2-3mL of CSF will be sent to the Correlative Studies Laboratory (CSL). Refer to provided study specific lab manual for tube and shipping requirements.
- If peripheral blasts are present in the apheresis sample, they may be frozen for use in future in vitro or in vivo experiments to evaluate the ability of the modified T cells to target the patient's primary leukemic cells.
- Any materials in excess of that needed for prescribed correlative studies will be retained for potential additional analysis predicated on trial findings that cannot be anticipated in advance (see section 15).
- If patient undergoes a tissue biopsy, additional slides or tissue will be requested by the Sponsor and if obtained, sent to CSL to be examined for the presence of CAR modified T cells and/or CD19+ cells either using immunohistochemical analysis and/or PCR based assays.

4.5.4 Long-Term Follow-Up

Research participants who receive engineered T cells will be required to participate in long-term follow-up (LTFU) per the guidelines set forth by the FDA's Biologic Response Modifiers Advisory Committee that apply to gene transfer studies to capture delayed adverse events related to the use of lentivirally transduced T cells.

Screening for Delayed Adverse Events

For the first 5 years following treatment with CAR T cells, patients will be seen yearly. The visit will include an interval medical history, including disease response evaluation if applicable and

physical exam, specifically eliciting for the development of any new clinical conditions, including malignancy, neurologic disorder, rheumatologic or autoimmune disorder, or hematologic disorder. Unexpected medical problems that are felt to be related to the CAR T cells will be collected. If patients are not able to be seen at study site, they may be seen by their primary physician for a physical exam and a blood draw courier kit will be provided to obtain research samples. The physician will be provided with adverse event screening guidelines and will be requested to notify the study team of all new malignancies and unexpected illness felt to be related to the CAR T cells.

Adverse Event reporting will be compliant with 21 CRF 312.32.

Following the first 5 years of LTFU, patients who do not have evidence of persisting gene modified T cells will be contacted yearly by phone for an additional 10 years (for a total of 15 years of LTFU). For those patients who have continued persistence of transferred T cells, they will continue to be seen yearly. For LTFU for at least a total of 15 years, with the expectation that more frequent medically monitoring will be conducted to adequately care for patients who have B cell aplasia and require immunoglobulin replacement therapy.

Research Tests

At time points specified in section 7 LTFU tables, for the first five years after T cell infusion, up to 15mL of blood will be collected and then if required, analyzed for persistence of T cells and replication-competent lentivirus (RCL) at the CSL according to the guidance from the FDA regarding Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events. At year 5, continued annual blood specimens will be obtained for an additional 10 years only from patients with evidence of persistent engraftment of gene modified T cells or a prior positive RCL test.

If a patient receives a repeat CAR T cell infusion on this protocol after beginning LTFU research testing, the testing schedule will restart with the most recent infusion.

If a patient receives a different lentivirus gene therapy product following treatment on PLAT-02, they will be expected to have RCL and persistence testing for that product on the subsequent gene therapy protocol and will no longer have such specimens collected on PLAT-02.

Persistence: PCR for the transgene vector sequence and/or flow cytometric analysis may be done on circulating mononuclear cells to determine the persistence of transferred T cells. Persistence assay testing will be discontinued if patient has had 2 successive negative tests.

Clonality: Patients who at any time point in long term follow up have an absolute increase of >5% of cells positive for the CAR transduction marker will have a repeat test in 1 month. If the % of cells positive for the CAR transduction marker continues to increase, additional testing for clonality will be performed. Clonality may be measured by either examining integration sites or TCR diversity among CAR transduction marker positive cells. If there is evidence of clonality, repeat testing will be performed no later than 3 months.

Replication Competent Lentivirus (RCL): Evidence for RCL will be done using VSVg qPCR at each post-infusion time point during the first year (pre-infusion, 3, 6 and 12 months). If all post-treatment assays are negative during the first year, no additional samples will be collected.

Subsequent myeloablative HCT patients

For the subset of patients that do go on to a subsequent myeloablative allogeneic HCT, the only relevant information for delayed adverse event monitoring will be the research tests, given that the spectrum of concomitant medical issues common to allo-HCT patients such as graft-versus-host disease will preclude an accurate attribution to the gene therapy component of this protocol. For this reason, HCT patients will only be asked to provide a blood sample for required LTFU timepoints.

If the research participant dies while on study, then an autopsy will be requested.

4.6 Concomitant Therapy

All standard supportive care measures for patients undergoing experimental therapies will be used at the discretion of the patient's treating oncologist.

After undergoing leukapheresis, patients are eligible to receive additional chemotherapy per the treating oncologist while their therapeutic cell product is being generated. This includes both intrathecal and systemic, although there is not a requirement for either to be given. Specific regimens to be used will be at the discretion of the treating physician and based on patient specific considerations.

The following agents are <u>not</u> allowed while on study through day +30:

- Supra physiologic dosing of systemic corticosteroids (except as outlined for management or prevention of T cell therapy toxicity OR when given during the time of cell generation when the patient receives non-study specified chemotherapy and must not be given within the 7 days prior to the infusion of the T cell product)
- CD19 directed immunotherapy (for example- murine monoclonal antibodies or derivatives there of; blinatumomab)
- T cell growth factors (such as IL-2, IL-7 or IL-15) interferons, vaccines, and other cellular products), pentoxifylline
- Other investigational agents.
- G-CSF is permitted prior to CAR T cell infusion or when delayed neutrophil engraftment is encountered beyond d+21 following T cell infusion and at any time for life-threatening infections. Pegylated G-CSF and GM-CSF are NOT permitted.

Active infections due to anti-leukemia chemotherapy that occur after study enrollment will be treated according to the clinical standard of care.

If the T cell product has a positive culture that is discovered after being infused into the patient, the treating physician will be immediately notified, blood cultures will be drawn and appropriate antibiotic coverage will be initiated.

5 MANAGEMENT OF TOXICITIES AND COMPLICATIONS

5.1 Management of Symptoms Associated with T cell Infusion

Mild transient symptoms have been observed with adoptive T cell therapy including fevers, chills, rigors, headache, and rarely nausea, vomiting, hypotension and pulmonary toxicity. The management of these symptoms is outlined below.

- Fever, chills and temperature elevations >38.2°C will be managed with additional acetaminophen as clinically indicated, and 1 mg/kg Demerol I.V. for chills (max 50mg). Additional methods such as cooling blankets may be employed for fevers resistant to these measures. All patients who develop fever or chills should have a blood culture drawn with an admission for IV antibiotics and supportive care.
- Headache may be managed with acetaminophen. If unresponsive to acetaminophen, manage with good clinical judgment.
- Nausea and vomiting may be treated with ondansetron IV or PO as well as additional standard anti-emetic treatments per patient preference.
- Transient hypotension will initially be managed by intravenous fluid administration, however, patients with persistent hypotension will require transfer to the intensive care unit for definitive medical treatment.
- If significant hypotension occurs during the infusion, the infusion should be immediately suspended. Significant hypotension is defined as symptomatic and/or systolic blood pressure < 80 mm/hg for age >12 years old, <70 mm/hg for age <12 years old, or a 15% drop from baseline, whichever value is lower.
- Treatment algorithm for significant hypotension: begin with normal saline 10mL/kg over 30 minutes; repeat additional boluses, up to a total of 60ml/kg. Alternatively, may use PRBC in place of normal saline if patient's HCT is <25%. After receiving 40ml/kg, consideration of vasopressors should be prioritized.
- Hypoxemia will be managed with supplemental oxygen and an etiology for hypoxemia will be worked up per standard clinical practice.
- If the T cell infusion is stopped before completion, and the symptoms causing cessation of the infusion have returned to baseline within 30 minutes, the T cell infusion may be restarted at 50% of the rate it was previously infusing, and all products should be completed prior to the expiration date and time listed on the bag label.
- For any acute toxicity occurring during the infusion for which a decision is made to terminate the infusion, the residual T cells should be returned to the TCPC for analysis. Investigation of possible causes of observed signs should proceed and, if necessary, additional medical treatment will be instituted.

5.2 Management of Acute Adverse Event(s) Attributable to Expansion of the Infused T Cells

In managing patients with adverse events during the time of in vivo expansion of genetically modified T cells, additional research labs may be requested. All patients for whom there is

concern of cytokine release syndrome will be admitted to the hospital for observation. During Phase 1 if a patient is suspected of having a cytokine release syndrome, blood samples will be requested up to once per day during the time of illness in order to do cytokine analysis as well as T cell analysis. Patients may receive cytokine directed therapy for symptom control including but not limited to tocilizumab. The use of steroids may be given in an effort to prevent a more serious toxicity.

Table 5-1 Recommended symptom management of CRS

Symptom related to CRS	Suggested Intervention
Fever of > 38.2°C	Acetaminophen (12.5mg/kg) PO/IV up to every 4 hours
Persistent fever of ≥ 39°C for 10 hours that is unresponsive to acetaminophen	Tocilizumab (8-12mg/kg) IV
Persistent fevers ≥ 39°C after Tocilizumab	Dexamethasone 5-10 mg IV/PO up to every 6-12 hours with continued fevers
Recurrence of symptoms 48 hours after initial dose of Tocilizumab	Tocilizumab (8-12mg/kg) IV
Hypotension	Fluid bolus, target hematocrit >24%
Persistent/recurrent hypotension after initial fluid bolus (within 6 hours)	Tocilizumab (8-12mg/kg) IV
Use of low dose pressors for hypotension for longer than 12 hours	Dexamethasone 5-10 mg IV/PO up to every 6 hours with continued use of pressors
Initiation of higher dose pressors or addition of a second pressor for hypotension	Dexamethasone 5-10 mg IV/PO up to every 6 hours with continued use of pressors
Initiation of oxygen supplementation	Tocilizumab (8-12mg/kg) IV
Increasing respiratory support with concern for impending intubation	Dexamethasone 5-10 mg IV/PO up to every 6-12 hours
Recurrence/Persistence of symptoms for which tocilizumab was given ≥ 48 hours after initial dose was administered	Tocilizumab (8-12mg/kg) IV

5.2.1 Ablation of T cells:

Following start of T cell infusions, the following rules for initiation of corticosteroid therapy will be applied:

Research participants experiencing a new grade 3 or higher non –hematologic toxicity with an attribution of ≥4 (probable or definite) to the infused T cells that does not meet the definition of the exclusion criteria for dose limiting toxicity detailed in Section 4.2.1.1 that last for greater than 48 hours and is not controlled by medical intervention (including cytokine directed therapy), will commence with corticosteroid treatment outlined below.

Research participants experiencing a new grade 3 or higher non-hematologic toxicity with an attribution of ≥4 (probable or definite) to the infused T cells, regardless of the exclusion criteria for dose limiting toxicity detailed in Section 4.2.1.1, that in the opinion of the protocol principal investigator or designee, cannot be controlled and puts the research participant in significant risk of an untoward outcome if measures are not taken to ameliorate the toxicity, will commence with corticosteroid treatment outlined below.

Table 5-2 Recommended Steroid dose schedule

Treatment Day	Treatment Type and Dosage
Day 1	Intravenous methylprednisolone at 2 mg/kg/day divided q 8hr
Day 2	Intravenous methylprednisolone at 1 mg/kg/day divided q 8hr
Day 3-4	Prednisone at 1 mg/kg/day divided q 12 hours (begin wean if symptom improvement)
Day 4-5	Prednisone at 0.5 mg/kg/day divided q 12 hours
Day 5-6	Prednisone at 0.25 mg/kg/day divided q 12 hours
Day 6-9	Prednisone at 0.1 mg/kg/day divided q 12 hours

Methylprednisolone may be substituted for prednisone at an 80% dose equivalent. Alternatively, dexamethasone may be given in place of prednisone. All research participants will be hospitalized for at least the first 72 hours of receiving corticosteroids. Such steroid treatment strategies have been clinically shown to reverse the side effects of adoptively transferred cytolytic immune cells such as T cells [155-157].

Cetuximab will be administered to study subjects under the following circumstances:

- Subjects who meet the criteria for corticosteroid treatment (5.2.2.1) and who fail to improve after 48 hours as outlined in 5.2.2.2
- Subjects who exhibit clinically significant GVHD that is unresponsive to corticosteroids and have evidence of EGFRt+ T cell engraftment
- Subjects whose molecular studies indicate a lymphoproliferative disorder arising from a infused genetically modified T cell

- Prolonged B cell aplasia in patients with persistence of infused EGFRt+ T cells who require chronic IVIG replacement in the setting of a durable complete disease remission of >3 years. Remission status must include either multiparameter flow or PCR negative.
- Subjects preparing to undergo a myeloablative HCT who have evidence of EGFRt+ T cell engraftment will receive cetuximab in order to generate additional data as to the efficacy of cetuximab to ablate the EGFRt+ T cells.

Cetuximab shall only be given after conferring with the study PI. Prior to administering, patients will be made aware of potential toxicities associated with the treatment and will be given written documentation.

Dosing recommendations are as follows:

Subjects Age ≥ 18 years based FDA approved dosing:

Loading dose of 400mg/m^2 IV, followed by 250mg/m^2 IV weekly for a total of 4 doses.

Subjects Age <18 years based on phase I data in children [158]:

Dose of 250mg/m^2 IV administered over 1 hour weekly for a total of 4 doses.

Pretreatment with IV diphenhydramine is recommended 30-60 minutes before each dose of cetuximab.

For all subjects receiving cetuximab, prior to each subsequent dose, T cell persistence shall be determined. Subjects should have up to 15 mL of blood drawn for this research study pre-ablation and 1, 3, 7, 10, 14, and 28 days after. If after 2 doses there is no appreciable decrement in the genetically modified T cell burden, no further doses should be given. For research subjects that have documented response to cetuximab, up to a total of 4 weekly doses may be given. Any further cetuximab therapy, if deemed necessary by the investigative team will be administered only after consultation with the FDA.

Additional measures may also be taken to resolve the toxicity associated with T cell infusion such as, but not limited to, immunosuppressive medications such as ATG and calcineurin inhibitors, or chemotherapy agents with immunosuppressive properties the administration of other immunosuppressive agents or cytokine directed therapy.

In managing patients who have evidence of tumor lysis, standard guidelines of care should be followed

6 DRUG INFORMATION

T cell product manufacturing will be conducted in the SCRI Therapeutic Cell Production Core (TCPC). At the end of the culture, cells from both expansion bags will be harvested, washed and formulated in cryopreservation media, and transferred to cryopreservation storage. Cryopreserved cells will be stored in vapor phase in a controlled access LN_2 freezer maintained in the TCPC facility until released for clinical use. Cell product which does not meet release criteria will be reviewed for release on a case-by-case basis by the sponsor representative, study chair and principal investigator at a minimum, in collaboration with the relevant regulatory bodies. On the day of T cell product re-infusion cryopreserved unit(s) will be thawed at the bedside and will be immediately available for infusion.

6.1 Possible Side Effects of Gene Modified T Cells

Cytokine release syndrome and symptoms of neurotoxicity have been associated with the death of patients receiving CAR modified T cells that recognize the same protein we are targeting, CD19. The role of CRS and neurotoxicity as a cause of death has not been ruled out. The risks and side effects may be related to the dose given and are unpredictable. The additional risk of development of graft versus host disease exists in those patients who are receiving donor T cells.

For complete safety information please see the SCRI-CAR19v1 Investigator's Brochure.

7 EVALUATIONS/MATERIAL AND DATA TO BE ACCESSIONED

7.1 Required and Recommended Clinical, Laboratory and Disease Evaluations

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to +/- 3 days until Day +35, and then a +/- 2 week window) from protocol directed therapy and/or disease evaluations for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are permitted (except where explicitly prohibited within the protocol).

Required Evaluations - Phase 1 Table

	Screen (must be within 7 days of enrollment)*	Pre- apheresis	Pre-T cell infusion #1*	Day +1	Day +2	Day +3	Day +4, 5 and 6	Day +7	Day + 8 and 9	Day +10	Day +11, 12, and 13	Day +14	Day +21	Day +28 **	Day +35 **	Day +42 **	Day +63 **
Demography	X																
Inclusion/ exclusion criteria	X	X	X														
Performance status [a]	X																
History and physical	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Review of medical status and systems	X		X	X	X	X	X	X	X	X	X	X	X	X		X	X
Pregnancy test	X																
Height	X		X														
Weight	X	X	X	X	X			X				X	X	X		X	X
BSA	X		X														
Vital signs (temp, pulse, BP, resp rate)	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Pulse oximetry	X	X	X														
GVHD assessment	X		X					X				X	X	X		X	X
Neuro exam			X					X				X					
Review pathologic specimens	X																
Echo or MUGA	X		X [b]														
Hematology [c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Comprehensive metabolic panel [d]	X		X	X	X	X	X	X	X	X	X	X	X	X		X	
Serum IgG			X										X			X	X
Serum Ferritin			X					X				X		X			X
Infectious Disease Testing [e]	X																
BM Unilateral Aspirate [f]	X		X					X					X			X	X
Lumbar Puncture [g]	X		X										X			X	X
Correlative Studies Peripheral Blood [h]		X	X	X		X		X		X		X	X	X	X	X	X

Continued next page

Required Evaluations - Phase 1 Table, *continued*

	Screen (must be within 7 days of enroll-	Pre- apheresis	Pre-T cell infusion #1*	Day +1	Day +2	Day +3	Day +4, 5 and 6	Day +7	Day + 8 and 9	Day +10	Day +11, 12, and 13	Day +14	Day +21	Day +28**	Day +35**	Day +42**	Day +63**
BM evaluation [i]	OBTAINED PER THE TREATING PHYSICIAN'S DISCRETION																
Adverse Events [j]	CONTINUOUS																
Concomitant medications	CONTINUOUS																

* Bone marrow aspirate, lumbar puncture, ECHO/MUGA can be done up to 14 days prior

** If subject has had no detectable CAR T cells for two successive peripheral blood checks after day +14, only samples necessary for RCL detection will continue to be taken. Blood persistence assays will be discontinued; however BM samples will continue to be analyzed.

a Performance status will be assessed using Karnofsky performance status for patients ≥16 years of age or Lansky score for patients < 16 years of age (see Appendix I of the protocol)

b Echo or MUGA should only be repeated if the patient has received anthracyclines following most recent echocardiogram, can be done up to 2 weeks prior to time point

c Complete Blood Count (CBC), Manual Differential and Platelet Count

d Electrolytes, BUN/urea, creatinine, total and conjugated bilirubin, ALT, LDH, Uric Acid, Calcium, Magnesium, Phosphorus

e Can be done up to 3 months prior to enrollment to include HIV, Hepatitis B surface antigen and Hepatitis C Antibody and PCR if required

f Up to 5mL to CSL lab for correlative studies to be sent on all marrow aspirates EXCEPT for the screening marrow; standard morphology and multiparameter flow to be done and/or reviewed at UW or SCH; cytogenetics must be done on a marrow pre-enrollment, can be done at any time time point over the course of the current relapse and does not need to be repeated for the study

g Up to 2-3mL to CSL lab for correlative studies; cell count, glucose, protein, and cytology on all specimens performed as a clinical lab.

h Up to 1mL/kg (not to exceed 40mL) to CSL for correlative studies.

i Any additional bone marrow evaluations done for clinical indications prior to patients undergoing HCT, would request up to5 mL sent to CSL lab for correlative studies

j CTCAE version 4

Required Evaluations - For patients who receive additional T cell infusions

	Pre-T cell infusion #2	Day +1	Day +3	Day +7	Day +10	Day +14	Day +21	Day +28*
Inclusion/exclusion criteria	X							
History and physical	X	X	X	X	X	X	X	X
Review of medical status and systems	X	X	X	X	X	X	X	X
Height	X							
Weight	X	X		X		X	X	X
BSA	X							
Vital signs (temp, BP, pulse, resp rate)	X	X	X	X	X	X	X	X
Pulse oximetry	X							
GVHD assessment	X			X		X	X	X
Neuro exam	X			X		X		
Cardiology (Echo or MUGA)	X[a]							
Hematology [b]	X	X	X	X	X	X	X	X
Comprehensive metabolic panel [c]	X	X	X	X	X	X	X	X
Serum immunoglobulin G level	X						X	
Serum Ferritin	X			X		X		X
Bone Marrow Unilateral Aspirate[d]				X			X	
Lumbar Puncture [e]							X	
Correlative Studies Peripheral Blood [f]	X	X	X	X	X	X	X	X
Concomitant medications	X	CONTINUOUS						
Adverse Events		CONTINUOUS						
Bone marrow evaluation		OBTAINED PER TREATING PHYSICIAN'S DISCRETION						

***** If subject has had no detectable CAR T cells for two successive peripheral blood checks after day +14, only samples necessary for RCL detection will continue to be taken. Blood persistence assays will be discontinued

a ECHO or MUGA should only be repeated if the patient has received anthracyclines following most recent echocardiogram, can be done up to 2 weeks prior to time point

b Complete Blood Count (CBC), Manual Differential and Platelet Count

c Electrolytes, BUN/urea, creatinine, total and conjugated bilirubin, ALT, LDH, Uric Acid, Calcium, Magnesium, Phosphorus

d 5mL to CSL lab for correlative studies to be sent on all marrow aspirates EXCEPT for screening marrow; standard morphology and multiparameter flow; cytogenetics must be done on a marrow pre-enrollment, can be done at any timepoint over the course of the current relapse and does not need to be repeated for the study

e Up to 2-3mL to CSL lab for correlative studies; as well as clinical lab testing for cell count, glucose, protein, and cytology on all specimens performed as a clinical lab.

f Up to 1mL/kg (not to exceed 40mL) to CSL lab for correlative studies.

g Any additional bone marrow evaluations done for clinical indications prior to patients undergoing HCT, would request 5 mL sent to CSL lab for correlative labs

h CTCAE version 4

Required Evaluations - Phase 2 Table

	Screen (must be within 7 days of enrollment)*	Pre- apheresis	Pre-lympho-depletion	Pre-T cell infusion #1	Day +1	Day +7	Day +10	Day +14	Day +21	Day +63
Demography	X									
Inclusion/exclusion criteria	X	X		X						
Performance status [a]	X									
History and physical	X	X	X	X	X	X	X	X	X	X
Pregnancy test	X									
Height	X			X						
Weight	X	X	X	X	X	X		X	X	X
BSA	X			X						
Vital signs (temp, BP, pulse, resp rate)	X	X	X	X	X	X	X	X	X	X
Pulse oximetry	X	X		X						
GVHD assessment	X			X		X		X	X	X
Neuro exam				X		X		X	X	
Cardiology (Echo or MUGA)	X		X [b]							
Hematology[c]	X	X	X	X	X	X	X	X	X	X
Comprehensive metabolic panel [d]	X		X	X	X	X	X	X	X	
Serum IgG				X					X	X
Serum Ferritin				X		X		X		X
Infectious disease testing [e]	X									
Bone Marrow unilateral aspirate [f]	X		X						X	X
Lumbar Puncture [g]	X		X						X	
PET Scan [h]			X						X	X
Correlative Studies Peripheral Blood [i]		X		X			X	X	X	X
CRS labs [j]					Daily when grade 2-4 CRS is occurring					
Concomitant medications	X	X			CONTINUOUS until Day+ 30					
Adverse Events [k]					CONTINUOUS until Day +30					

***** Bone marrow aspirate, lumbar puncture, ECHO/MUGA can be done up to 14 days prior

a Performance status will be assessed using Karnofsky performance status for patients ≥16 years of age or Lansky score for patients < 16 years of age (see Section 17 Appendix I of the protocol)

b Echo or MUGA should only be repeated if the patient has received anthracyclines following most recent echocardiogram, can be done up to 2 weeks prior to time point

c Complete Blood Count (CBC), Manual Differential and Platelet Count

d Electrolytes, BUN/urea, creatinine, total and conjugated bilirubin, ALT, LDH, Uric Acid, Calcium, Magnesium, Phosphorus

e Can be done up to 3 months prior to enrollment to include HIV, Hepatitis B surface antigen and Hepatitis C Antibody and PCR if required

f 5mL to CSL lab for correlative studies to be sent on all marrow aspirates EXCEPT for the screening marrow; standard morphology and multiparameter flow required with each marrow

footnotes continued next page

g up to 2-3mL to CSL lab for correlative studies as well a clinical lab testing for glucose and protein except for screening LP; cell count and cytology on all specimens performed as a clinical lab. Refer to protocol section 4.5.1.8 for obtaining additional LP if grade 2 or higher neurotoxicity occurs

h Only for subjects with known extramedullary, non-CNS, non-testicular disease involvement. Once negative, it does not need to be repeated.

i Up to 1mL/kg (not to exceed 40mL) to CSL lab for correlative studies. See Lab Manual for full details

j CRS labs include CRP, LDH, d-dimer, fibrinogen, ferritin and absolute lymphocyte count.

k CTCAE version 4

Required Evaluations - LTFU (Years 1-5)

Evaluations may occur within a 2 month window

Time Point after most recent T cell infusion (months)	3	4*, 5*	6	7*, 8* 9* 10* 11*	12	15*	18*	24	30*	36	42*	48	54*	60
Medical History [a]			X		X			X		X		X		X
Physical Exam			X		X			X		X		X		X
T cell persistence [b] **	X	X	X	X	X	X	X	X	X	X	X	X	X	X
RCL [c]	X		X		X									
Serum IgG [d]			X		X		X	X	X	X	X	X	X	X
Adverse Event Monitoring [e]	CONTINUOUS													
Bone Marrow Aspirate [f]	OBTAINED PER TREATING PHYSICIAN'S DISCRETION													

* Only applicable if suspected ongoing T cell persistence at last check or required for confirmation of loss of persistence. For subjects with a CD19 negative relapse or a stem cell transplant, samples are required at 6 month intervals rather than monthly, and should be done in conjunction with required RCL time points when applicable.

** Persistence assays will be discontinued if patient has had 2 successive negative tests.

a May be obtained by local physician or treating oncologist. If relevant, records pertaining to disease response and/or relapse will be collected.

b Up to 15mL peripheral blood will be collected at each time point for all research studies (T cell persistence, RCL detection).

c Further time points will be determined by sponsor after review with FDA if RCL testing is positive at any of the time during the 1st year.

d If Serum IgG levels are checked, results will be collected.

e Adverse Event Monitoring will be continuous for the development of a new malignancy, neurologic disorder, rheumatologic or autoimmune disorder or hematologic disorder and the primary physician will be requested to notify the study team.

f Any additional bone marrow evaluations done for clinical indications prior to patients undergoing HCT, would request 5 mL sent to CSL lab for correlative studies

Required Evaluations - LTFU (Years 6-15)

Evaluations may occur within a 2 month window

Time point after most recent T cell infusion (years)	6	7	8	9	10	11	12	13	14	15
Medical History [a]	X	X	X	X	X	X	X	X	X	X
Physical Exam [b]	X	X	X	X	X	X	X	X	X	X
T cell Persistence [c *]	X	X	X	X	X	X	X	X	X	X
Serum IgG [d]	X	X	X	X	X	X	X	X	X	X
Adverse Event Monitoring [e]	CONTINUOUS									

* Persistence assays will be discontinued if patient has had 2 successive negative tests.

a Will be conducted by phone unless subject has evidence of T cell persistence, then may be obtained by local physician or treating oncologist. If relevant, records pertaining to disease response and/or relapse will be collected.

b Only required for those patients who have evidence of persistence of transferred T cells

c If required, up to 15mL peripheral blood will be collected for all research studies (T cell persistence).

d If Serum IgG levels are checked, results will be collected.

e Adverse Event Monitoring will be continuous for the development of a new malignancy, neurologic disorder, rheumatologic or autoimmune disorder or hematologic disorder and the primary physician will be requested to notify the study team.

7.2 Optional Studies

There is no optional portion of the study.

8 SUPPORTIVE CARE

These are provided for institutional consideration. Investigator discretion should be used, and individual considerations made for specific patient situations and institutional practices.

Necessary supportive measures for optimal medical care will be given throughout the study as indicated by the treating physician and the patient's medical need.

8.1 Central Venous Access

See section 4.4.2

8.2 Blood Product Support

- All blood products should be irradiated, leukoreduced prior to transfusion, additional requirements per patient needs
- Efforts should be made to maintain a platelet count > 50,000 mm3 during periods of grade 2-4 CRS unless clinically the patient has a higher threshold
- Packed RBC transfusions should be used to keep hematocrit level ≥ 25% during periods of grade 2-4 CRS, unless clinically the patient has a higher threshold

8.3 Hospitalization

See section 4.4.1

8.4 Antimicrobial Prophylaxis

- PJP prophylaxis should be given according to institutional standards.
- Prevention of fungal infection should be given according to institutional standards.
- IVIG or substitute should be administered when quantitative gamma immunoglobulins (IgG) are below 400 mg/dL or according to institutional standards.

8.5 Diet

An immunosuppressive diet should be maintained while the patients are on protocol.

9 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

9.1 Criteria for Removal from Protocol Prescribed Therapy

A subject is considered to be receiving protocol prescribed therapy for the duration of time during which they have persistence of the CAR T cell product or through Day 63 after the first infusion, whichever occurs later. A subject may be discontinued from study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. All subjects who have received a T cell product per this protocol and subsequently lose T cell persistence will still be expected to adhere to long-term follow up guidelines as set forth by the FDA and this protocol.

The following is a list of possible reasons for study treatment discontinuation:

- The judgment of the principal investigator that the patient is too ill to continue prior to T cell infusion.
- Patient/family noncompliance with study therapy and/or clinic appointments.
- Pregnancy
- Voluntary withdrawal; a patient or his/her parents/legal guardians may remove himself/herself from the study at any time without prejudice.
- Significant and rapid progression of leukemia requiring medical interventions or treatment for leukemia including chemotherapy and stem cell transplant.
- Subject is enrolled onto PLAT-03 cohort A
- Patient is unable to undergo apheresis
- Technical difficulties are encountered in the T cell genetic modification, cloning, and expansion procedure precluding the release of T cell product for infusion.
- If a patient had successful generation of a therapeutic T cell product but was removed from the study prior to receiving the infusion, they will be eligible to re-enroll on the trial at a future date if they meet the eligibility criteria and a space is available.
- Meets off study criteria

All subjects who prematurely discontinue study treatment should come in for an early discontinuation visit as soon as possible. All subjects who have received a CAR T cell product on this protocol and are subsequently removed from protocol therapy will be required to adhere to long-term follow-up adverse event and sample collection requirements for 15 years from the time of final CAR T cell product administration, as mandated by the FDA. Subjects who receive subsequent lentiviral gene modified cells will only be followed on this trial for survival and disease status (see Schedule of Procedures).

A reasonable attempt will be made by the investigator to provide a reason for subject withdrawals; if obtained, the reason for the subject's withdrawal from the study will be documented in the subject's source documents.

9.2 Off Study Criteria

- Death.
- Lost to follow-up.

- Withdrawal of consent for any further data submission.
- 5 year anniversary of T cell infusion and has been enrolled onto a LFTU protocol for gene therapy
- 15 year anniversary of T cell infusion
- Never received CAR T cell product

9.3 Replacement of Subjects

Subjects who are removed/withdraw from the study prior to receiving T cell therapy will be replaced.

Subjects who are removed/withdrawn from the study prior to day + 30 for a reason other than toxicity, that preclude them from a full toxicity evaluation and who do not meet the definition of a DLT, will be replaced.

Subjects in the Phase 2 cohort who are removed/withdrawn from study prior to Day +63 for reason other than disease progression or toxicity, without obtaining MRD negative response, will be replaced.

Subjects in the Phase 2 who received products manufactured with ExpAct Beads will be replaced.

9.4 Temporary Suspension of T Cell Infusion

T cell infusions will be immediately halted and not re-initiated until proper consultation with the DMC, IRB, IBC, FDA for:

- Unexpected patient death due to infused T cells
- If there is unexpected Grade 5 toxicity definitely, probably or possibly attributed to study treatment while on study.
- Lack of Improvement of SAE requiring corticosteroid and/or cetuximab administration.
- If any research participant receiving systemic corticosteroids and/or cetuximab for ablation of T cell-mediated side effects does not show an improvement of the toxicity that prompted corticosteroid ablation to a toxicity grade of <3 within 14 days of initiating ablation procedures.
- Leukemia lymphoproliferative syndrome involving gene modified T cells or detection of RCL. The occurrence of a malignancy attributed to lentiviral modified T cells or detection of RCL would result in termination of the protocol.
- Study Termination

The study may be terminated at any time by the Sponsor or the F DA.

9.5 Protocol Violations

When a protocol violation occurs, it will be reported to the IRB either in real time or with the annual status report, according to severity and according to institutional policy.

- A protocol violation occurs when the subject or investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary

endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication
- Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The study chair will determine if a protocol violation will result in withdrawal of a subject.

10 STATISTICAL CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

10.1 Primary Objectives

The primary objectives are to determine the feasibility of deriving a therapeutic cell product, the safety of the T cell product infusion and to define the full toxicity profile as well as the response rate and persistence of T cell product for the Phase 2 cohorts.

10.1.1 Feasibility

Feasibility will be assessed by looking at the number of products successfully manufactured versus those that were unsuccessful.

Feasibility Criteria will be defined as follows: No therapeutic product could be generated for the patient after two attempts using a single apheresis product for starting material.

Unsuccessful products will be defined as:

- Cell product did not meet QC release criteria and could not be released for infusion.
- Cell product that was not manufactured do to expansion failure

10.1.2 Safety

Safety assessment will be analyzed and the following data will be obtained:

Toxicity Assessment (the evaluation taking place with 24-hrs of the initial T cell infusion will serve as baseline measurements)

- History and physical exams occurring on study after the T cell infusion.
- Vital signs during and at least weekly x 2 weeks after each infusion
- Pulse oximetry before and during the infusion
- Laboratory/radiographic evaluations taking place on study and occurring after the T cell infusion
- B cell reconstitution
- Serum immunoglobulin levels
- Graft versus host disease assessment
- Adverse event reporting

Response rates will be analyzed with bone marrow aspirates taken at day +7 (phase 1 only), 21, 42 (phase 1 only), and 63.

- Endpoint is MRD negative rate by day +63

- Bone marrow studies will be evaluated and responses graded per standard ALL criteria (see section 11.2)
- Response rate for NHL patients will be analyzed separately with bone marrow aspirates and PET scan taken at day +21 and day +63 and will be evaluated and responses graded per standard NHL criteria (see section 11.3)

10.1.3 Persistence

Persistence of CAR+ T cells will be analyzed with peripheral blood and bone marrow samples.

- Endpoint is persistence of CAR+ T cells past day +63
- Persistence of CAR+ T cells is defined as either detection of the CAR+ T cells by flow or PCR above the lower limit of detection, or B cell aplasia (defined as <1% CD19+ cells in the lymphocyte subset as determined by flow cytometry) that cannot be attributed to other B cell targeting agents.

10.2 Secondary Objectives

The secondary objectives are to determine the duration and magnitude of the in vivo persistence of the adoptively transferred T cells as well as to assess the accumulation of the transferred T-cells in the bone marrow and the development/persistence of B cell aplasia. Additionally the efficacy of cetuximab to ablate $EGFRt^{+}$ T cells will be analyzed:

- PCR and MPF from bone marrow and peripheral blood as a measure of magnitude and presence of transferred T cells.
- MPF from bone marrow and peripheral blood as a measure of B cell aplasia.
- The pre and post cetuximab T cell persistence data will be analyzed to measure the response rate of EGFRt+ T cells to ablation with the endpoint of absence of detectable EGFRt+ T cells 28 days after initiation of cetuximab.

10.2.1 Duration of overall response-leukemia:

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that treatment failure is objectively documented

10.2.2 Survival

- Overall: Defined as time from T cell infusion to death from any cause. If a patient is alive at the last evaluation time period, survival time is censored at the time of last follow-up.
- Disease Free: Defined as the time from T cell infusion to the first observation of disease or death from any cause, whichever occurs first. If the patient has not relapsed or died, disease-free survival is censored at the time of last follow-up.
- Non-Relapse Mortality: Defined as time to death event where cause of death is not attributable to underlying disease.

10.3 Cohort size and Rules for Cohort Advancement

The Phase 1 part of the study will follow a 3-patient cohort up-and-down design, with rules very similar to the algorithm known as 'conventional 3+3' [159, 160]. Phase 2 will employ a non-sequential, fixed-dose, fixed-sample size design. Safety and toxicity will be continually monitored during both phases.

10.3.1 Sample size

Phase 1 is expected to require 24-36 evaluable patients, however depending upon DLT responses and dose de-escalation rules it might require between 5 and 48 evaluable patients. Phase 2 is designated for 72 additional patients on Cohort 2A (mandated lymphodepletion) with additional patients on cohort 2B (alternative lymphodepletion). Cohort 2B is expected to have <10 patients but there is no maximum accrual for this cohort. The inevaluability rate is expected to be about 10%. Due to replacement of subjects receiving ExpAct beads, total accrual of phase 2 subjects is expected to be around 150.

10.3.2 Cohort advancement rules for phase 1 – applies to dose escalation cohorts

1. During Phase 1, dose-escalation decisions will be based on cohorts of the 3 most recent evaluable patients receiving the same dose.
2. Treatment of **the first cohort at each new dose level** will be staggered by a minimum of 14 days between patients.
3. The trial commences at Dose Level 1 (see Section 4.1 for dose level definitions).
4. Each patient's DLT status will be evaluated on the day specified in Section 4.2, or upon the recording of a DLT event, whichever occurs earlier. See Section 4.2 for a complete description.
5. **Ordinary Dose-Escalation Rules:** After each cohort's DLT status is available, dose escalation proceeds according to Table 10-1. These rules will be applied separately to govern transition between dose levels in Phase I Escalation, and between dose levels in Phase I Extension.

Table 10-1 Phase 1 ordinary dose escalation rules.

Current Cohort's DLT Count	Escalation Decision Rule
0 out of 3	**Escalate:** Treat the next 3 patients one dose level higher.
≥ 2	**De-Escalate:** Treat the next 3 patients one dose level lower. If the first 2 patients both experience DLTs, stop the cohort at 2 patients and de-escalate.
1 out of 3	**Repeat:** Enter 3 more patients at the current dose level, and consult this table again based on their DLT count.

6. **Dose Re-Escalation:** This protocol does allow for **one** re-escalation to a dose level de-escalated from, with the following constraints:

a. In the re-escalation cohort, patients will be staggered by 30 days.

b. If the first patient at the re-escalated cohort experiences a DLT, the dose level will be closed to further accrual and the study will de-escalate one level down.

c. If a second de-escalation from the dose level is mandated according to Table 10.1 (i.e., 2 or more DLTs in the current cohort), the dose level and all levels above it are closed to further accrual.

7. Boundary Conditions:

a. If the current dose is the highest one currently allowed for accrual, and further escalation is mandated, the dose will be repeated for the next 3-patient cohort.

b. If the current dose is the lowest one and further de-escalation is mandated, the dose can be repeated once as a re-escalation, with the second cohort staggered and evaluated according to the re-escalation rules above.

8. **Stopping Rules:** Phase I will stop after 9 patients have been evaluated for DLTs at any single dose level. **Early stopping will take place:**

a. If, at any point, a dose level meets the Biologically Effective Dose (BED) criteria (see below for BED criteria).

b. If the lowest dose level (Dose Level -1) is disallowed due to safety, according to the dose re-escalation rules above.

9. **No prior Allo-HSCT Rules during phase 1**: these subjects will not be used in the dose escalation calculation, but will be counted towards the dose de-escalation. The exclusion of them in the dose escalation calculation is due to the possibility that the post Allo-HSCT patients have a higher level of toxicity, therefore it is most appropriate to escalate based on the post allo-hsct subjects alone.

10.3.3 Definition of MTD and BED, and dose selection for phase 1 extension cohort and phase 2

The MTD at the end of Phase 1 dose escalation cohorts is defined as the highest T cell dose, with at least 6 toxicity-evaluable patients and a cumulative DLT rate under 34%. See section 4.2 for the definition of a DLT, and Section 10.6 for the definition of toxicity-evaluable subjects.

The Biologically Effective Dose (BED) is the lowest dose with at least 6 patients evaluable for both safety and efficacy, and having:

- A cumulative DLT rate under 34%;
- Over 80% of patients experiencing persistence of CAR modified T cells in the bone marrow at day 63, with no detection of CD19+ cell in the bone marrow at day 63.

If Phase1 ends with no dose meeting either the BED or the MTD, the DMC will convene to consult upon the next steps. *(enrollment onto Phase 1 was completed on 11 May 2016)*

10.3.4 Statistical design for phase 2

After analysis of the first 36 evaluable subjects on the phase 1, there was an improved disease-free survival (DFS) in pts with ongoing BCA beyond day 63 (HR: 0.25 [95% CI: 0.08, 0.81], P = 0.01). A complete response does benefit patients, but persistence provides an even greater benefit. In order to incorporate this distinction into simple and realistic trial rules, one persistence response will be equivalent to two efficacy-without-persistence responses. This will enable interpretation and analysis of all trial data within a single self-consistent framework. Therefore, the main endpoint of the phase 2 design has 3 levels of efficacy:

1. Incomplete response;
2. Complete response (MRD-CR by Day 63), but no persistence of CAR T cells at day 63;
3. Complete response and CAR T-cell persistence beyond Day 63

Phase 2 Efficacy Rules

Phase 2 is designed as a hypothesis test for efficacy, with a three-level endpoint described above. Success is defined as an acceptable combination of efficacy and persistence. The hypothesis is composite, involving direct measurement of the persistence rate, as well as a combined **efficacy-persistence score** in which efficacy only (level 2 endpoint) responses add 1 point, while persistence responses (level 3) add 2 points. The Null hypothesis, representing insufficient response, is

Persistence beyond Day 63 ≤25%, **or** persistence + efficacy ≤0.95 points per subject

Successful response rate for Phase II will be declared if the Null is rejected at $\alpha<0.05$, using a one-sided Multinomial test. With n=72 patients, the rejection region is defined as 25 or more patients experiencing persistence, **and** a combined efficacy-persistence score of 83 points or more.

The decision rule is outlined by a bold red line in the diagram below (*Figure 2*). With 72 patients, this rule will reject the Null at a power of 80% or greater, for true persistence as low as 39%, provided that true efficacy is high enough. Given true persistence of 62.5% or higher, the

Null will be rejected with 80% or more power, regardless of additional efficacy-only outcomes.

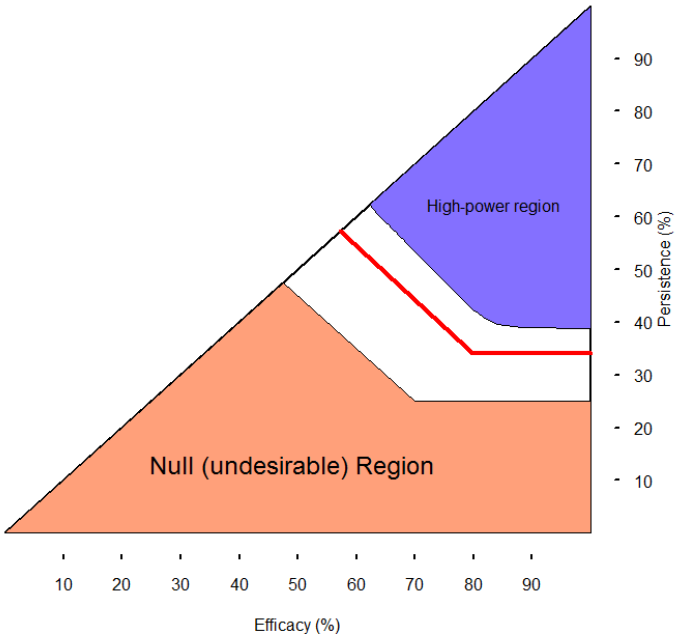


Figure 2: Phase 2 decision rules with n=72. The x and y axes indicate the true underlying proportions of efficacy and persistence responses, respectively. The orange region indicates conditions included under the definition of the Null hypothesis. The red line demarcates the observed proportions lying on the decision boundary. The purple-blue region consists of conditions under which successful trial completion is expected with probability 80% or greater.

There is no statistical design for cohort 2B, for subjects with <5% circulating CAR+ T cells at the time of enrollment or for the NHL patients of cohort 2A and 2B who will be analyzed separately. It is anticipated that both groups will be a very small. These patients will be enrolled as necessary with descriptive analysis of persistence and efficacy.

Subjects treated with products manufactured with ExpAct beads will be analyzed separately and reported descriptively with regards to efficacy, persistence, and toxicity.

10.4 Study Analysis Plan

The primary goal of the Phase 1 study is to evaluate the toxicity and feasibility results associated with cellular immunotherapy that utilizes patient-derived T cells that have been genetically modified to express a CD19-specific chimeric immunoreceptor for patients with relapsed $CD19^{+}$ acute lymphoblastic leukemia following allogeneic HCT.

In addition to safety and feasibility, the primary objective of the Phase 2 study is to evaluate the efficacy associated with cellular immunotherapy that utilizes patient-derived T cells that have been genetically modified to express a CD19-specific chimeric immunoreceptor for patients with relapsed $CD19^{+}$ acute lymphoblastic leukemia.

Baseline information (e.g., the extent of prior therapy) and demographic information will be summarized using means, standard deviations, ranges, counts and percents. The toxicities

observed at each dose level will be summarized in terms of type (organ affected or laboratory determination such as absolute neutrophil count [ANC]), severity (by NCI CTCAE v4.0 and nadir or maximum values for the laboratory measures), date of onset and attribution. Tables will be created to summarize these toxicities and side effects by dose level.

All disease responses will be reported. Objective response for all subjects will be summarized at each dose level, and the number and percent responding combined across dose levels. Survival and time to relapse/progression or HCT will be summarized both by pooling across dose levels and within each dose level. All results for the summary statistics noted above will be tabulated and reported.

10.5 Data and Safety Monitoring

10.5.1 Quality Assurance

Quality assurance will be monitored by the sponsor/sponsor designee at a minimum of twice per year. The Fred Hutchinson/University of Washington Cancer Consortium Clinical Research Support office may collaborate with the sponsor to provide the QA monitor for this program at Seattle Children's Hospital in accordance with the Fred Hutch/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan (DSMP). In addition, the sponsor may delegate monitoring responsibilities to an external CRO. Under the provisions of this plan, Fred Hutch Clinical Research Support (CRS) coordinates with data and compliance monitoring conducted by consultants, contract research organizations, or Fred Hutch employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state and federal guidelines.

10.5.2 Weekly Safety Review

The Study Chair, Investigators, study statistician (if needed), Clinical Research Associate (CRA), research nurses and other site staff will meet weekly (or as needed when there are active participants) to review subject enrollment and conduct patient safety review. The group is responsible for monitoring the data and safety of this study, including implementation of the stopping rules for safety and efficacy. The group will review as applicable enrollment, adverse events from each participant, DLTs, protocol compliance, CAR T-cell persistence analysis, feasibility data and follow-up information for each subject.

10.5.3 Aggregate and Real-Time Safety Review

In addition to the weekly safety review, at any time of required or clinically indicated observations, the patient will have real-time assessment of toxicity and adverse events. These events will be reported to the Principal Investigator, research nurses and clinical research associates for attribution and reporting as applicable (see section 12).

10.5.4 Data Monitoring Committee

The study will be monitored by a Data Monitoring Committee (DMC). This is an independent committee with no affiliation to the protocol. The DMC will meet prior to the study opening to

review and approve the study protocol and DMC charter. The DMC will review all toxicity data occurring from start of T cell infusion through day 30 after T-cell infusion after accrual has been completed at the open dose level. The DMC will make recommendations regarding the opening of the next dose level. During the phase 2 portion, the DMC will review all toxicity and efficacy data approximately every 6 months. In addition, the DMC will review study conduct including accrual, drop-outs, data completeness, any inability to generate T cell product that meets all Quality Control criteria, positive bacterial cultures from the T-cell product, subjects not meeting eligibility criteria for T-cell infusion, protocol compliance and treatment efficacy measures.

DMC meetings may be called at any time by the DMC chair or sponsor for additional safety review if indicated. Following any temporary suspension of accrual for a safety event, the DMC will be convened and will further review the safety data within 48 hours of notification of the event to recommend if continuation of accrual is appropriate. If the DMC agrees that the study treatment is likely to be safe for additional subjects, they may recommend accrual to resume. Applicable regulatory agencies will receive copies of the DMC's recommendations as they become available.

The DMC will meet within 2 weeks of the availability of the report at each review point. Further enrollment into the cohort or progression to the next cohort will not occur until receipt of DMC recommendations and if necessary, approval from regulatory agencies.

10.5.5 Study Stopping Rules

Refer to Section 9.4: Study Closure, and Section 10.1.2: Safety with the addition of below criteria:

- If the feasibility endpoint analysis shows <50% successful product generation after at least six patients have been enrolled, the study will be suspended pending a detailed review by the Sponsor and DMC.
- If either success or failure in Phase II response (Section 10.3.4) has been arithmetically guaranteed before the full 72-patient sample has been evaluated, the research team will be entitled, but not compelled, to end the study early, or to continue in order to examine differences in response rates between risk groups identified in Phase I.

10.6 Data Sets Analyzed

All eligible patients who enrolled into the study and receive T cell therapy will be included in the safety analysis.

10.7 Demographics and Baseline Characteristics

The following demographic variables at screening will be summarized by dose level: race, gender, age, height and weight.

10.8 Evaluability for Toxicity

A patient will be considered evaluable for toxicity if the patient receives the prescribed dose of genetically modified T cell product, whether during Phase I or Phase II, **and meets <u>at least one</u> of the following conditions numbered 1-3:**

1. Experiences a DLT within the first 30 days following the infusion regardless of whether s/he remains on the trial subsequently;

2. Is still available for follow-up on Day 30 following the infusion, and has not received additional infusions or treatment in the meantime
3. Prior to pursing additional treatments prior to day 30, there was absence of detectable T cells

10.9 Evaluability for Efficacy

A patient will be considered evaluable for disease response if: (1) the patient meets the eligibility criteria for pre-infusion criteria; (2) the patient receives the T cell infusion; and (3) the patient is under follow-up for a sufficient period to evaluate the disease as defined below.

All patients who received a specified dose of genetically modified T cell product are efficacy-evaluable for that dose if they meet at least one of the following conditions:

1. Exited the trial due to a DLT and/or disease progression within the first 63 days following infusion;
2. Exited the trial due to efficacy within the first 63 days following infusion;
3. Are still available for follow-up on Day 63 following infusion.

A patient who dies as a result of toxicity after receiving T cell product will be considered a non-responder. For patients who receive a 2nd dose of T cells in the Phase 1 portion before obtaining a complete response, the response to dose 1 will be used for the efficacy analysis.

11 EVALUATION CRITERIA

11.1 Common Terminology Criteria for Adverse Events (CTCAE)

This study will utilize the CTCAE of the NCI for toxicity and performance reporting. The descriptions and grading scales found in the revised CTCAE version 4 will be utilized for reporting except for CRS on the phase 2 portion of the study, for which study-specific grading will be used (Section 12.3 AE Grading and Relationship to Investigation agent). All appropriate treatment areas should have access to a copy of the CTCAE version 4, which can be downloaded from the CTEP web site (http://ctep.cancer.gov). Additionally, toxicities are to be reported on the appropriate data collection forms.

11.2 Disease Response Criteria Leukemia

11.2.1 MRD negative Complete Remission (MRD-CR)

Attainment of M1 marrow with negative MRD (<0.01%) with no evidence of circulating leukemic blasts or extramedullary disease and with recovery of peripheral counts (ANC ≥ 500/μL and PLT count ≥ 50,000 μL). Qualifying marrow and peripheral counts should be performed within 1 week of each other.

11.2.2 MRD positive Complete Remission (MRD+CR)

Attainment of M1 marrow with positive MRD (≥0.01%) with no evidence of circulating leukemic blasts or extramedullary disease and with recovery of peripheral counts (ANC ≥ 500/μL and PLT count ≥ 50,000 μL). Qualifying marrow and peripheral counts should be performed within 1 week of each other.

11.2.3 MRD negative Complete Remission without count recovery (MRD-CRi)

Insufficient recovery of platelets (< 50,000/ μL) or ANC (<500/μL) but otherwise meets the criteria of MRD-CR.

11.2.4 MRD positive Complete Remission without count recovery (MRD+CRi)

Insufficient recovery of platelets (< 50,000/ μL) or ANC (<500/μL) but otherwise meets the criteria of MRD+CR.

11.2.5 Complete Remission, MRD unknown (CR)

Attainment of M1 marrow with no available MRD results, with no evidence of circulating leukemic blasts or extra medullary disease and with recovery of peripheral counts (ANC ≥500/μL and PLT count ≥50,000 μL). Qualifying marrow and peripheral counts should be performed within 1 week of each other.

11.2.6 Complete Remission without count recovery, MRD unknown (CRi)

Insufficient recovery of platelets (<50,000/ μL) or ANC (<500/μL) but otherwise meets the criteria of CR, MRD unknown.

11.2.7 Partial Remission (PR)

This requires a decrease of at least 50% in the percentage of blasts to a post-treatment value of 5% to 25% in the bone marrow aspirate. (If the pre-treatment blast percentage was 50-100%, this must decrease to a value between 5-25%. If the pre-treatment blast percentage was 20-49%, this must decrease by at least half to a value greater than 5%) with recovery of peripheral counts (ANC > 500/µL and platelet count> 50,000/µL).

11.2.8 Partial Remission Cytolytic (PRCL)

Complete disappearance of circulating blasts and achievement of at least 50% reduction in bone marrow blast count from baseline.

11.2.9 Minimal Response Cytolytic (MRCL)

≥ 50% reduction in the peripheral blast count from baseline with no increase in peripheral white blood cell count.

11.2.10 Stable Disease (SD)

This is present when the patient fails to qualify for either MRD-CR, MRD+CR, PR, PRCL, MRCL, or progressive disease (PD).

11.2.11 Progressive Disease (PD)

An increase of at least 25% in the absolute number of circulating leukemic cells from baseline, development of extramedullary disease, or other laboratory or clinical evidence of PD following delivery of chemotherapy.

11.2.12 Definitions of Relapse

All relapses will be classified as $CD19^+$ or $CD19^-$.

Bone Marrow Relapse is defined as M3 marrow after previous remission from ALL.

MRD relapse is defined as an increase in MRD level of at least 1 log.

CNS Relapse is defined as:

- At least 5 WBC's/µL in CSF with blasts present on cytospin.

or

- Any number of WBC's in CSF with immunophenotypic proof of leukemic relapse. Immunophenotypic proof of relapse is defined as identifiable blasts plus (for B-lineage) TdT or CD-10 positivity on 2 consecutive CSF samples four weeks apart or (for T-lineage) TdT and CD-7 or TdT positivity alone on 2 consecutive CSF samples four weeks apart.

Testicular Relapse is defined as:

- Unilateral or bilateral testiculomegaly with biopsy-proven testicular involvement.

or

- Unilateral or bilateral testiculomegaly with concurrent relapse in the bone marrow and/or CNS.

An extramedullary relapse will be considered as a relapse event.

Combined Relapse is defined as relapse at 2 or more of the above sites.

11.3 Disease Response Criteria Lymphoma

Complete Remission is defined as:

11.3.1 CR

Imaging reveals complete disappearance of all measurable or evaluable lesions (except bone), no malignant blasts in the bone marrow nor in the CSF

11.3.2 CRu

Residual mass is negative by FDG-PET, BM and CSF morphologically free of disease

11.3.3 Partial Response (PR)

50% decrease in SPD (sum of product of greatest perpendicular diameters) on imaging (FDG-PET may be positive(Deauville score of 4 or 5 with reduced lesional uptake compared to baseline, no new and /or PD: morphologic evidence of disease may be present in BM or CSF if present at diagnosis, however there should be 50% reduction in the percentage of lymphoma cells

11.3.4 Minor Response (MR)

>25% but <50% decrease in SPC on imaging; no new and/orPD; morphologic evidence of disease may be present in BM or CSF if present at diagnosis, however there should be 50% reduction in the percentage of lymphoma cells

11.3.5 No Response (NR)

Does not meet CR, PR, MR, or PD

11.3.6 Progressive Disease (PD)

>25% increase in SPD on imaging, Deauville score of 4 or 5 on FDG-PET with increase in lesional uptake from baseline or development of new morphologic evidence of disease in BM or CSF

11.3.7 Definitions of Relapse

All relapses will be classified as $CD19^{+}$ or $CD19^{-}$.

Relapse: appearance of new lesions, appearance or reappearance of tumor cells in bone marrow or CSF

12 ADVERSE EVENT REPORTING REQUIREMENTS

12.1 Purpose

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Certain adverse events must be reported in an expedited manner to allow for timelier monitoring of patient safety and care. The following sections provide information about expedited reporting.

The investigative team will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug. AE's will be reported to all institutional and federal regulatory entities in compliance with current statutes.

12.2 Definitions

Adverse Event: An AE is any untoward medical occurrence in a clinical investigation of a patient administered a T cell product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product.

An unexpected AE is one of a type not identified in nature, severity, or frequency in the current IRB approved protocol or investigator brochure or of greater severity or frequency than expected. The investigator will determine whether it is unexpected according to the Investigator's Brochure, the underlying disease and concomitant treatment and the relationship of the investigational drug to all AEs

A Serious Adverse Event (SAE) is any adverse event (experience) occurring in a patient who is administered a T cell product at any dose that results in ANY of the following outcomes:

- death;
- life threatening (A life-threatening event is defined as having placed the research participant, in the view of an Investigator, at immediate risk of death from the adverse event as it occurred. It does not include an adverse event that had it occurred in a more serious form, might have caused death);
- requires an unanticipated inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity;
- a congenital anomaly/birth defect;
- secondary malignancy, or
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions

that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

All adverse events that do not meet at least one of the above criteria are defined as non-serious. Assessment of the cause of the event will have no bearing on the evaluation of the event's severity.

Additional events not considered serious are hospitalizations occurring under the following circumstances:

- planned before entry into the clinical study
- elective treatment of a pre-existing condition
- occur on an outpatient basis and do not result in overnight hospitalization
- part of the normal treatment or monitoring of the research participant and not associated with any deterioration in condition.

Additionally, a hospitalization/prolongation of a hospitalization for monitoring patients with non-serious adverse events or for management of concomitant medical care issues will not be considered an AE for expedited reporting nor be a factor in MTD determination.

12.3 AE Grading and Relationship to Investigation agent

CTCAE version 4.0 will be used to evaluate all toxicities except for phase 2 reporting which will use study specific CRS grading (see table below). Neurological toxicities should not be reported as CRS and should be reported separately for each symptom observed. Any toxicity reported by research participants during the protocol designated time frames for which there is no specific CTC designation will be graded on the following scale: Grade 0- no toxicity, Grade 1- mild toxicity, usually transient, requiring no special treatment and generally not interfering with usual daily activities, Grade 2- moderate toxicity that may be ameliorated by simple therapeutic maneuvers, and impairs usual activities, Grade 3- severe toxicity which requires therapeutic intervention and interrupts usual activities. Hospitalization may be required or may not be required, Grade 4- life-threatening toxicity that requires hospitalization. Attribution will be unrelated, unlikely, possible, probably, or definite.

Table 12-1: Grading Criteria for CRS for Phase 2

Grade	Description of Symptoms
1: Mild	Not life-threatening, require only symptomatic treatment such as antipyretics and anti-emetics (e.g., fever, nausea, fatigue, headache, myalgia, malaise)
2: Moderate	Require and respond to moderate intervention: Oxygen requirement for nasal cannula or simple face mask, or Hypotension responsive to fluids or low dose of a single vasopressor, or Grade 2 organ toxicity (by CTCAE v4.0) attributed to CRS
3: Severe	Require and respond to aggressive intervention: Oxygen requirement for non-rebreather or CPAP/BiPAP, or Hypotension requiring high dose of a single vasopressor (e.g., norepinephrine ≥ 20 µg/min, dopamine ≥ 10 µg/kg/min, phenylephrine ≥ 200 µg/min, or epinephrine ≥ 10 µg/min), or Hypotension requiring multiple vasopressors (e.g., vasopressin + one of the above agents, or combination vasopressors equivalent to ≥ 20 µg/min norepinephrine), or Grade 3 organ toxicity or Grade 4 transaminitis (by CTCAE v4.0) attributed to CRS
4: Life-threatening	Life-threatening: Requirement for ventilator support, or Grade 4 organ toxicity (excluding transaminitis)
5: Fatal	Death

Adapted from (Lee 2014)

12.4 Event Collection

All AE's that occur following infusion of modified T cells and up to and including 30 days after the administration of T cells will be captured and documented. **Phase 2 will only capture and document grade ≥2 AEs following infusion of modified T cells and up to and including 30 days. In addition, grade 1 CRS and grade 1 neurotoxicity are considered AEs of interest and will be captured and documented during Phase 2.** Any SAE that is designated possibly, probably, or definitely related to the modified T cells that occurs greater than 30 days will also be captured. Related SAEs will continue to be reported for as long as the patient is being followed for any reason.

Baseline AEs: Although a pertinent positive finding identified on baseline assessment is not an AE, when possible it is to be documented as "Course Zero" using CTCAE terminology and grade. An expedited AE report is not required if a patient is entered on a protocol with a pre-existing condition (e.g., elevated laboratory value, diarrhea). The baseline AE must be re-assessed throughout the study and reported if it fulfills expedited AE reporting guidelines (12.5).

- If the pre-existing condition worsens in severity, the investigator must reassess the event to determine if an expedited report is required.
- If the AE resolves and then recurs, the investigator must re-assess the event to determine if an expedited report is required.
- No modification in grading is to be made to account for abnormalities existing at baseline.

Persistent AE's: A persistent AE is one that extends continuously, without resolution.

12.5 Serious Adverse Event Reporting and Study-Specific Reporting

Serious adverse events will be evaluated by the institutional PI and must be reported in the eCRF database and the CRO managing the safety database as instructed and according to reporting guidelines detailed below. All unexpected and related serious adverse events will be sent to the DMC. The institutional PI will report all unanticipated problems to the sponsor, institutional IRB and IBC according to federal and local guidelines. If there is a question regarding attribution of the SAE, the study chair will submit the relevant data to the DMC for review on an ad hoc basis.

Written notification of an SAE occurring within 30 days of T cell infusion, regardless of attribution, must be sent to the Sponsor or Sponsor's designee within 24 hours of the site's knowledge of the event. Additionally, SAEs that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting within 24 hours. The following events, if meeting SAE criteria in Section 12.2 constitute exceptions and do not require reporting to the Sponsor or Sponsor's representative; however they must be entered into the eCRF database.

Table 12-2 Exceptions to SAE Reporting Guidelines

Grade:	Adverse Event
≤ 4	Myelosuppression (includes white blood cell decreased)
≤ 4	Anemia
≤ 4	Lymphocyte count decreased
≤ 4	Neutrophil count decreased
≤ 4	Platelet count decreased
≤ 3	Catheter-related infection
≤ 3	Febrile Neutropenia
1	Fever

The institutional PI is responsible for submitting follow-up reports for all SAEs until the SAE has resolved or until the patient's condition stabilizes (in case of persistent impairment), or the patient dies.

The Study Sponsor along with the CRO is responsible for assessing each SAE that occurs, and deciding whether or not that SAE meets the criteria specified for reporting to the Food And Drug Administration (in accordance with 21 CRF 312) and NIH-OBA (in accordance with the NIH guidelines for research involving recombinant DNA molecules).

13 RECORDS AND REPORTING

13.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) OR paper CRF when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the Sponsor (or designee), but will be identified by a site number, subject number and initials.

For eCRFs: If a correction is required for an eCRF, the time and date stamps track the person entering or updating eCRF data and creates an electronic audit trail. *For paper CRFs:* If a correction is made on a CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. A copy of the CRF will remain at the Investigator's site at the completion of the study.

13.2 Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

13.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. *For Electronic Data Capture (EDC) studies:* Queries are entered, tracked, and resolved through the EDC system directly. *For paper studies:* Query reports (Data Clarification Requests) pertaining to data omissions and discrepancies will be forwarded to the Investigators and study monitors for resolution. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

13.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

13.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years following marketing of the investigational product or for two years after centers have been notified that the IND has been discontinued. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

13.6 Subject Confidentiality

In order to maintain subject confidentiality, only a site number, subject number and subject initials will identify all study subjects on CRFs and other documentation submitted to the Sponsor. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

14 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

14.1 Protocol Amendments

Any amendment to the protocol will be written by the study chair. Protocol amendments can not be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRB is notified within five working days.

14.2 Institutional Review Boards and Independent Biosafety Committees

The protocol and consent form will be reviewed and approved by the institutional IRB/IBC prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IBC in accordance with the standard operating procedures and policies of the IRB/IBC, and the Investigator will keep the IRB/IBC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IBC compliance with regulations.

Any documents that the IRB/IBC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IBC. The IRB/IBCs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated.

Protocol and/or informed consent modifications may not be initiated without prior written IRB approval except when necessary to eliminate immediate hazard to the patient. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained before implementation.

The IRB must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

Research modifications will be submitted to the IBC according to NIH guidelines, standard operating procedures and policies of the IBC.

14.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form (ICF), assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonization and will also comply with local regulations. The Investigator will send an IRB-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB, assent from the subject will also be obtained. If a subject is unable to sign the ICF and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

14.4 Publications

The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

14.5 Investigator Responsibilities

By signing the Investigator Signature Page the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AE's that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.

6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

15 RESEARCH PARTICIPANT-SPECIFIC BIOLOGICAL MATERIALS

Unused T cell product, biologic samples and cryopreserved PBMC will not be returned to the research participant or their family/families. They will become property of the Sponsor and may be used in non-therapeutic research.

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APPENDICES

Appendix I: Performance Status Scales/Scores

Appendix II: GVHD Staging and Grading Assessment; Diagnosis of Chronic GVHD

Appendix III: Highly Effective Contraception

APPENDIX I: PERFORMANCE STATUS SCALES/SCORES

Performance Status Criteria Karnofsky and Lansky performance scores are intended to be multiples of 10					
ECOG (Zubrod)		**Karnofsky**		**Lansky***	
Score	Description	Score	Description	Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
		90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.	80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
		70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours	60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
		50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
		30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
		10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

*The conversion of the Lansky to ECOG scales is intended for NCI reporting purposes only.

APPENDIX II GVHD STAGING, GRADING, AND DIAGNOSIS INFORMATION

Appendix II, Table 1: Staging of Acute GVHD

Category	Time of symptoms after HCT or DLI †	Presence of Acute GVHD Features	Presence of Chronic GVHD Features*
Acute GVHD			
Classic acute GVHD	≤ 100 days	Yes	No
Persistent, recurrent or late onset acute GVHD	> 100 days	Yes	No
Chronic GVHD			
Classic chronic GVHD	No time limit	No	Yes
Overlap syndrome	No time limit	Yes	Yes

† DLI (donor lymphocyte infusion)

Appendix II, Table 2: Grading of Acute GVHD

Severity of Individual Organ Involvement		
Skin	+1	a maculopapular eruption involving less than 25% of the body surface
	+2	a maculopapular eruption involving 25-50% of the body surface
	+3	generalized erythroderma involving >50% of the body surface
	+4	generalized erythroderma with bullous formation and often with desquamation
Liver	+1	bilirubin (2.0-2.9 mg/100ml)
	+2	bilirubin (3-5.9mg/100ml)
	+3	bilirubin (6-14.9mg/100ml)
	+4	bilirubin > 15mg/100ml
Gut	Diarrhea is graded +1 to +4 in severity. Nausea and vomiting and/or anorexia caused by GVHD is assigned as +1 in severity. The severity of gut involvement is assigned to the most severe involvement noted. Patients with visible bloody diarrhea are at least stage +2 gut and grade +3 overall	
Diarrhea	+1	≤ 1000 ml of liquid stool/day* (≤ 15ml of stool/kg/day)†
	+2	>1,000 ml of stool/day* (> 15ml of stool/kg/day)†
	+3	>1,500 ml of stool/day* (> 20ml of stool/kg/day)†
	+4	2,000 ml of stool/day* (≥ 25ml of stool/kg/day)†

**In the absence of infectious/medical cause*
†For pediatric patients

Appendix II, Table 2: Grading of Acute GVHD, *continued*

Severity of GVHD	
Grade I	+1 to +2 skin rash
	No gut or liver involvement
Grade II	+1 to +3 skin rash and/or
	+1 gastrointestinal involvement and/or +1 liver involvement
Grade III	+4 skin involvement and/or
	+2 to +4 gastrointestinal involvement and/or
	+2 to +4 liver involvement with or without a rash
Grade IV	Pattern and severity of GVHD similar to grade 3 with extreme constitutional symptoms or death

From "Graft-vs-host disease" Sullivan, Keith M. *Hematopoietic Cell Transplantation* Ed: D. Thomas, K. Blume, S. Forman, Blackwell Sciences; 1999, pages 518-519.

Appendix II, Table 3: Diagnosis of chronic GVHD

Table3a: Classification of manifestations for the clinical diagnosis of chronic GVHD

Organ or site	**Diagnostic** *sufficient to establish a chronic GVHD diagnosis*	**Distinctive** *insufficient alone to establish a chronic GVHD diagnosis*	**Common** *seen with both acute and chronic GVHD*
Skin	Poikiloderma Lichen planus-like Sclerotic morphea-like Lichen sclerosis-like	Depigmentation	Erythema Maculopapular Pruritus
Nails		Dystrophy Longitudinal ridging, splitting or brittleness Onycholysis Pterygium ungus Nail loss (usually symmetric and affects most nails)	
Scalp and body hair		New alopecia not due to chemoradiotherapy Scaling, papulosquamous lesions	
Mouth	Lichenoid Hyperkeratotic plaques Diminished oral cavity opening from sclerosis	Xerostomia Mucocele Mucosal atrophy Pseudomembranes [1] Ulcers [1]	Gingivitis Mucositis Erythema Pain
Eyes		New onset of sicca (dryness, gritty or painful [2]) Cicatricial conjunctivitis Keratoconjunctivitis sicca [2] Confluent areas of punctate keratopathy	
Genitalia	Lichen planus-like Vaginal scarring / stenosis	Erosions [1] Fissures [1] Ulcers [1]	

continued

Table3a: Classification of manifestations for the clinical diagnosis of chronic GVHD, *continued*

Organ or site	**Diagnostic** *sufficient to establish a chronic GVHD diagnosis*	**Distinctive** *insufficient alone to establish a chronic GVHD diagnosis*	**Common** *seen with both acute and chronic GVHD*
GI tract	Esophageal web Esophageal strictures or stenosis in upper to mid third [1]		Anorexia, nausea, vomiting, diarrhea Failure to thrive / weight loss
Liver [3]			Bilirubin > 2x ULN [1] Alk phos > 2x ULN [1] AST or ALT >2x ULN[1]
Lung	Bronchiolitis obliterans [4] (biopsy confirmed)	Bronchiolitis obliterans [4] (based on PFTs and CT scan imaging)	Cryptogenic organizing pneumonia (COP/BOOP)
Muscles, fascia, joints	Fasciitis Joint stiffness or sclerotic contractures	Myositis or polymyositis [2]	

AIHA: autoimmune hemolytic anemia; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BOOP: bronchiolitis obliterans with organizing pneumonia; COP: cryptogenic organizing pneumonia; CT: computerized tomography; ITP: immune thrombocytopenia; PFTs: pulmonary function tests; ULN, upper limit or normal

1 In all cases, infection, drug effects, malignancy, and other cause must be excluded.

2 Diagnosis of cGVHD requires biopsy or radiographic confirmation (or Schirmer test or slit lamp examination for eyes)

3 Because liver histology in acute and chronic GVHD is not distinguishable the diagnosis of cGVHD cannot be made on the basis of biopsy alone and requires a distinctive manifestation in at least one other organ system.

4 Criteria for diagnosing bronchiolitis obliterans: Forced expiratory volume in 1 second/forced (or slow) vital capacity ratio <0.7 and forced expiratory volume in 1 second <75% of predicted and evidence of air trapping or small airway thickening or bronchiectasis

Table 3b: Manifestations acknowledged as chronic GVHD if the diagnosis is already confirmed

Organ or Site	Manifestation
Skin	Sweat impairment, ichthyosis, keratosis pilaris, hypopigmentation, hyperpigmentation
Hair	Thinning scalp hair not otherwise explained (typically patchy, coarse or dull), premature graying
Eyes	Photophobia, periorbital hyperpigmentation, blepharitis (eyelid erythema with edema)
GI tract	Exocrine pancreatic insufficiency
Muscle/Joint	Edema, muscle cramps, arthralgia or arthritis
Hematology	Thrombocytopenia, eosinophilia, lymphopenia
Immune	Hypo-or hyper- gammaglobulinemia, autoantibodies (AIHA, ITP)
Other	Ascites, pericardial or pleural effusions, peripheral neuropathy, nephrotic syndrome, myasthenia gravis, cardiac conduction abnormality, cardiomyopathy

AIHA: autoimmune hemolytic anemia; ITP: immune thrombocytopenia

APPENDIX III: HIGHLY EFFECTIVE CONTRACEPTION

Highly effective contraception is defined as either:

- Total abstinence: When this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.
- Sterilization: Patient has had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks before taking study treatment. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment
- Male partner sterilization (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate). [For female study patients, the vasectomized male partner should be the sole partner for that patient]
- Use a combination of the following (both a+b):

a. Hormonal contraception (oral or injected) or placement of an intrauterine device (IUD) or an intrauterine system (IUS)

b. Barrier method of contraception: Condom or Occlusive cap (diaphragm or cervical vault caps) with spermicidal foam/gel/film/cream/vaginal suppository.

Note: Woman are considered post-menopausal and not child bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or six months of spontaneous amenorrhea with serum FSH levels > 40 mIU/mL and estradiol <20 pg/mL or have had surgical bilateral oophorectomy (with or without hysterectomy) at least six weeks ago. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of child bearing potential.

Male patient must use highly effective (double barrier) methods of contraception (e.g., spermicidal gel plus condom) A condom is required to be used also by vasectomized men in order to prevent delivery of the study treatment via seminal fluid.