Abbreviated Title: Anti-CD30-CAR T cells

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Title: Anti-CD30 CAR T Cells with Fully-human Binding Domains for Treating CD30-expressing Lymphomas including Anaplastic Large Cell Lymphomas

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Commercial Agents: Cyclophosphamide, Fludarabine

TABLE OF ABBREVIATIONS

Abbreviation	Full Terminology
ABVD	Doxorubicin-bleomycin-vinblastine-dacarbazine
AE	Adverse event
ALCL	Anaplastic large cell lymphoma
ALK- ALCL	Anaplastic lymphoma kinase negative anaplastic large cell lymphoma
ALL	Acute lymphocytic leukemia
alloHSCT	Allogeneic hematopoietic stem cell transplant
ALT	Alanine aminotransferase
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase
autoHSCT	Autologous hematopoietic stem cell transplant
AVD	Doxorubicin-vinblastine-dacarbazine
β-HCG	Beta-human chorionic gonadotropin
BEACOPP	Bleomycin-etoposide-doxorubicin-cyclophosphamide-vincristine-procarbazine-prednisone
BSA	Body surface area
BUN	Blood urea nitrogen
C3D	Cancer Center Clinical Data System
CAR	Chimeric antigen receptor
CBC	Complete Blood Count
CCR	Center for Cancer Research
cDNA	Complementary Deoxyribonucleic acid

Abbreviation	Full Terminology
CFSE	carboxyfluorescein diacetate,succinimidyl ester proliferation assay
CHOEP	Cyclophosphamide-doxorubicin-vincristine-etoposide-prednisone
CHOP	Cyclophosphamide-doxorubicin-vincristine-prednisone
CIT	Center for Information Technology
CK	Creatine kinase
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration formula
Cl	Chloride
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
CMV	Cytomegalovirus
CNS	Central nervous system
CO_2	Bicarbonate
CPT	Cell preparation tube
CR	Complete remission
CRIS	Clinical Research Information System
CRO	Central Registration Office
CRP	C-reactive peptide
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse events
DA-EPOCH-R	Dose adjusted etoposide-prednisone-doxorubicin-vincristine-doxorubicin-rituximab
DLBCL	Diffuse large B cell lymphoma
DLI	Donor lymphocyte infusion

Abbreviation	Full Terminology
DLT	Dose limiting toxicity
DTM	Department of Transfusion Medicine
EATL	Enteropathy-associated T-cell lymphoma
EBV	Epstein-Barr Virus
ECOG	Eastern Cooperative Oncology Group
EFS	Event-free survival
EKG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
ENKTL	Extranodal natural killer/T-cell lymphoma
SB	Surgery Branch
FDA	Food and Drug Adminstration
FDG	Fludeoxyglucose
FSH	Follicle stimulating hormone
G6PD	Glucose-6-phosphate dehydrogenase
GnRH	Gonadotropin-releasing hormone
GVHD	Graft versus host disease
GVL	Graft versus lymphoma effect
GVM	Graft versus malignancy
Hb	Hemoglobin
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
HL	Hodgkin lymphoma
HLA	Human leukocyte antigen

Abbreviation	Full Terminology
HRPP	Human Research Protections Program
HSV	Herpes simplex virus
HTLV-1	Human T-cell Lymphotropic virus 1
IBC	Institutional Biosafety Committee
IFNγ	Interferon gamma
IL	Interleukin
IND	Investigational new drug
IRB	Institutional review board
iRIS	Integrated Research Information System
K	Potassium
LAM-PCR	Linear amplification mediated polymerase chain reaction
LDH	Lactate dehydrogenase
Mg	Magnesium
MTD	Maximum tolerated dose
MRI	Magnetic resonance imaging
Na	Sodium
NCI	National Cancer Institute
NIH	National Institutes of Health
OSP	Office of Science Policy
ORR	Overall response rate
OS	Overall survival
OHSRP	Office for Human Subjects Research Protections
PBMC	Peripheral blood mononuclear cells

Abbreviation	Full Terminology
PD	Progressive disease
PE	Physical Exam
PET	Positron emission tomography
PI	Principal Investigator
PICC	Peripherally inserted central catheter
PMBL	Primary mediastinal B cell lymphoma
PR	Partial remission
PT	Prothrombin time
PTT	Partial thromboplastin time
PTCL NOS	Peripheral T cell lymphoma not otherwise specified
qPCR	Quantitative polymerase chain reaction
R-CHOP	Rituximab-cyclophosphamide-doxorubicin-vincristine-prednisone
RCL	Replication competent lentivirus
RNA	Ribonucleic acid
RPR	Rapid plasma reagin
RT-PCR	Real-time polymerase chain reaction
SPD	Sum of the product of diameters
scFv	Single chain variable fragment
SD	Stable disease
SMC	Safety Monitoring Committee
SOP	Standard operating procedure
SST	Serum separator tube
TBI	Total body irradiation

Abbreviation	Full Terminology
TBNK	Refers to peripheral blood lymphocyte phenotyping as T, B, or NK cells.
TCRs	T cell receptors
TNFR	Tumor necrosis factor receptor
TRAF	Tumor necrosis factor receptor associated factors

PRÉCIS

Background:

- Improved treatments for a variety of treatment-resistant, CD30-expressing malignancies including anaplastic large cell lymphoma, and other CD30-expressing lymphomas are needed.
- T cells can be genetically modified to express chimeric antigen receptors (CARs) that specifically target malignancy-associated antigens.
- Autologous T cells genetically modified to express CARs targeting the B-cell antigen CD19 have caused complete remissions in a small number of patients with lymphoma. These results demonstrate that CAR-expressing T cells can have anti-lymphoma activity in humans.
- CD30 expression can be easily detected by immunohistochemistry on lymphoma cells, which allows selection of CD30-expressing malignancies for treatment.
- CD30 is not known to be expressed by normal cells except for a small number of activated lymphocytes.
- We have constructed a novel fully-human anti-CD30 CAR that can specifically recognize CD30-expressing target cells in vitro and eradicate CD30-expressing tumors in mice.
- This particular CAR has not been tested before in humans.
- Possible toxicities include cytokine-associated toxicities such as fever, hypotension, and neurological toxicities. Elimination of a small number of normal activated lymphocytes is possible, and unknown toxicities are also possible.

Objectives:

Primary

- Determine the safety and feasibility of administering T cells expressing a novel fully-human anti-CD30 CAR to patients with advanced CD30-expresssing lymphomas.

Eligibility:

- Patients must have anaplastic large cell lymphoma, angioimmunoblastic T-cell lymphoma, peripheral T-cell lymphoma not otherwise specified, diffuse large B-cell lymphoma not otherwise specified, primary mediastinal B-cell lymphoma, grey zone lymphoma, enteropathy-associated T-cell lymphoma, or extranodal NK/T-cell lymphoma, nasal type
- Patients must have malignancy that is both measurable on a CT scan with a largest diameter of at least 1.5 cm and possessing increased metabolic activity detectable by PET scan. Alternatively patients with lymphoma detected by flow cytometry of bone marrow are eligible.
- Patients must have a creatinine of 1.6 mg/dL or less and a normal cardiac ejection fraction.
- An ECOG performance status of 0-2 is required.

- No active infections are allowed including evidence of active HIV, hepatitis B, or hepatitis C. At the time of protocol enrollment, patients must be seronegative for CMV by antibody testing or must have a negative blood CMV PCR.
- Absolute neutrophil count≥1000/μL, platelet count≥55,000/μL, hemoglobin ≥ 8g/dL
- Serum ALT and AST less or equal to 3 times the upper limit of the institutional normal unless liver involvement by malignancy is demonstrated.
- At least 14 days must elapse between the time of any prior systemic treatment (including corticosteroids above 5 mg/day of prednisone or equivalent corticosteroid dose) and initiation of required leukapheresis.
- Clear CD30 expression must be detected on 75% or more of malignant cells from either bone marrow or lymphoma mass by flow cytometry or immunohistochemistry. The patient's malignancy will need to be assessed for CD30 expression by flow cytometry or immunohistochemistry performed at the NIH. If unstained, paraffin-embedded bone marrow or lymphoma sections are available from prior biopsies, these can be used to determine CD30 expression by immunohistochemistry; otherwise, patients will need to come to the NIH for a biopsy to determine CD30 expression. The sample for CD30 expression can come from a biopsy obtained at any time before enrollment, unless the patient has received a prior anti-CD30 monoclonal antibody, in which case the sample must come from a biopsy following completion of the most recent anti-CD30 monoclonal antibody treatment.
- Eligible patients with diffuse large B-cell lymphoma or primary mediastinal B-cell lymphoma must have received 2 prior treatment regimens at least 1 of which included an anthracycline and an anti-CD20 monoclonal antibody.
- Patients who have never had an allogeneic hematopoietic stem cell transplant as well as patients who have had a 9/10 or 10/10 HLA-matched sibling or a 9/10 or 10/10 HLA-matched unrelated donor hematopoietic stem cell transplant are potentially eligible.
- Women who are pregnant or plan to become pregnant will be excluded.

Design:

- This is a phase I dose-escalation trial.
- Patients will undergo leukapheresis.
- T cells obtained by leukapheresis will be genetically modified to express an anti-CD30 CAR.
- Patients will receive a lymphocyte-depleting chemotherapy conditioning regimen with the intent of enhancing the activity of the infused anti-CD30 CAR-expressing T cells.
- A chemotherapy conditioning regimen of cyclophosphamide and fludarabine will be administered prior to all CAR T-cell infusions Fludarabine will be given on the same days as the cyclophosphamide.
- Two days after the chemotherapy ends, patients will receive an infusion of anti-CD30-CAR-expressing T cells.

- The initial dose level of this dose-escalation trial will be $0.3x10^6$ CAR^+ T cells/kg of recipient bodyweigh for Cohort 1. The initial dose level will be $1 x 10^6$ CAR^+ T cells/kg for Cohort 2.
- The cell dose administered will be escalated until a maximum tolerated dose is determined.
- Following the T-cell infusion, there is a mandatory 9-day inpatient hospitalization to monitor for toxicity.
- Outpatient follow-up is planned for 2 weeks and 1, 2, 3, 4, 6, 9, and 12 months after the CAR T-cell infusion. Long-term gene-therapy follow-up consisting of yearly visits to a doctor near the patient's home for 4 more years and then yearly telephone contact for 10 additional years will be required.
- As of Amendment E (Protocol version: 08/03/2018), repeat treatments consisting of the conditioning chemotherapy followed by a CAR T-cell infusion at the MTD for the patient's cohort are allowed for eligible patients with any best responses except continuing complete remission or progressive malignancy.
- Re-enrollment will be allowed for a small number of subjects.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

Determine the safety and feasibility of administering T cells expressing a novel fully-human anti-CD30 chimeric antigen receptor (CAR) to patients with advanced CD30-expressing hematologic malignancies.

1.1.2 Secondary Objectives

- Evaluate the in vivo persistence and peak blood levels of anti-CD30 CAR T cells after CAR T-cell infusions.
- Assess for evidence of anti-lymphoma activity by anti-CD30 CAR T cells.
- Assess the immunogenicity of the CAR used in this protocol.

1.2 BACKGROUND AND RATIONALE

1.2.1 Introduction

We have developed a fully-human anti-CD30 CAR, and we have demonstrated that T cells expressing this CAR have CD30-specific activity in vitro and in vivo. Anti-CD30-CAR-expressing T cells expressing this CAR can eradicate tumors in mice. We propose to conduct a phase I clinical trial of anti-CD30-CAR-expressing T cells. This clinical trial will enroll patients with advanced CD30-expressing malignancies. Patients enrolled on the trial will receive a single cycle of chemotherapy that is designed to decrease endogenous lymphocyte counts because extensive evidence demonstrates that depleting endogenous lymphocytes, and possibly other cells, with chemotherapy or total body irradiation dramatically increases the anti-tumor activity of adoptively transferred T cells.[1-3] After the lymphocyte-depleting chemotherapy, patients will receive an infusion of autologous anti-CD30-CAR T cells. The T-cell dose will escalate with sequential cohorts of patients until a maximum tolerated dose is determined.

1.2.2 Biology of CD30

CD30 (TNFRSF8) is a type I transmembrane receptor that belongs to the TNF receptor (TNFR) superfamily. CD30 is strongly expressed by neoplastic cells of Hodgkin lymphoma (HL) and anaplastic large cell lymphoma (ALCL)[4]. Further studies described expression of CD30 in several types of non-Hodgkin lymphoma, embryonal cell carcinoma, and some mesenchymal cell tumors.[5] Expression of CD30 in T-cell lymphomas is heterogenous, but has been reported in up 57% of patients with these types of tumors.[6-8] In addition, studies have shown that 14-21% of diffuse large B cell lymphomas can express CD30. [9,10] CD30 expression was found to be largely absent from non-lymphoid tissues.[5,6] However, expression of the receptor has been reported in human decidual cells, the endometrial cells that form the uterine lining during pregnancy.[11] Positive staining of Ganglion and Purkinje cells of the brain and exocrine pancreatic cells were later excluded as an artifact of paraffin embedded tissues.[5,6] Although expression is very low during resting conditions, CD30 can be upregulated by activated B cells and $CD4^+$ and $CD8^+$ T cells in a variety of settings including viral infections, such as Epstein Barr Virus and HIV-1, as well as during some

autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus.[5,12] CD30 can also be released as a soluble receptor (sCD30), and serum levels of sCD30 have correlated with worsened outcomes of HL and ALCL.[13,14] However, serum levels of sCD30 increase during some viral diseases, so it is unlikely to be a good diagnostic marker.[5,15,16] Because of its high expression on some lymphoid malignancies and limited expression on non-hematopoietic tissues, CD30 is an excellent candidate for the development of targeted therapeutics.

The ligand for CD30 is CD30 ligand (CD30L), which is expressed on numerous cell types including T cells, B cells, monocytes, neutrophils, and eosinophils.[12] Like many members of the TNFR superfamily, the cytoplasmic portion of CD30 contains binding sites for several TNF receptor associated factors (TRAF), adaptor proteins involved in the signaling cascade. CD30 has been shown to interact with TRAF-1,2,3 and 5, and these signaling molecules can then lead to the activation of the transcription factor NF-κB.[17-20] CD30 has co-stimulatory functions that can alter T-cell effector functions through several actions ranging from inducing cellular proliferation to pro-apoptotic activity to lymphocyte trafficking. Studies using CD30 deficient ($CD30^{-/-}$) mice have suggested that CD30 may have roles in regulating T-cell responses. In a model of graft versus host disease, blocking CD30/CD30L interactions reduced migration of $CD4^+$ T cells to the gastrointestinal track but did not affect proliferation or apoptosis.[21] However, another study showed that CD30 signaling limits the expansion of autoreactive $CD8^+$ T cells in a mouse model of autoimmune diabetes.[22] Furthermore, there is some controversy over the role of CD30 in negative selection of T cells in the thymus.[23,24] It has also been suggested that CD30 could function in memory $CD8^+$ T cell production.[25] In ALCL cells, CD30 activation induces apoptosis, but HL cells were insensitive to CD30 activation.[26] These pleotropic effects of CD30 signaling are likely context-dependent and are determined by the availability of secondary signaling molecules.

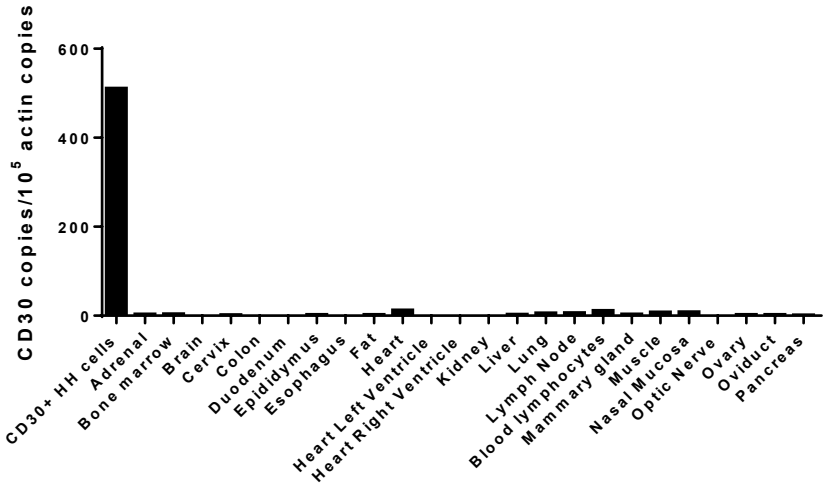
Brentuximab vedotin is an antibody drug conjugate that consists of the monoclonal anti-CD30 antibody, cAC10, conjugated to the antimitotic chemotherapeutic, monomethyl auristatin E.[27,28] The success of Brentuximab vedotin in the treatment of HL and ALCL further supports the notion that therapies targeting CD30 could improve disease outcomes for $CD30^+$ malignancies, and there has been great interest in developing new therapeutics.[29] Previous work has shown progress towards development of a fully human anti-CD30 monoclonal antibody, clone 5F11(MDX-060), that improved survival in a mouse model of HL likely through antibody-dependent cellular cytotoxicity.[30] In a clinical trial of 72 patients treated with MDX-060 objective clinical responses were seen in 6 patients, and importantly toxicity was rare.[31]

Figure 1 shows that CD30 had a restricted pattern of expression. CD30 cDNA copies were measured in samples of the indicated normal tissues by a very sensitive qPCR assay that was capable of detecting CD30 cDNA levels as low as 10 copies per 10^5 actin cDNA copies. In addition, qPCR was performed on cDNA from HH, a $CD30^+$ lymphoma cell line as a positive control. Actin copies were also measured by qPCR in all of the samples, and the results were expressed as the number of CD30 cDNA copies per 10^5 actin cDNA copies. Extensive immunohistochemistry studies of CD30 expression on normal tissues have shown that the only normal tissues that express CD30 are activated lymphocytes and, according to 1 report, endometrial decidual cells of the lining of the pregnant uterus and some deciduoid cells of the lining of the endometrium during the secretory phase of the menstrual cycle.[6,11,15] In experiments performed in the NCI Laboratory of Pathology for this protocol, CD30 was carefully assessed by immunohistochemistry on a large panel of endometrial samples. The proliferative phase

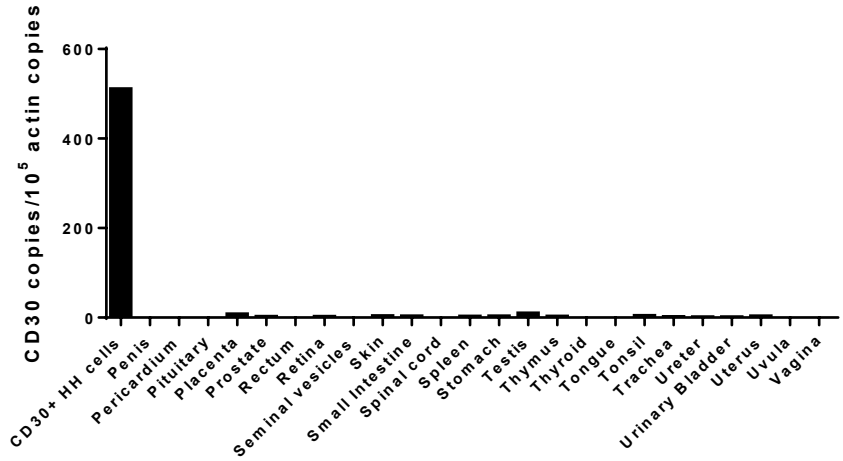
endometrium was completely negative for CD30 expression in agreement with other reports. The secretory phase of the endometrium was assessed at the following time-points of the menstrual cycle, day 16-18, day 19-20, day 21-22, day 23-24, day 25-26, and day 27-28. All samples were negative for CD30 except for dim and very rare CD30 expression (<1% of total cells) at the day 27-28 time point in 2/14 patients. Because the endometrium is shed at or shortly after day 28 and because of the extremely rare and weak CD30 expression in only 2/14 patients, we do not anticipate any significant toxicity will be caused by anti-CD30 CAR T cells. Importantly, CD30 expression was assessed for and found to be absent in all major organs including brain, heart, lungs, kidney, skin, liver, intestines (large and small), blood vessels, skin, and testes.[6,11,15]

Figure 1 ***CD30 RNA expression in normal tissues***

A.



B.



Immunohistochemical staining of normal human tissues for CD30 performed at the Laboratory of Pathology at the NIH shows that all normal tissues tested; uterus, left atrium, left ventricle, skeletal muscle, tail of the pancreas, and lung; did not express CD30 by immunohistochemistry. A concurrently tested positive control, splenic tissue involved by Hodgkin lymphoma in an HIV positive patient, consistently expressed CD30 by immunohistochemistry (Dr. Stefania Pittaluga, unpublished data).

1.2.3 Clinical background of CD30$^+$ lymphomas

Hodgkin lymphoma (HL) is an aggressive malignancy that is curable with anthracycline-based regimens, such as ABVD or BEACOPP, and with radiation therapy in cases of localized disease. Yet 15-27% of patients with advanced or unfavorable HL are not cured with first-line therapy and develop relapsed or refractory disease.[32] Of that subset of patients, only approximately 40% achieve sustained remissions with standard salvage chemotherapy followed by autologous hematopoietic stem cell transplantation (autoHSCT).[33-35] Those who have relapsed disease following autoHSCT have a 5-year overall survival (OS) of only 32%.[36] Overall, about 20% of patients with HL will not be cured with current therapies.[37] To investigate non-cytotoxic approaches in this chemotherapy-refractory group, a phase I trial was conducted in a heavily pre-treated group of patients with HL, 78% of whom had relapsed following autoHSCT. The anti-PD-1 antibody nivolumab resulted in an 87% response rate, though follow-up was short and most responses were only partial responses.[38]

Diffuse large B cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma.[39] DLBCL is often curable by anthracycline-based chemotherapy combined with rituximab (R-CHOP or DA-EPOCH-R) in the first-line setting, but at least 40% of patients have relapsed or refractory disease.[40] Patients with DLBCL that is refractory to first salvage chemotherapy have response rates to second salvage therapy of only 14-43%.[41-45] Patients with DLBCL refractory to second line chemotherapy have very poor prognoses with a reported median survival of only 4 months and a one year survival rate of only 4%.[45] Primary mediastinal B-cell lymphoma (PMBL) is a subtype of DLBCL that is closely related to nodular sclerosis HL. In one report, PMBL was shown to be highly responsive to the DA-EPOCH-R regimen, with a 5-year event-free survival (EFS) of 93%, with report of successful salvage radiotherapy in patients with refractory localized disease.[46] Conversely, mediastinal grey zone lymphoma is a rare disease with pathologic features intermediate between nodular sclerosis HL and PMBL, with reported 5-year EFS of only 62% with DA-EPOCH-R.[47] Yet there is no agreed upon standard therapy for non-localized relapsed or refractory PMBL or grey zone lymphoma, and novel approaches are needed.[48]

Peripheral T-cell lymphomas are a heterogeneous group of T-cell malignancies, including anaplastic large cell lymphoma (ALCL), peripheral T-cell lymphoma not otherwise specified (PTCL-NOS), enteropathy-associated T-cell lymphoma (EATL), and extranodal natural killer/T-cell lymphoma (ENKT). Standard first-line therapy for the most common PTCL subtypes, ALCL, PTCL-NOS, EATL, and advanced ENKTL, consists of anthracycline-based regimens, such as CHOP, CHOEP, or DA-EPOCH. Unfortunately the response to first-line therapy is not as robust as in B-cell malignancies. The 3-year EFS estimates for patients with anaplastic lymphoma kinase negative anaplastic large cell lymphoma (ALK$^-$ ALCL) and PTCL-NOS are 46% and 41%, respectively.[49] Five year overall survival for EATL has been estimated at 10-20%,[50,51] and median survival for advanced ENKTL with anthracycline based regimens is less than a year.[52] The role of

auto-HSCT following first remission in peripheral T-cell lymphomas remains controversial, with the best single arm data supporting the practice in ALK- ALCL, in which five-year OS was 70%.[53] A minority of patients with relapsed or refractory PTCL following first-line systemic therapy achieves responses with histone deacetylase inhibitors or the antifolate pralatrexate. PTCL overall response rates (ORR) have been reported as 25% with romidepsin,[54] 31% with belinostat,[55] and 29% with pralatrexate.[56]

1.2.4 **Proof of concept of CD30 as a therapeutic target: Brentuximab and MDX-060**

Brentuximab vedotin is an antibody-drug conjugate, consisting of an anti-CD30 antibody conjugated by a protease-cleavable linker to monomethyl auristatin E (MMAE), a microtubule-disrupting agent.[57] In a phase I trial of brentuximab in relapsed and refractory histologically CD30+ HL, ALCL, and AITL, the ORR was 38% with median duration of response being 9.7 months.[58] In a phase II trial of brentuximab in patients with CD30+ HL relapsed or refractory following auto-HSCT, the ORR was 75% with a CR rate of 34%,[59] and long term follow-up showed a median PFS of only 9.3 months.[60] Brentuximab has been further shown to produce responses in HL both prior to auto-ASCT[61] and following autoHSCT as consolidation therapy,[57] as well as in the up-front setting in combination with AVD.[62] Robust responses were also seen in relapsed and refractory ALCL in the phase 2 setting, with 86% objective response and median duration of response 13 months.[63] Responses in relapsed and refractory PTCL-NOS have been less impressive, with ORR of 33%,[64] though the ORR was 85% in the front-line setting when brentuximab was given sequentially with CHOP.[65] Activity of brentuximab has also been demonstrated in CD30+ DLBCL, with ORR of 44% in a mostly chemotherapy refractory population.[66] Reported grade 3-4 toxicities of brentuximab include peripheral neuropathy (likely due to the MMAE), neutropenia, thrombocytopenia, anemia, tumor lysis syndrome, progressive multifocal leukoencephalopathy, pancreatitis, acute renal failure, pyrexia, dyspnea, arthralgia, nausea, diarrhea, and fatigue.[57-59,61,63,64,67,68] In the setting of combination therapy with ABVD, but not in combination with AVD, brentuximab was associated with grade 3-4 pulmonary toxic effects and resulted in two deaths related to pulmonary toxicity.[62] Loss of CD30 expression has not been shown in tissue biopsy samples from patients with HL or ALCL who had progressive disease despite prior brentuximab, in which cases CD30 continues to be expressed.[69] This suggests that novel approaches to CD30 targeted therapy could benefit patients with relapsed disease following brentuximab therapy.

MDX-060 is a fully human anti-CD30 monoclonal antibody that, in a trial of 72 patients with relapsed or refractory histologically CD30+ lymphomas, resulted in 2 partial and 4 complete responses, with median duration of CR being 5 months.[31] MDX-060 is also known as 5F11. Grade 3-4 toxicities included dyspnea, acute respiratory distress syndrome, anemia, tamponade, epistaxis, increased liver transaminases, and psoriasis. These toxicities are quite rare, and in patients with very advanced Hodgkin disease, it is unclear if they are truly related to the anti-CD30 monoclonal antibodies administered.

1.2.5 Clinical results with anti-CD30 CAR T cells

Limited results of use of anti-CD30 CAR T cells in humans are available. In recent data presented at the 2015 annual meeting of the American Society for Hematology, results from nine patients (7 with HL and 2 with ALCL) receiving anti-CD30 CAR T cells were reported. One patient achieved a CR and one patient achieved a PR. No cytokine-storm related toxicities were observed.[70] In another study, a partial response was reported in a single patient with relapsed HL who received

three daily doses of fludarabine 25 mg/m^2 and cyclophosphamide 250 mg/m^2 followed by an infusion of 3.2 x 10^8 anti-CD30 CAR cells, which were 5% CAR positive.[71] This patient did not experience any infusion related toxicities.

1.2.5 Role of allogeneic hematopoietic stem cell transplant in CD30$^+$ lymphomas

Allogeneic hematopoietic stem cell transplant (alloHSCT) has been shown to result in a graft versus lymphoma (GVL) effect in a select group of patients with HL with second relapse, following autoHSCT in most patients, with 3-year OS of 63% in one retrospective series.[72] GVL effect in relapsed and refractory DLBCL has resulted in a 5-year OS of 18-26% with alloHSCT in a large retrospective series[73] and in a 3-year OS of 45-54% in two other reports,[74,75] again demonstrating that sustained remissions are possible in a small subset of patients. Results of alloHSCT in PTCL subtypes show that in a relatively young, fit group, 5-year OS was 50-57%.[76,77] The GVL effect of allo-HSCT in each of these lymphoid malignancies suggests that T-cell mediated immunotherapy can result in sustained remissions in a minority of cases.

1.2.6 Donor Lymphocyte Infusion (DLI)

Donor lymphocyte infusions (DLI) are infusions of unmanipulated lymphocytes used to treat malignancy after allo-HSCT. DLI can induce remissions in 70-80% of patients with chronic myeloid leukemia (CML) that relapses into chronic phase after alloHSCT.[78-80] Susceptibility of hematologic malignancies to DLI after alloHSCT varies depending on disease histology.[81]

The most important toxicity of DLI is GVHD. Grade II-IV acute GVHD has been estimated to occur in 28% to 44% of patients.[82-84] A recent review of DLI found that grade II-IV acute GVHD occurred after DLI in 34% to 48% of patients receiving DLI for various diseases and grade III-IV acute GVHD occurred after DLI in 20-35% of patients.[85] Chronic GVHD occurs commonly after DLI with an incidence of 33% to 83% and highly variable severity.[82-85] When DLI is used to treat CML, the incidence of GVHD increases as the number of T cells contained in the DLI increases.[86,87] For CML, DLIs with higher numbers of T cells had an equivalent anti-leukemia effect as DLIs containing lower numbers of T cells.[86] In another study, DLIs that contained greater than or equal to 1x10^7 T cells/kg of recipient bodyweight were associated with an increased incidence of GVHD compared to DLIs containing lower doses of T cells.[88] Miller and coworkers induced profound lymphodepletion with fludarabine and cyclophosphamide chemotherapy before DLI in an attempt to increase GVM. [89] This therapy was associated with high levels of severe acute GVHD.[89] Forty-seven percent of patients treated with fludarabine and cyclophosphamide prior to a DLI of 1x10^8 T cells/kg of recipient bodyweight developed grade III-IV acute GVHD, and 5 of 15 patients suffered fatal outcomes directly attributable to GVHD.[89] Because many patients with relapsed malignancy after alloHSCT require urgent treatment for rapidly progressive disease, many patients receive chemotherapy prior to DLI.[82,83,90] The chemotherapy regimens that are administered to patients for disease control prior to DLI are generally not as immunosuppressive as the fludarabine plus cyclophosphamide regimen administered by Miller and coworkers. Patients with relapsed myeloid malignancy after alloHSCT were treated with the combination of chemotherapy and DLI. These patients received DLIs with a median T cell dose of 1x10^8 T cells/kg of recipient bodyweight, and 44% of patients developed grade II-IV acute GVHD.[91] The anti-leukemia activity of chemotherapy plus DLI appeared to be greater than the anti-leukemia activity achieved in historical control patients that were treated with DLI alone.[91] Porter and coworkers treated patients with a conventional DLI followed by an infusion of T cells

that had been activated ex vivo with beads that were conjugated to anti-CD3 and anti-CD28 antibodies.[92] The number of ex vivo activated T cells that was administered to each patient was escalated from $1x10^6$ to $1x10^8$ T cells per kg of recipient bodyweight. The rate of GVHD observed after this therapy was not different from the rate that would be expected with conventional DLI alone.[92]

1.2.7 Mechanism of the graft-versus-malignancy (GVM) effect

The prolonged remissions of advanced B-cell malignancies that have often occurred after non-myeloablative allo-HSCT demonstrate that there is an immunologic GVM effect against these diseases.[93-96] The GVM effect against B-cell malignancies is clearly demonstrated by the remissions that have sometimes occurred when these diseases were treated with DLI in the absence of other therapies after allo-HSCT.[78-80,82,83,97] For CML, acute myeloid leukemia (AML) and ALL, the GVM effect is closely associated with GVHD. When relapse rates of leukemia patients with and without GVHD were compared, relapse rates were lower for patients with GVHD than for patients without GVHD.[98,99] Comparisons of relapse rates of T cell-depleted allo-HSCT and T-replete allo-HSCT indicated that transplantation of donor T cells prevented relapse after allo-HSCT for ALL, AML and CML.[98,100] There was also a decreased rate of relapse for patients with AML and CML that received transplants from allogeneic donors compared to patients that received transplants from syngeneic (identical twin) donors,[98] indicating an important role for allogeneic immunity in the GVM effect against these leukemias. In contrast, no differences in relapse rates for lymphoma were detected when allogeneic T-cell replete, allogeneic T-cell-depleted, and syngeneic transplants were compared.[101] However, this study only assessed patients that were treated with myeloablative transplants between the years 1985 and 1998. Nonmyeloablative transplants were excluded.[101] Because of these limitations it is possible that this study missed a subtle yet important GVM effect.

Although the cellular mechanism of the GVM effect has not been defined and may be quite heterogeneous, substantial clinical evidence points to an important role for T cells and minor histocompatibility antigens.[102] Most importantly, when T cell-replete alloHSCT and T cell-depleted allo-HSCT were compared, there was an increased relapse risk in ALL, AML and CML patients that received T cell-depleted allo-HSCT.[98,100] In addition, a patient with CML that had relapsed after standard DLI was treated with T cells that specifically recognized the patient's CML cells.[103] The CML-specific T cells were derived by culturing T cells from the patient's allogeneic donor with CML cells from the patient. The patient entered a CR that lasted two years after treatment with these CML-specific T cells.[103] An important role for minor histocompatibility antigens in GVM was suggested by a study that showed a decreased rate of relapse among male patients with CML who received allogeneic transplants from female donors compared to all other donor/recipient sex combinations.[104] The transplant combination consisting of a female donor with a male recipient is the only transplant combination in which female donor T cells that are specific for minor histocompatibility antigens encoded by the Y-chromosome might make a contribution to GVM.[104]

CML is clearly very susceptible to an immunologic GVM effect.[78-80,97] The results obtained when acute leukemia and lymphoma are treated with DLI demonstrate that a weaker GVM effect is active against these diseases.[78-80,82,83,105] The strong GVM effect that is present when CML is treated with T-cell-replete allo-HSCT demonstrates that T cells can play an important role in mediating a clinically significant GVM effect. Augmentation of the less powerful GVM effect

associated with allo-HSCT for lymphomas might improve the outcomes of patients with these diseases. One possible way to augment the GVM effect against lymphomas is to genetically engineer donor T cells to express receptors that specifically recognize antigens expressed by malignant cells.

1.2.8 Definition and Assessment of Graft-versus-host Disease

Graft-versus-host disease (GVHD) is an attack against normal recipient tissues that is mediated by the cells transferred with the transplant graft.[106] For GVHD to occur, there must be antigenic differences between the recipient and the allograft donor.[106] GVHD is divided into two broad categories, acute GVHD and chronic GVHD. Characteristic features of acute GVHD include maculopapular rash, gastrointestinal disorders (nausea, diarrhea, or ileus), and cholestatic liver disease.[107] Chronic GVHD can manifest with a wide variety of signs and symptoms.[107] Formal definitions of four subsets of GVHD have been published.[107]

1. Classic acute GVHD: GVHD occurring less than or equal to 100 days after transplantation or DLI with characteristic features of acute GVHD and without characteristic features of chronic GVHD.
2. Persistent, recurrent, or late-onset acute GVHD: GVHD occurring greater than 100 days after transplantation or DLI with characteristic features of acute GVHD and without characteristic features of chronic GVHD.
3. Classic chronic GVHD: GVHD occurring at any time that has characteristic features of chronic GVHD but that does not have characteristic features of acute GVHD.
4. Overlap syndrome: GVHD occurring at any time that has characteristic features of both acute and chronic GVHD.

Acute GVHD is graded according to standard grading systems as grades I through IV with grade IV being the most severe (see Section **1.1**).[108,109] Chronic GVHD is given a global score of mild, moderate, or severe according to the report of the NIH Consensus Development Project on Criteria for Clinical Trials in Chronic GVHD.[110] Based on organ scores detailed in Jagasia et al. Chronic GVHD can be staged as described here. Mild chronic GVHD involves only 1 or 2 organs or sites (except the lung), with no clinically significant functional impairment (maximum of score 1 in all affected organs or sites). Moderate chronic GVHD involves (1) at least 1 organ or site with clinically significant but no major disability (maximum score of 2 in any affected organ or site) or (2) 3 or more organs or sites with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites). A lung score of 1 will also be considered moderate chronic GVHD. Severe chronic GVHD indicates major disability caused by chronic GVHD (score of 3 in any organ or site). A lung score of 2 or greater will also be considered severe chronic GVHD.

1.2.9 T-cell gene therapy

In an attempt to develop effective immunotherapies for cancer that are less toxic than allogeneic stem cell transplantation, many investigators have developed T-cell gene therapy approaches to specifically target T cells to tumor-associated antigens.[111] T cells can be prepared for adoptive transfer by genetically modifying the T cells to express receptors that specifically recognize tumor-associated antigens.[111-118] Genetic modification of T cells is a quick and reliable process, and

clinical trials of genetically modified T cells targeting a variety of malignancies have been carried out.[111,119-122] Genetically modified antigen-specific T cells can be generated from peripheral blood mononuclear cells (PBMC) in sufficient numbers for clinical treatment within 10 days.[120] Genetically modifying T cells with gammaretroviruses consistently causes high and sustained levels of expression of introduced genes without in vitro selection,[119,122-124] and genetic modification of mature T cells with gammaretroviruses has a long history of safety in humans.[125-127] There are two general approaches for generating antigen-specific T cells by genetic modification: introducing genes encoding natural αβ T cell receptors (TCRs) or introducing genes encoding chimeric antigen receptors (CARs).[111,113,115,117] CARs are fusion proteins incorporating antigen recognition moieties and T cell activation domains.[116,128-130] The antigen-binding domains of most CARs currently undergoing clinical and preclinical development are antibody variable regions.[113,116,128,130] TCRs recognize peptides presented by human leukocyte antigen (HLA) molecules; therefore, TCRs are HLA-restricted, and a particular TCR will only be useful in patients expressing certain HLA molecules,[111,113,115,128] which limits the number of patients who could be treated with T cells genetically modified to express a TCR. In contrast, CARs recognize intact cell-surface proteins and glycolipids, so CARs are not HLA-restricted, and CARs can be used to treat patients regardless of their HLA types.[111,113,131-133]

1.2.10 **Chimeric antigen receptors preclinical background**

Preclinical experiments evaluating CAR-expressing T cells as cancer therapy were initiated in 1993.[134,135] These experiments led to a clinical trial of CAR-transduced T cells targeting the α-folate receptor on ovarian cancer cells; no tumor regressions were observed during this clinical trial.[136] Preclinical studies have assessed a wide variety of factors that could affect in vivo function of CAR-expressing T cells. Multiple approaches for inserting CAR genes into T cells by using gammaretroviruses,[119,122-125,137-139] lentiviruses,[120,140-143] or transposon systems[144,145] have been assessed. Because all methods of T-cell genetic modification require a period of in vitro culture, various T-cell culture techniques have been evaluated.[120,137,146] Different portions of CARs including antigen-recognition moieties, extracellular structural components, costimulatory domains such as the cytoplasmic portion of the CD28 protein, and T-cell-activation moieties such as the signaling domains of the CD3ζ protein can all be important to the in vivo function of CAR-expressing T cells, and all of these portions of CARs remain the subject of intensive investigation.[128,137,141,147-149]

Much of the preclinical work evaluating CARs has been performed with CARs targeting the B-cell antigen CD19.[137-139,144,150-152] Data suggesting that T-cell costimulation played an important role in the activity of CAR-expressing T cells in vivo led investigators to add signaling moieties from the costimulatory molecule CD28 to CARs.[139,148] These studies showed that adding CD28 moieties to CARs enhanced antigen-specific cytokine production and proliferation by anti-CD19 CAR T cells.[148,153,154] T cells expressing CARs with CD28 signaling moieties and CD3ζ signaling domains were more effective than T cells expressing CARs without CD28 moieties at eradicating human leukemia cells from mice.[153,154] Subsequently, CARs incorporating other signaling domains from costimulatory molecules such as 4-1BB (CD137) were developed.[140] Anti-CD19 CARs containing the signaling domains of both 4-1BB and CD3ζ were superior to CARs containing the signaling domains of CD3ζ without any costimulatory domains at eradicating

human malignant cells from mice.[141,147] Similar to CD28, including 4-1BB signaling moieties in CARs led to increased CD19-specific proliferation and enhanced in vivo persistence.[141] In contrast to T cells expressing a CAR with a CD28 moiety, the increased in vitro proliferation and prolonged in vivo persistence of T cells expressing a 4-1BB-containing CAR occurred whether or not the T cells were exposed to the antigen that the CAR recognized.[141,147]

1.2.11 **Clinical results with anti-CD19 CAR T cells**

Because the vast majority of clinical results with CAR T-cell therapies are with anti-CD19 CARs a summary of clinical results with anti-CD19 CARs is included here. Of course, clinical results with anti-CD30 CARs might be better or worse than the results obtained with anti-CD19 CARs. Results from several clinical trials of anti-CD19 CAR T cells have been reported to date in peer-reviewed papers.[119,120,122-124,155-159] The first evidence of antigen-specific activity of anti-CD19 CAR T cells in humans was generated during a clinical trial at the National Cancer Institute in a patient who experienced a dramatic regression of advanced follicular lymphoma.[123] This clinical trial utilized a gammaretroviral vector to introduce an anti-CD19 CAR containing the signaling domains of the CD28 and CD3ζ molecules.[123] The anti-CD19 CAR-transduced T cells were prepared by using a 24-day in vitro culture process. The clinical treatment regimen consisted of lymphocyte-depleting chemotherapy followed by an infusion of anti-CD19 CAR T cells and a course of high-dose interleukin-2 (IL-2). The first patient treated on this protocol had a large disease burden of follicular lymphoma. This first patient experienced no acute toxicities except for a low grade fever that lasted for 2 days, and he obtained a partial remission (PR) that lasted for 32 weeks after treatment.[123] Bone marrow biopsies revealed a complete elimination of extensive bone marrow lymphoma that was present before treatment; in addition, normal B-lineage cells were completely eradicated from the bone marrow.[123] The bone marrow B-cell eradication was confirmed by flow cytometry, and it persisted for over 36 weeks.[123] B cells were also completely absent from the blood during this time, while T cells and other blood cells recovered rapidly.[123] Seven months after the anti-CD19 CAR T cell infusion, progressive lymphoma was detected in the patient's cervical lymph nodes. The lymphoma remained $CD19^+$, so the patient was treated a second time with anti-CD19 CAR T cells. The first and second treatment regimens were the same except the patient received a higher dose of cells with the second treatment. After the second treatment, the patient obtained a second partial remission that is ongoing over 5 years post-treatment.[122]

Seven more patients were subsequently treated with the same regimen of chemotherapy, anti-CD19 CAR T cells, and high-dose IL-2.[122] In 4 of 7 evaluable patients on the trial, administration of anti-CD19 CAR T cells was associated with a profound and prolonged B-cell depletion.[122,123] In all 4 patients with B-cell depletion, the depletion lasted for over 36 weeks. The B-cell depletion could not be attributed to the chemotherapy that was administered because blood B-cells recovered to normal levels in 8 to 19 weeks in patients receiving the same chemotherapy plus infusions of T cells targeting NY-ESO or gp100, which are antigens that are not expressed by B cells.[123] Because normal B cells express CD19, prolonged normal B-cell depletion after anti-CD19 CAR T-cell infusions demonstrated that CAR-expressing T cells had a powerful ability to eradicate $CD19^+$ cells in humans. All of the patients with long-term B-cell depletion obtained either complete or partial remissions of their malignancies, and the 4 patients with long-term B cell depletion also developed hypogammaglobulinemia. Hypogammaglobulinemia in these patients was routinely treated with infusions of intravenous immunoglobulins. Of the eight patients treated, seven

patients were evaluable for malignancy response; the one patient who was not evaluable died with pneumonia caused by influenza A.[122] Six of the seven evaluable patients had remissions of their malignancies. Two of the remissions were complete remissions (CRs) of CLL.[122] Both of these CRs were confirmed by multicolor flow cytometry of bone marrow cells.[122] One of these CRs lasted 24 months, and the other is ongoing at 48 months.[122] Most patients treated with this regimen of chemotherapy, anti-CD19 CAR T cells, and IL-2 experienced significant acute toxicities including fever, hypotension, and neurological toxicities such as delirium and obtundation.[122] All of these toxicities peaked within 10 days after the cell infusion and resolved less than 3 weeks after the cell infusion.[122] These acute toxicities correlated with serum levels of the inflammatory cytokines tumor necrosis factor and interferon-γ, and T cells producing these inflammatory cytokines in a CD19-specific manner were detected in the blood of patients after the anti-CD19 CAR T cell infusions.[122] In our experience, patients with CLL and ALL tended to have more violent cytokine release syndromes than did patients with lymphoma. The severity of cytokine-release syndrome in patients' leukemia tends to correlate with disease burden in our experience. Patients with lymphoma tend to have a more varied clinical course with some patients experiencing hypotension and tachycardia, while others had isolated neurological toxicity.

We continued studies using the same CAR as in our previously reported anti-CD19 CAR T-cell reports. In these more recent studies, IL-2 was not administered to patients and the T-cell production process was shortened from 24 days to 10 days. The elimination of IL-2 administration was done in an attempt to lessen toxicity, and the shortening of the cell production process was done in an attempt to both simplify the cell production and to increase in vivo T-cell persistence and proliferation. We reported the results of this modified clinical protocol in a very recent paper.[160] In summary this paper reported treatment of 15 patients with advanced B-cell malignancies. Nine patients had diffuse large B-cell lymphoma (DLBCL), 2 patients had indolent lymphomas, and 4 patients had chronic lymphocytic leukemia (CLL). Patients received a conditioning chemotherapy regimen of cyclophosphamide and fludarabine followed by a single infusion of anti-CD19-CAR T cells. Of 15 patients, 8 obtained complete remissions (CRs), 4 patients obtained partial remissions, 1 patient had stable lymphoma, and 2 patients were not evaluable for response. CRs were obtained by 4 of 7 evaluable patients with chemotherapy-refractory DLBCL; 3 of these 4 CRs are ongoing with durations ranging from 9 to 22 months. Acute toxicities including fever, hypotension, delirium, and other neurological toxicities occurred in some patients after infusion of anti-CD19-CAR T cells; these toxicities resolved within 3 weeks after cell infusion. One patient died suddenly of an unknown cause 16 days after cell infusion. CAR T cells were detected in the blood of patients at peak levels ranging from 9 to 777 CAR^+ T cells/μL. Elimination of exogenous IL-2 from our protocol did eliminate the toxicity that is known to occur with administration of high-dose IL-2, but cytokine-release type toxicity attributable to the CAR T cells still remained.

In an attempt to further reduce the overall toxicity of our anti-CD19 CAR treatment protocol, we substantially reduced the dose of the chemotherapy regimen administered before CAR T-cell infusions. We treated 9 patients with B-cell lymphoma who received a single infusion of $1x10^6$ anti-CD19-CAR-expressing T cells/kg bodyweight preceded by a low-dose chemotherapy regimen of 3 daily doses of cyclophosphamide 300 mg/m^2 and fludarabine 30 mg/m^2 administered on the same days. Eight of the 9 patients had DLBCL that was refractory to chemotherapy (chemo-

refractory) or had relapsed less than 1 year after autologous stem cell transplantation. Both of these clinical situations carry a grim prognosis, with median survivals of only a few months. Despite the very poor prognosis of our patients, one patient with DLBCL obtained a CR and 4 DLBCL patients obtained PRs. The PRs included complete resolution of large lymphoma masses. Compared to our previous experience with anti-CD19 CAR T cells preceded by high-dose chemotherapy, toxicity was reduced when CAR T cells were infused after low-dose chemotherapy. None of the 9 patients treated with low-dose chemotherapy and CAR T cells required vasopressor drugs or mechanical ventilation, although some patients did experience short-term neurological toxicity. As expected, the severity of neutropenia and thrombocytopenia was reduced with the low dose chemotherapy compared to high-dose chemotherapy. Blood anti-CD19 CAR T cell levels have been assessed in 6 patients with a quantitative PCR assay that can detect cells containing the CAR gene; we detected CAR+ cells in the blood of all 6 patients. These results demonstrate that anti-CD19 CAR T cells administered after low-dose chemotherapy have significant activity against chemo-refractory DLBCL and could potentially become a standard treatment for patients with lymphoma.

The effectiveness of anti-CD19 CAR T cells against advanced B-cell malignancies, particularly CLL and ALL has been reported by multiple groups.[142,157-159,161] Multiple groups have recently demonstrated the 70% to 90% complete remission rates when anti-CD19 CAR T cells are used to treat ALL.[157,159,161] These impressive response rates have been associated with significant toxicity that can be divided into 2 main categories. The first category is "cytokine-release syndrome" that consists mainly of fever, tachycardia, hypotension, fatigue, and in some cases myocardial dysfunction; these toxicities typically last for a few days to approximately 2 weeks before resolving.[157,159,161] The second main category is neurological toxicity that sometimes occurs in patients not suffering from the typical cytokine-release syndrome toxicities. Commonly observed neurological toxicities include aphasia, tremor, seizures, and ataxia; similar to other toxicities, the neurological toxicities typically last from 1 or two days to 3 weeks before resolving.[159,161]

This clinical trial will also enroll patients who have had allogeneic hematopoietic stem cell transplants alloHSCT in the past. The cells for producing anti-CD30 CAR T cells will be obtained from the transplant recipients (patients with malignancy) rather than the transplant donors. In prior experience, administration of anti-CD19 CAR T cells, either T cells derived from normal transplant donors or from transplant recipients, graft-versus-host disease incidence has been extremely low.[157,162,163] In our own experience with donor-derived allogeneic anti-CD19 CAR T cells, no patient has developed acute GVHD out of a total of 19 patients treated (unpublished data and [162]). Despite the low GVHD rate, many alloHSCT patients with persisting B-cell malignancies have obtained remissions on clinical trials of anti-CD19 CAR T cells. [157,161-163]

1.2.12 **Anti-CD30 CAR development and preclinical testing**

We designed a CAR that incorporated a single chain variable fragment (scFv) from the fully-human anti-CD30 antibody 5F11. The 5F11 antibody was derived by immunizing mice transgenic for human immunoglobulin genes. This CAR also contained the hinge, transmembrane, and cytoplasmic regions of the human CD28 molecule, and the cytoplasmic region of the CD3-zeta molecule. The CAR gene is encoded by a self-inactivating lentiviral vector. The CAR was designated LSIN-5F11-28Z. LSIN stands for self-inactivating lentivirus. A diagram of the CAR

is shown in **Figure 2**. After transductions, we found high levels of cell surface expression of the anti-CD30 CAR on the transduced T cells (**Figure 3**).

Figure 2

Figure 3

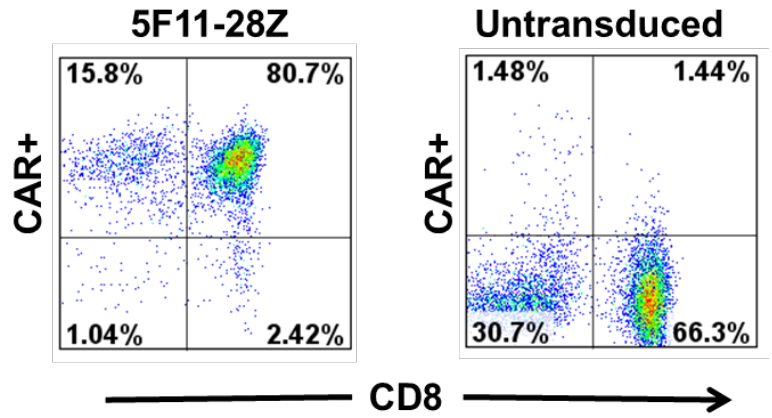
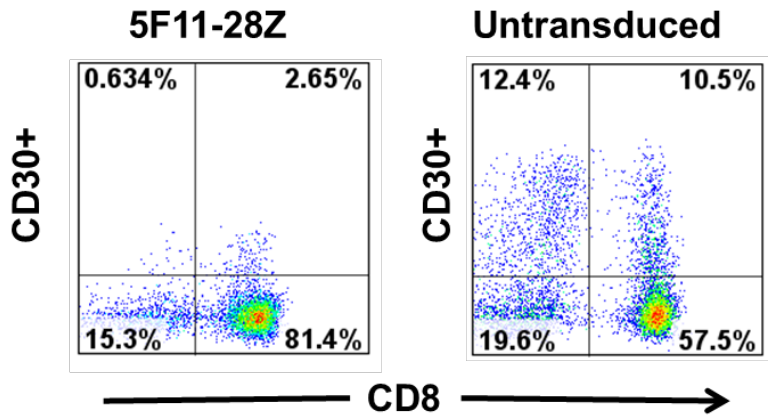


Figure 3 shows anti-CD30 CAR expression on T cells from Donor 1 six days after transduction with lentiviruses encoding the LSIN-5F11-28Z CAR. Transductions were carried out 1 day after the cultures were started, so the T cells had been in culture for a total of 7 days at the time of this analysis. The plots are gated on live, $CD3^+$ lymphocytes.

We also performed a series of in vitro assays to assess the function of anti-CD30-CAR-expressing T cells, and we found that CD30-CAR-expressing T cells exhibit CD30-specific activities including CD107a upregulation and cytokine production in vitro. These experiments showed that anti-CD30-CAR-expressing T cells are activated in an antigen-specific manner.

Figure 4



Because activated T cells express CD30, we hypothesized that the 5F11-28Z CAR T cells might eliminate CD30$^+$ activated T cells from cultures of anti-CD30 CAR-transduced T cells. In multiple experiments we found that cultures of 5F11-28Z-transduced T cells had only residual CD30$^+$ T cells that usually expressed low levels of CD30. In contrast, untransduced T-cell cultures contained sizable populations of CD30$^+$ T cells, some of which had high levels of CD30 expression. For the same cultures of Patient 1's T cells shown in **Figure 3**, **Figure 4** shows CD30 staining of T cells. The plots in **Figure 4** are gated on live CD3$^+$ lymphocytes.

Figure 5

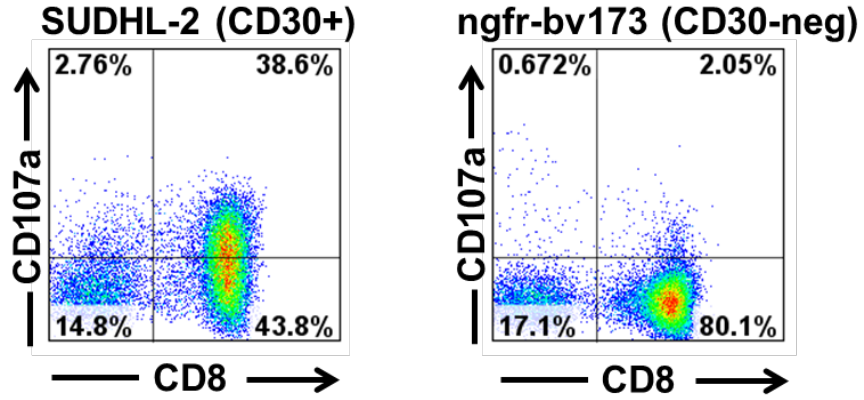


Figure 5 shows upregulation of CD107a, which indicates degranulation and correlates with cytotoxicity,[164] when anti-CD30 CAR-expressing T cells from Donor 2 were cultured with the CD30-expressing cell line SUDHL-1. CD107a was not upregulated when anti-CD30-CAR-expressing T cells from Donor 2 were cultured for 4 hours with the negative control cell line ngfr-

bv173, which does not express CD30. The plots show that the 5F11-28Z CAR T cells recognize CD30 in an antigen-specific manner.

It is critical to test any new CAR for specificity. To test for specificity, we cultured CAR-expressing T cells or untransduced T cells from the same patient with target cells overnight, and then performed a standard IFNγ (enzyme-linked immunosorbent assay) ELISA to see if T cells are activated, as indicated by IFNγ release, when the T cells are cultured with particular target cells (**Table 1**). Ideally, the anti-CD30 CAR T cells should only react with $CD30^+$ target cells. We performed ELISA assays on culture supernatant from overnight co-cultures of T cells plus either $CD30^+$ or CD30-negative target cells. T cells transduced with the anti-CD30 CAR produced large amounts of IFNγ when they were cultured overnight with the CD30-expressing cell lines but only small amounts of IFNγ when cultured with CD30-negative cell lines or CD30-negative primary human $CD34^+$ hematopoietic stem cells (**Table 1**). In all of the experiments reported in **Table 1**, effector T cells from a patient were either transduced with 5F11-28Z or left untransduced (UT). All numbers in the table are IFNγ levels in picograms/mL. The $CD30^+$ target cells used in these experiments were the $CD30^+$ lymphoma cell lines SU-DHL-1, HH, and HDLM-2. A chronic myeloid leukemia cell line called bv173 was transduced with the gene for CD30 to provide another $CD30^+$ target. These CD30-negative cell lines were used: the leukemia cell line ngfr-bv173 (bv173 transduced with the low-affinity nerve growth factor gene); the T-cell leukemia cell line CCRF-CEM, Saos-2 (a bone sarcoma cell line); A549 (a lung carcinoma cell line); MDA-231 (a breast cancer cell line); 293GP (a human embryonic kidney cell line); TC71 (a Ewings sarcoma cell line); COLO205 (a colon carcinoma cell line); U251 (a glioblastoma cell line) and Panc10.05 (a pancreatic carcinoma cell line). Primary human $CD34^+$ hematopoietic stem cells were also used as targets. In each experiment, the result for effector T cell cultured alone was also given. Experiment 3 in **Table 1** makes the important point that soluble CD30 protein added to ELISA co-cultures along with 5F11-28Z T cells and target cells only slightly reduced the amount of IFNγ produced by the CAR T cells. This indicates that CD30 protein does not block CAR recognition of target cells. This is important because CD30 can be shed by lymphomas, and some patients with $CD30^+$ lymphomas have soluble CD30 in their serum and presumably in their lymphoma masses.

***Table 1** Specificity of 5F11-28Z-transduced T cells*

Experiment 1

	Targets						
CAR expressed by T cells	**SUDHL-1**	**HH**	**CD30-bv173**	**NGFR-bv173**	**CCRF-CEM**	**T cells alone**	**% T cells expressing CAR**
5F11-28Z	25468	78624	3781	33	31	13	93.6
Un-transduced	17	29	39	26	16	<12	0

T cells expressing the indicated CARs in the far left column were cultured with the indicated target cells overnight, and then a standard IFNγ ELISA was performed. SUDHL-1, HH, and CD30-bv173 are $CD30^+$ cell lines. NGFR-bv173 and CCRF-CEM are CD30-negative cell lines. The

numbers are pg/mL of IFNγ released at the end of the culture period. The percent of T cells expressing each CAR was determined by staining CAR-transduced and Untransduced T cells with Protein L and subtracting the % protein L staining of untransduced T cells from the % protein L staining of each CAR-transduced T-cell population.

Targets							
CAR expressed by T cells	**SUD HL-1**	**HDLM-2**	**NGFR-bv173**	**CCRF-CEM**	**293GP**	**T cells alone**	**% of T cells expressing CAR**
5F11-28Z	**3489**	**3534**	**14**	**23**	**176**	**<12**	**95.8**
Un-transduced	**13**	**20**	**30**	**<12**	**137**	**<12**	**0**

Experiment 2

T cells expressing the indicated CARs in the far left column were cultured with the indicated target cells overnight, and then a standard IFNγ ELISA was performed. SUDHL-1, and HDLM-2 are $CD30^+$ cell lines. NGFR-bv173, CCRF-CEM, and 293GP are CD30-negative cell lines. The numbers are pg/mL of IFNγ released at the end of the culture period. The percent of T cells expressing each CAR was determined by staining CAR-transduced and untransduced T cells with Protein L and subtracting the % protein L staining of untransduced T cells from the % protein L staining of each CAR-transduced T-cell population.

Experiment 3

Targets								
CAR expressed by T cells	**HH0μg/mL CD30**	**HH 5 μg/mL CD30**	**HH 1 μg/mL CD30**	**HH 0.2 μg/mL CD30**	**U251**	**Colo-205**	**T cells with no target**	**Tcells with no target 5 μg/mL CD30**
5F11-28Z	**23021**	**18690**	**19920**	**18403**	**15**	**<12**	**<12**	**278**
Un-transduced							**143**	**123**

T cells expressing the 5F11-28 CAR or left un-transduced as indicated in the far left column were cultured with the indicated target cells overnight, and then a standard IFNγ ELISA was performed. HH are $CD30^+$ cell lines. The numbers followed by pg/mL refers to the concentration of human CD30 protein added to the media during the entire time that the T cells and target cells were cultured together. U251 and Colo-2015 are CD30-negative cell lines. The numbers are pg/mL of IFNγ released at the end of the culture period. 94.8% of the 5F11-28Z T cells expressed the CAR as measured by flow cytometry.

Experiment 4

Targets								
CAR expressed by T cells	**HH**	**A549**	**TC71**	**Sol8**	**Panc10.05**	**MDA231**	**Primary CD34+**	**T cells alone**
5F11-28Z	126692	107	773	92	48	354	153	26
Un-transduced	508	20	28	33	12	163	71	<12

T-cells expressing the 5F11-28 CAR or left un-transduced as indicated in the far left column were cultured with the indicated target cells overnight, and then a standard IFNγ ELISA was performed. HH is a $CD30^+$ lymphoma cell line. A549, TC71, Sol8, Panc10.05, and MDA231 are CD30-negtive cells lines. Primary $CD34^+$ hematopoietic stem cells were also included. 95.8% of the 5F11-28Z T cells expressed the CAR as measured by flow cytometry. 5F11-28Z-transduced T cells also proliferated in a CD30-specific manner.

Figure 6

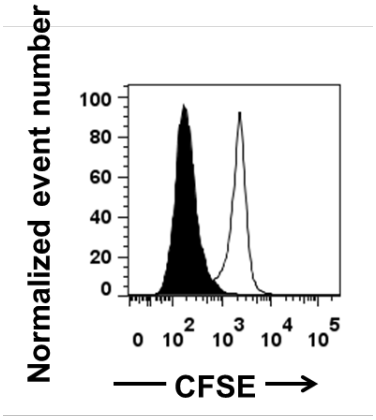


Figure 6shows a carboxyfluorescein diacetate,succinimidyl ester (CFSE) proliferation assay in which anti-CD30-CAR-transduced T cells were cultured for 4 days with either CD30-bv173 cells or CD30-negative ngfr-bv173 cells. CFSE was diluted to a greater degree, indicating more proliferation, compared to when 5F11-28Z-transduced T cells were cultured with CD30-negative ngfr-bv173target cells. The assay was conducted as described previously.[165]

Figure 7

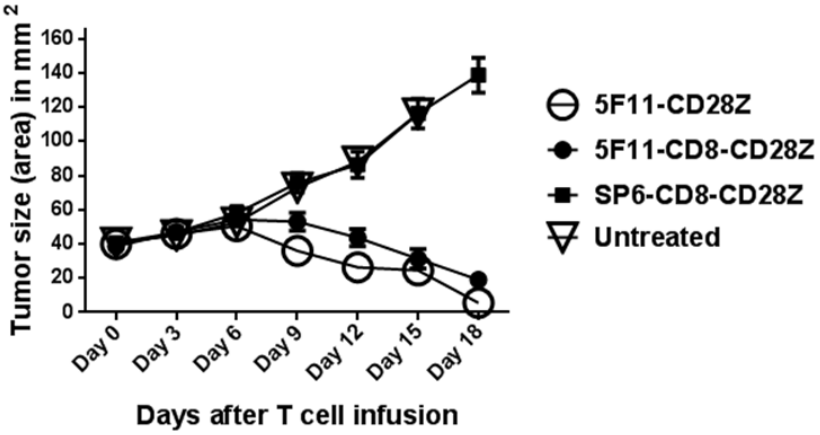
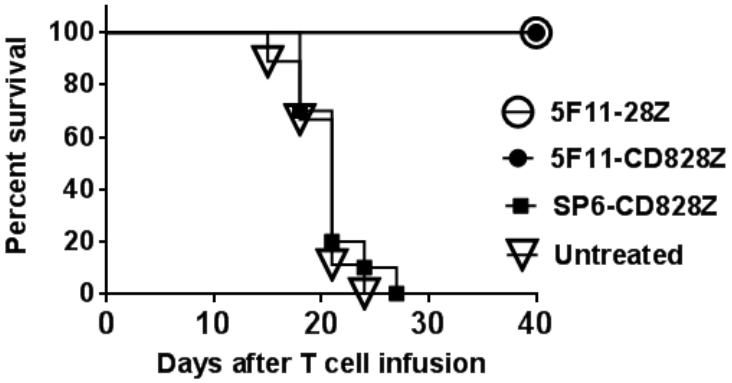


Figure 7 shows an experiment in which immunocompromised NSG mice were engrafted with HH lymphoma cells. The cells grow rapidly, and after 4 days, solid masses were present. Mice were then either left untreated or treated with a single infusion of 8 million T cells that were transduced with either 5F11-CD28Z or 5F11-CD828Z or the negative-control CAR SP6-CD828Z. T cells expressing either 5F11CAR were able to eliminate tumors in the mice, and clear superiority of one CAR over the other has not been established in this model.

Figure 8



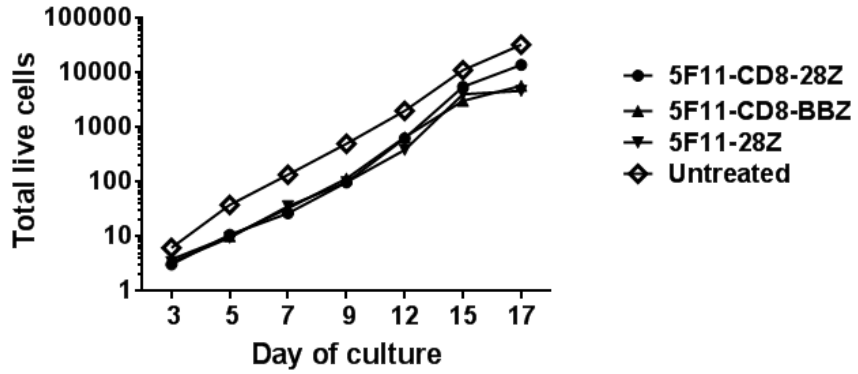
The overall survival of mice in the same experiment from **Figure 7**is shown in **Figure 8**. None of the mice treated with either 5F11-28Z or 5F11-CD828Z had a recurrence of their tumors. Of note, the HH cell line sheds substantial amounts or soluble CD30, and mice with HH tumors have CD30 detectable in their serum by ELISA. This shows that anti-CD30 CARs can be effective even in the presence of soluble CD30.

In the experiments depicted in **Figure 7** and **Figure 8**, CAR T cells were infused on Day 0, and no other treatments were administered. In the experiments reported in **Figure 7** and **Figure 8**,

there were 10 mice in each group except the untreated group, which had 9 mice. The experiment was terminated at day 40 because NSG mice receiving infusions of human T cells eventually develop graft-versus-host disease, which confounds experimental results.

As shown in **Figure 4**, $CD30^+$ T cells are eliminated from cultures of 5F11-28Z-transduced T cells. This raises the concern that T-cell cultures of anti-CD30 CAR-expressing T cells might not yield enough T cells to conduct a clinical treatment. We have performed multiple experiments in which the proliferation and overall increase in T-cell number were assessed in cultures of anti-CD30 CAR-transduced T cells. Results of one of 4 experiments with similar results are shown in **Figure 9**. 5F11-containing CARs did proliferate well with an increase in T-cell number compatible with obtaining enough cells for a clinical treatment in cultures of 3 different anti-CD30 CARs including 5F11-28Z, 5F11-CD828Z, and 5F11-CD8BBZ (which has a 4-1BB costimulatory domain). We conclude from this that fratricide of $CD30^+$ T cells by anti-CD30 CAR-containing T cells will not preclude generation of a clinical anti-CD30 CAR T-cell product.

Figure 9



1.2.13 **Rationale for immunosuppressive chemotherapy and selection of lymphocyte-depleting chemotherapy regimen.**

We plan to administer a conditioning chemotherapy regimen of cyclophosphamide and fludarabine before infusions of CAR-expressing T cells because substantial evidence demonstrates an enhancement of the anti-malignancy activity of adoptively-transferred T cells when chemotherapy or radiotherapy are administered before the T cell infusions.[1,2,166] In mice, administering chemotherapy or radiotherapy prior to infusions of tumor-antigen-specific T cells dramatically enhanced the anti-tumor efficacy of the transferred T cells.[1-3,111,115,166] Administering chemotherapy or radiotherapy enhances adoptive T-cell therapy by multiple mechanisms including depletion of regulatory T cells and elevation of T-cell stimulating serum cytokines including interleukin-15 (IL-15) and interleukin-7 (IL-7), and possibly depletion of myeloid suppressor cells and other mechanisms.[1-3,167] Removal of endogenous "cytokine sinks" by depleting endogenous T cells and natural killer cells caused serum levels of important T-cell stimulating cytokines such as IL-15 and IL-7 to increase, and increases in T-cell function and anti-tumor activity were dependent on IL-15 and IL-7.[1] Experiments in a murine xenograft model showed that regulatory

T cells could impair the anti-tumor efficacy of anti-CD19 CAR T cells.[168] Myeloid suppressor cells have been shown to inhibit anti-tumor responses.[167] Experiments with a syngeneic murine model showed that lymphocyte-depleting total body irradiation (TBI) administered prior to infusions of anti-CD19-CAR-transduced T cells was required for the T cells to cure lymphoma.[152] In these experiments, some mice received TBI, and other mice did not receive TBI. All mice were then challenged with lymphoma and treated with syngeneic anti-CD19-CAR T cells. Mice receiving TBI had a 100% cure rate and mice not receiving TBI had a 0% cure rate.[152]

Strong suggestive evidence of enhancement of the activity of adoptively-transferred T cells has been generated in humans.[119,169,170] Very few clinical responses have occurred and very little evidence of in vivo activity has been generated in clinical trials of autologous anti-CD19-CAR T cells administered without lymphocyte-depleting chemotherapy.[119,124] In contrast, many regressions and evidence of long-term B-cell depletion have occurred in clinical trials in which patients received anti-CD19-CAR T cells after lymphocyte-depleting chemotherapy.[120,122,123,142] The chemotherapy regimen that best increases the anti-malignancy efficacy of CAR-expressing T cells is not known, but the most commonly used chemotherapy regimens that have been used in clinical trials and that convincingly demonstrate persistence and in vivo activity of adoptively transferred T cells have included cyclophosphamide and/or fludarabine.[120-123,169,170] Both cyclophosphamide and fludarabine are highly effective at depleting lymphocytes.[169,170] One well-characterized and commonly used regimen is the combination of 300 mg/m^2 of cyclophosphamide administered daily for 3 days and fludarabine 30 mg/m^2 administered daily for three days on the same days as the cyclophosphamide.[171] Multiple cycles of this regimen can be tolerated by heavily pretreated leukemia and lymphoma patients.[171] As of amendment E (protocol version: 08/03/2018), the chemotherapy regimen is changed to cyclophosphamide 500 mg/m^2 and fludarabine 30 mg/m^2 daily for three days starting with Cohort 1 Dose Level 3 and Cohort 2 Dose Level 2 as described in Section 3.1.6 and Section 3.4.4.1. This is based on suboptimal lymphocyte depletion seen in 7 of the first 8 patients treated. This regimen was used successfully in a report of anti-CD19 CAR T-cell therapy [172].

1.2.14 **Rationale for dose-escalation**

The clinical trial described in this protocol is planned as a dose escalation in which the number of anti-CD30-CAR T cells administered to patients will be increased with sequential dose levels. The rationale for conducting a dose-escalation trial of a cellular therapy is based on two main sources of evidence. First, the anti-tumor efficacy of adoptively-transferred T cell treatments increases as the dose of T cells administered to mice increases.[173-175] Second, in the setting of allogeneic transplantation, relapsed malignancy is often treated with infusions of allogeneic donor lymphocytes (DLIs).[176,177] The incidence of graft-versus-host disease, which is caused by T cells attacking allogeneic antigens on host tissues, increases as the dose of T cells administered in DLIs increases.[176,177] Previous experience with autologous anti-CD19 CAR T cells has shown that a cell dose of 5 x 10^6 cells/kg may be too toxic, but a dose of 1 x 10^6 cells/kg is tolerable.[160] In this trial, the first dose level for patients in Cohort 1 has been set at less than a third of this tolerable dose, at 0.3 x 10^6 cells/kg. Based on the favorable safety profile found at the 0.3 x 10^6 cells/kg and at the 1 x 10^6 cells/kg doses for patients in Cohort 1, this protocol was amended to allow 1 x 10^6 cells/kg to be the starting dose for patients in Cohort 2 with amendment D (Protocol version: 05/03/2018).

1.2.15 **Rationale for a fully-human CAR**

Immune responses against genetically-modified T cells have previously been reported.[156,178,179] Cytotoxic T-cell responses against suicide-gene-modified T cells have previously been documented.[178] The CARs used in all published CAR studies to date used antibody components that were derived from murine antibodies. It would be expected that in at least some patients these murine components would be immunogenic. As with other aspects of the CAR field, most work concerning CAR immunogenicity has been conducted with anti-CD19 CARs. Supporting this belief are data from a small number of patients that indicate that T-cell responses can develop against the FMC63 single chain variable fragment (scFv) that is a part of most anti-CD19 CARs that are currently being tested. Jensen and coworkers and Lee and coworkers have reported T-cell responses against the FMC63 murine scFv.[156,179] Riddell and coworkers have reported cytotoxic T-cell responses against the FMC63 scFv (Riddell, Presentation at the American Society of Hematology Annual Meeting, 2013). Because this protocol intends to give patients multiple doses of CAR-modified T cells, the risk of developing anti-CAR immune responses is possibly even higher than in most previous clinical trials that only administered a single infusion of CAR T cells. All in all, use of a fully-human CAR will decrease the risk of anti-CAR T-cell responses that we hypothesize could interfere with CAR T-cell efficacy especially when multiple infusions of T cells are given.

The use of a fully-human CAR might increase persistence of CARs after a first dose of CAR T cells. In our ongoing trials of murine anti-CD19 CARs, persistence of CAR T cells in the blood has been limited.[160] We hope that the fully-human anti-CD30 CAR will have longer persistence.

1.2.16 **Summary of risks and potential benefits**

This clinical trial is being performed to evaluate a genetically-modified T-cell therapy for advanced $CD30^+$ lymphomas, which are often incurable diseases. Only patients with malignancies persisting despite at least 2 prior lines of therapy will be enrolled. The risks of the study fall into 6 general categories. First, chemotherapy that is part of the protocol treatment could cause cytopenias. As with any chemotherapy that causes neutropenia and thrombocytopenia, this chemotherapy could cause toxicities such as infections and bleeding. The second category of toxicity is cytokine-release-type toxicities such as high fevers, tachycardia, transient abnormalities of liver function, and hypotension. These cytokine-release-type toxicities have been detected in other clinical trials of CAR T cells during the first 2 weeks after anti-CD19 CAR T cells were infused.[122,158] A third category of potential toxicities are neurological toxicities such as delirium, obtundation, myoclonus, seizures, headache, and transient focal neurological toxicities including aphasia and focal paresis. In previous anti-CD19 CAR trials, cytokine-release toxicities and neurological toxicities have been limited in duration with toxicities generally resolving within 2 days to 3 weeks. Of course, with anti-CD30 CAR T cells, toxicity could be more or less severe than the toxicity that occurs after infusion of anti-CD19 CAR T cells.

The fourth possible category of toxicity is direct damage to normal tissues by the CAR T cells. This could happen because of unexpected cross-reactivity of the anti-CD30 CAR with proteins other than CD30 in vivo. This trial will be the first of a CAR containing the fully-human 5F11 variable regions, so cross-reactivity with normal proteins is not inconceivable. We have performed extensive testing of 5F11 CARs by culturing 5F11-CD28Z CAR T cells with a variety of human cell lines, and we have not seen recognition of cell lines that did not express CD30. While we did

not witness this in our cell line data or immunohistochemistry data from the NIH Department of Pathology, CD30 has been reported to be expressed on the deciduoid cells of the uterine lining of the pregnant uterus and in the uterus in the secretory phase.[11] Therefore for safety reasons women having had menses in the last six months prior to enrollment will be excluded. Participating women must have a serum FSH in the post-menopausal range, greater than 22 units/Liter. The occurrence of post-autologous stem cell transplant amenorrhea in women under the age of 40 is substantial, with resumption of normal menses in only 45% of patients having received 2 or more lines of salvage therapy,[180] meaning a large portion of the population of pre-menopausal women eligible for this protocol will likely already have baseline amenorrhea. Participating women will also have to agree to continue appropriate contraception and not become pregnant for one year following the last CAR T cell infusion, as per the hypothetical risk of toxicity to the endometrium of the pregnant uterus. Additional possible toxicities are those reported with anti-CD30 monoclonal antibodies, as described in section **1.2.4**.

A fifth category of toxicity caused by anti-CD30 CAR T cells is impairment of normal immunity because CD30 is expressed on activated T cells and activated B cells. A 6th potential type of toxicity that has never actually occurred in a clinical trial of T-cell gene therapies is a gene therapy-specific toxicity such as occurrence of a replication-competent lentivirus or transformation of T cells caused by insertional mutagenesis.[125]

The potential benefits to subjects enrolling on this trial include the possibility that the anti-CD30-CAR T cells can cause a significant anti-malignancy effect. Many patients enrolled on trials of anti-CD19 CAR T cells obtained prolonged complete remissions of advanced malignancies,[120,122,181] so there is a chance that recipients of the anti-CD30 CAR T cells that are being evaluated in this protocol could derive a direct benefit from participation in this trial. In contrast, some patients did not obtain remissions on prior anti-CD19 CAR trials and in some patients the remissions were not lasting. Of course clinical results with anti-CD30 CAR T cells could be either better or worse than results with anti-CD19 CAR T cells. A small number of patients may have lymphomas that express both CD19 and CD30, specifically patients with DLBCL, PMBL, or grey zone lymphomas, and may be eligible for both the NCI SB autologous fully human anti-CD19 CAR T cell protocol and this protocol. In these cases, the patients would be referred first to the anti-CD19 CAR T cell protocol, as extensive data from multiple centers have already shown anti-CD19 CAR T cells to be active in chemorefractory DLBCL with expected cytokine release toxicities. Patients might also derive a benefit from knowing that they are contributing to the development of new cellular therapies for cancer. Aiding in the development of new therapies might help future patients.

As of amendment G (Protocol version: 08/12/2019) we are no longer enrolling Hodgkin lymphoma patients due to a lack of efficacy against Hodgkin lymphoma patients. This anti-CD30 CAR T-cell product appears to have modest activity in patients with Hodgkin lymphoma. Possible reasons for limited anti-malignancy efficacy in Hodgkin lymphoma include inhibition of the CAR T cells by the tumor microenvironment. Additionally, malignant Reed Sternberg cells, which express CD30, make up a minority of the cells in a Hodgkin lymphoma tumor mass; therefore, even in patients with significant burdens of lymphoma, the antigen stimulation of the CAR T cells by CD30 may be limited.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

Note: if a patient meets an eligibility requirement as outlined below, and is enrolled on the protocol but then is found to no longer meet the eligibility requirement after enrollment but before the start of protocol treatment, the treatment will be aborted or delayed.

2.1.1 **Inclusion Criteria**

2.1.1.1 Malignancy criteria

- Patients must have anaplastic large cell lymphoma, angioimmunoblastic T-cell lymphoma, peripheral T-cell lymphoma not otherwise specified, diffuse large B-cell lymphoma not otherwise specified, primary mediastinal B-cell lymphoma, grey zone lymphoma, enteropathy-associated T-cell lymphoma, or extranodal NK/T-cell lymphoma, nasal type
- Clear CD30 expression must be detected on 75% or more of malignant cells from either bone marrow or lymphoma mass by flow cytometry or immunohistochemistry. The patient's malignancy will need to be assessed for CD30 expression by flow cytometry or immunohistochemistry performed at the NIH. If unstained, paraffin-embedded bone marrow or lymphoma sections are available from prior biopsies, these can be used to determine CD30 expression by immunohistochemistry; otherwise, patients will need to come to the NIH for a biopsy to determine CD30 expression. The sample for CD30 expression can come from a biopsy obtained at any time before enrollment, unless the patient has received a prior anti-CD30 monoclonal antibody, in which case the sample must come from a biopsy following completion of the most recent anti-CD30 monoclonal antibody treatment.
- Eligible patients with with diffuse large B-cell lymphoma or primary mediastinal B-cell lymphoma must have received 2 prior treatment regimens at least 1 of which included an anthracycline and an anti-CD20 monoclonal antibody
- Patients must have measurable malignancy as defined by at least one of the criteria below.
  - Lymphoma mass that is measurable (minimum 1.5 cm in largest diameter) by CT scan is required unless bone marrow lymphoma is detectable.
  - For a lymphoma mass to count as measurable malignancy, it must have abnormally increased metabolic activity when assessed by positron emission tomography (PET) scan.
  - For lymphoma with only bone marrow involvement, no mass is necessary, but if a mass is not present, bone marrow malignancy must be detectable by flow cytometry.

2.1.1.2 **Other inclusion criteria**

- Greater than or equal to 18 years of age and less than or equal to age 73.
- Able to understand and sign the Informed Consent Document.
- Clinical performance status of ECOG 0-2

- Room air oxygen saturation of 92% or greater
- Male patients and must be willing to practice birth control from the time of enrollment on this study and for four months following the final CAR T cell infusion. Pre-menopausal patients (female patients who have had a menstrual period within the last year) must be willing to practice birth control from the time of enrollment and for one year following the final CAR T cell infusion.
- Seronegative for HIV antibody. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who are HIV seropositive can have decreased immune -competence and thus are less responsive to the experimental treatment and more susceptible to its toxicities.)
- Seronegative for HTLV-1.
- Negative for hepatitis B surface antigen. Positive hepatitis B tests can be further evaluated by confirmatory tests; and if confirmatory tests are negative, the patient can be enrolled.
- Seronegative for hepatitis C antibody unless antigen negative. If hepatitis C antibody test is positive, then patients must be tested for the presence of RNA by RT-PCR and be HCV RNA negative to be enrolled.
- A patient with a prior history of hepatits B or a prior history of hepatitis C may participate, as long as the patient's viral hepatitis has been treated, and the patient has no detectable HBV DNA or HCV RNA.
- At time of protocol enrollment, the patient should be negative for CMV by antibody testing or by PCR. In case of disagreement between these 2 CMV tests, the tests will be repeated and Dept. of Laboratory Medicine consulted.
- Absolute neutrophil count greater than or equal to 1000/mm^3 without the support of filgrastim or other growth factors
- Platelet count greater than or equal to 55,000/mm^3
- Hemoglobin greater than 8.0 g/dl. Transfusion support is allowed.
- Less than 5% malignant cells in the peripheral blood leukocytes
- Serum ALT and AST less or equal to 3 times the upper limit of the institutional normal unless liver involvement by malignancy is demonstrated.
- Serum creatinine less than or equal to 1.6 mg/dL.
- Total bilirubin less than or equal to 2.0 mg/dl.
- At least 14 days must have elapsed since any prior systemic therapy prior to apheresis and prior to the initiation of chemotherapy (including systemic corticosteroids at doses greater than prednisone 5 mg/day or the equivalent corticosteroid doses). Because this protocol requires collection of autologous blood cells by leukapheresis in order to prepare CAR T cells, systemic anti-malignancy therapy including systemic corticosteroid therapy at doses greater than prednisone 5 mg/day or the equivalent corticosteroid doses is not allowed within 14 days prior to the required leukapheresis. NOTE: Because of the long half-life and potential to affect CAR T cells, 30 days must elapse from the time of administration of anti-PD-1 or anti-PD-L1 antibodies or other agents that in the opinion of the PI can stimulate immune activity and infusion of CAR T cells.
- Normal cardiac ejection fraction (greater than or equal to 50% by echocardiography) and no evidence of hemodynamically significant pericardial effusion as determined by an echocardiogram within 4 weeks of the start of the treatment protocol.

- Patients must not take corticosteroids at doses higher than 5 mg/day of prednisone or the equivalent for 14 days before apheresis, and for 14 days prior to the conditioning chemotherapy regimen.
- Patients must be willing to undergo endotracheal intubation, mechanical ventilation, dialysis, CPR, and electrical defibrillation. Patients must be willing to receive vasopressor drugs and all other standard intensive care unit interventions. Any living will must be amended to allow these interventions or the patient will not eligible.
- Patients who have been treated on other protocols of genetically-modified T cells at the NIH only are potentially eligible under these conditions:
    - At least 6 months have elapsed since the last genetically-modified T-cell therapy that the patient received and there is no evidence of replication-competent retroviruses (evidence must be provided from prior NIH gene-therapy protocol Principal Investigator) and persisting genetically-modified T cells are not detectable in the patient's blood (evidence must be provided by prior NIH gene-therapy protocol Principal Investigator).

2.1.1.3 Additional inclusion criteria pertinent only for patients with prior allogeneic transplantation:

- Recipients must have received an 9/10 or 10/10 HLA-matched sibling allogeneic hematopoietic stem cell transplant, or a 9/10 or 10/10 HLA-matched unrelated donor (URD) allo-HSCT for an eligible $CD30^+$ lymphoma.

- Donor T cell engraftment after allo-HSCT (>90% donor chimerism of the T cell compartment).

- Patients must be at least 90 days post-transplant.

- Patients must be off all systemic immunosuppressive drugs including corticosteroids at doses of greater than 5 mg/day prednisone or equivalent, if given for treatment of graft versus host disease, for at least 28 days prior to protocol enrollment and must remain off immunosuppressive drugs while enrolled on the protocol. Patients must not be taking any systemic steroids at doses greater than 5 mg/day prednisone or equivalent for 14 days prior to apheresis and initiation of chemotherapy. Topical corticosteroid preparations applied to the skin such as solutions, creams, and ointments are allowed. Inhaled corticosteroids are allowed, and corticosteroid eye drops are allowed.

- Prior DLIs are not necessary.

- Either no evidence of GVHD or minimal clinical evidence of acute GVHD and chronic GVHD while off of systemic immunosuppressive therapy for at least 28 days. Minimal clinical evidence of acute GVHD defined as grade 0 to I acute GVHD (see Section **1.1**).[109] Minimal evidence of chronic GVHD is defined as mild global score chronic GVHD (as defined by the 2005 NIH consensus project) or no chronic GVHD (see Section **14.5**).[107]

Subjects with disease that is controlled to stage I acute GVHD or to mild global score chronic GVHD with local topical cutaneous steroids will be eligible for enrollment.

2.1.2 **Exclusion criteria**

- Patients with Hodgkin lymphoma are no longer eligible for participation (as of amendment G, Protocol version: 08/12/2019).
- Patients that require urgent therapy due to tumor mass effects or spinal cord compression.
- Patients with lymphoma masses 10.0 cm or larger in longest diameter will not be eligible.
- Patients that have active hemolytic anemia.
- Patients who are currently taking any medications for systemic anticoagulation other than aspirin will not be eligible.
- Patients with second malignancies in addition to their lymphoma are not eligible if the second malignancy has required treatment (including maintenance therapy) within the past 4 years or is not in complete remission. There are two exceptions to this criterion: successfully treated non-metastatic basal cell or squamous cell skin carcinoma.
- Women of child-bearing potential who are pregnant or breastfeeding because of the potentially dangerous effects of the preparative chemotherapy on the fetus or infant. Women of child-bearing potential are defined as all women except women who are post-menopausal or who have had a hysterectomy. Postmenopausal will be defined as women over the age of 55 who have not had a menstrual period in at least 1 year.
- Active uncontrolled systemic infections (defined as infections causing fevers and infections requiring intravenous antibiotics when the intravenous antibiotics have been administered for less than 72 hours); active coagulation disorders or other major uncontrolled medical illnesses of the cardiovascular, respiratory, endocrine, renal, gastrointestinal, genitourinary or immune system; history of myocardial infarction; history of idiopathic ventricular tachycardia or ventricular fibrillation in the past 12 months or history of ventricular tachycardia or ventricular fibrillation (VT/VF) associated with VT/VF risk factors (e.g., QT prolongation and cardiomyopathy); active cardiac arrhythmias (Active atrial fibrillation is not allowed, but resolved atrial fibrillation is allowed.); active obstructive or restrictive pulmonary disease; or active autoimmune diseases such as rheumatoid arthritis.
- Patients will not be seen for screening appointments or enrolled on the protocol if they have been hospitalized within the 7 days prior to the screening appointment or the date of protocol enrollment.
- Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease), unless the immunodeficiency has been cured by allogeneic stem cell transplant
- Systemic corticosteroid therapy at doses greater than 5 mg/day of prednisone or the equivalentdose is not allowed within 14 days prior to the required leukapheresis, or the initiation of the conditioning chemotherapy regimen. Corticosteroid creams, ointments, and eye drops are allowed.
- History of severe immediate hypersensitivity reaction to any of the agents used in this study.
- Patients with current CNS involvement by malignancy (either by imaging or cerebrospinal fluid involvement or biopsy-proven).
- Patients currently taking anticoagulants

2.1.3 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on the NIH websites and on NIH social media platforms. Recruitment flyers will also be distributed to oncologists who may refer patients. Recruitment flyers may also be emailed to individuals who have previously indicated their interest in being notified about NIH clinical trials through email. Participants will be recruited from the current patient population at NIH, and local community physicians.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed after a consent for screening has been signed

Note: Screening evaluation testing/procedures are conducted under the separate screening protocol, 01C0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols).

The following assessments must be completed within **4 weeks** prior to starting the chemotherapy conditioning regimen unless otherwise noted (if not, then the evaluation must be repeated):

- Complete history and physical examination, including, weight, height, and vital signs. Note in detail the exact size and location of any lesions that exist.
- Confirmation of diagnosis of lymphoma by the NCI Laboratory of Pathology and confirmation of clear CD30 expression on greater than 75% of the malignant cells from either bone marrow or a lymphoma mass by flow cytometry or immunohistochemistry. The sample used for this CD30 expression analysis can come from any time prior to enrollment on the protocol. The sample can be a fresh biopsy or paraffin-fixed slides.
- EKG
- MRI of the brain
- PET-CT if necessary to document measurable malignancy
- CT scan of neck, chest, abdomen, and pelvis if necessary to document measurable malignancy.
- Bone marrow biopsy with flow only if necessary to document measurable $CD30^+$ malignancy for enrollment purposes. If not necessary for enrollment and if not necessary clinically to investigate cytopenias, the bone marrow biopsy is not needed.
- Donor venous assessment (If has been performed with-in six months of apheresis, it does not need to be repeated)
- Antibody screen for Hepatitis B and C, HIV, HTLV-I/II, T. cruzi (Chagas agent), West Nile, CMV, and syphilis (RPR)
- Blood PCR for EBV, CMV, BK virus, and JC virus.
- Cardiac echocardiogram
- Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus,

Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid

- CBC with differential
- PT/PTT
- Glucose-6-phosphate dehydrogenase (G6PD) deficiency screening
- Thyroid Stimulating Hormone, T3
- Serum cortisol
- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential. Women of child-bearing potential are defined as all women except women who are post-menopausal or who have had a hysterectomy. Postmenopausal will be defined as women over the age of 55 who have not had a menstrual period in at least 1 year.

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found here.

2.3.1 Treatment Assignment Procedures for Registration Purposes Only:

Cohorts:

Number	Name	Description
1	Cohort 1	Dose escalation for patients who never had an alloHSCT
2	Cohort 2	Dose escalation for patients who have had an alloHSCT

Arm:

Number	Name	Description
1	CAR T-cells + Immuno therapy	All patients will be receiving starting dose: 0.3 x10^6 or 1.0 x10^6 CAR+ T cells/kg (weight based dosing)(up to a maximum dose of 18x10^6 CAR+ T cells/kg) infuse on day 0 + Cyclophosphamide: 300 or 500 mg/m2 IV infusion over 30 minutes on days -5, -4 and -3 + Fludarabine: 30 mg/m2 IV infusion over 30 minutes administered immediately following the cyclophosphamide on days -5, -4, and -3

Arm Assignment:

Patients in cohort 1 will be directly assigned to arm 1

Patients in cohort 2 will be directly assigned to arm 1

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

3.1.1 General study plan

This protocol is a phase I dose-escalation study of autologous T cells that are genetically modified to express a fully-human anti-CD30 CAR. The cell dose will be escalated unless occurrence of dose-limiting toxicities limits further escalation or until the maximum planned dose level is reached. The protocol will enroll patients who have never had an allogeneic hematopoietic stem cell transplant (allo-HSCT) and patients who have had allo-HSCT.

The protocol will enroll patients with malignancies that are resistant to standard therapies. Patients will be evaluated for general health with an emphasis on detecting cardiac and neurological abnormalities. An assessment of CD30 expression will be an important part of the eligibility screening. Patients enrolled on the study will undergo leukapheresis, and anti-CD30-CAR-expressing T cells will be generated by transducing the patient's T cells with a lentivirus encoding the anti-CD30 CAR. Patients will receive a conditioning chemotherapy regimen of cyclophosphamide 300 mg/m^2 or 500 mg/m^2 daily for 3 days and fludarabine 30 mg/m^2 IV daily for 3 days on the same days. This is an extensively-used chemotherapy regimen that can be easily administered on an outpatient basis. Results using similar lymphodepletion regimens incorporating varying doses of fludarabine and cyclophosphamide have been previously reported in anti-CD19 CAR T cell clinical trials.[157,160,161] Two days after the end of the conditioning chemotherapy, patients will receive a single infusion of anti-CD30-CAR-expressing T cells. A minimum 9-day hospitalization will be required after the cell infusion to monitor closely for acute toxicities. If acute toxicities occur or the patient remains at risk, it is anticipated that the patient will have a longer inpatient stay for medical management. Patients are required to stay within 60 minutes driving time from the Clinical Center until day 14 after the CAR T-cell infusion. Patients will then be evaluated for toxicity, and malignancy will be staged at 1, 2, 3, 4, 6, 9, and 12 months after the infusion.

A small number of subjects may be eligible for re-enrollment if a patient is removed from the protocol BEFORE completing protocol therapy, and would be required to meet all eligibility criteria at the time of re-enrollment. Patients will be assigned a new sequential study number for the reenrollment study period. Any cryopreserved anti-CD30 CAR T cells produced from a patient who was removed from the study can be used to treat that patient after re-enrollment. We do not anticipate changes in the risk profile for the initial versus re-enrollment

3.1.2 Planned repeat treatments

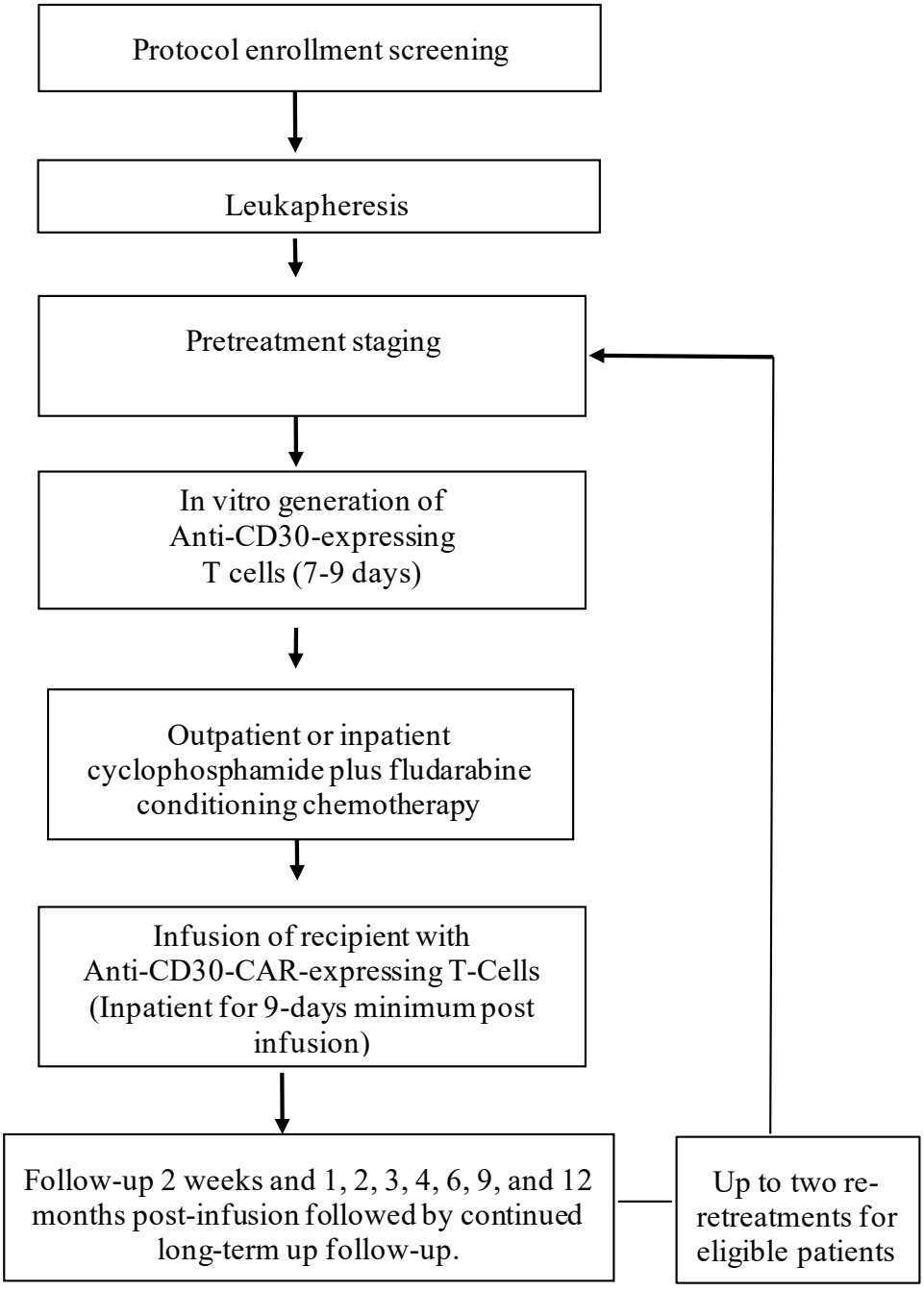
As of amendment E (Protocol version: 08/03/2018), patients will be allowed to receive multiple CAR T-cell infusions. Once the MTD has been determined for a cohort, patients in that cohort will be allowed to receive a second and potentially a third CAR T-cell treatment at the MTD for that patient's cohort. A maximum of 3 total CAR T-cell infusions per patient are allowed. Administration of second and third treatments will follow the same treatment plan as first treatments including the cyclophosphamide and fludarabine chemotherapy. Second and third

treatments will only be administered if the patient has measurable residual malignancy at least 2 months after the most recent prior treatment. Patients with a best response of progressive disease after a treatment are not eligible for re-treatment. Patient experiencing a DLT at any time are not eligible for retreatment. To be eligible for retreatment, a patient must have either cryopreserved CAR T cells or PBMC available that have never been exposed to a gene-therapy vector. If more than 1 of the first 3 patients, or 2 of the first 6 patients who are retreated have adverse events that meet criteria used to define DLTs, re-treatments will be stopped.

3.1.3 Inclusion of patients who have had allogeneic hematopoietic stem cell transplantation (allo-HSCT)

This trial will enroll patients with a history of allo-HSCT. Because patients who have had an allo-HSCT might be more prone to a variety of toxicities than patients who have not had a transplant and because the biology of adoptively-transferred T cells could be different in the allogeneic transplant setting, allo-HSCT patients will be enrolled on a separate cohort than patients who have not had an allo-HSCT. **Cohort 1** will enroll patients who have not had an allogeneic hematopoietic stem cell transplant, and **Cohort 2** will enroll patients who have had an 9/10 or 10/10 HLA-(human leukocyte antigen)-matched sibling or an 9/10 or 10/10-HLA-matched unrelated donor transplant.

3.1.4 **Protocol schema**



3.1.5 Dose Limiting Toxicity

Dose-limiting toxicities are defined as the following toxicities occurring within 30 days of CAR T-cell infusion (Note that DLTs will only be considered in patients who have received CAR T-cell infusions):

- Grade 3 toxicities possibly, probably, or definitely related to either the anti-CD30 CAR T cells or the fludarabine and cyclophosphamide chemotherapy and lasting more than 7 days
- Grade 4 toxicities possibly, probably, or definitely related to the anti-CD30 CAR T cells or the chemotherapy

The following specific toxicities will not be dose-limiting toxicities:

- Neutropenia (ANC<500/μL) lasting continuously 14 days or less
- Anemia (Hgb <8 g/dL) lasting continuously 10 days or less
- Transfusion-dependent thrombocytopenia lasting 25 days or less
- Thrombocytopenia that does not require transfusion support
- Asymptomatic hypophosphatemia of any grade
- All cytopenias except neutropenia, anemia, or thrombocytopenia
- Hypotension requiring treatment with vasopressors (norepinephrine dose of >2 mcg/minute or equivalent, doses less than or equal to 2 mcg/minute are not a DLT)for 72 hours or less. The 72 hours is measured from the first institution of vasopressors even if vasopressors are temporarily discontinued and then re-started.
- Fever
- Grade 4 elevation in alanine aminotransferase, or aspartate aminotransferase, or bilirubin that resolves to Grade 3 or less within 3 days or less
- Grade 4 creatinine kinase elevation that resolves to Grade 3 or less within 3 days or less
- Grade 4 prothrombin time (PT) or partial thromboplastin time (PTT) that resolves to Grade 3 in 3 days or less with no evidence of clinically-significant bleeding or thrombosis
- Asymptomatic electrolyte disturbances regardless of grade
- Prolongation of the QT_c interval of the ECG
- Infections controlled by antibiotics

For past recipients of allogeneic hematopoietic stem cell transplants only, the following will also be DLTs

- Grade IV acute GVHD by 60 days after infusion of anti-CD30 T cells (see Section **1.1)**
- Grade III acute GVHD by 60 days after infusion of anti-CD30 T cells that does not resolve to a Grade 0-1 with corticosteroid therapy within 30 days of onset (see Section **14.4**)

3.1.6 Dose Escalation

The trial will be a dose-escalation with 5 dose levels based on the patient's **actual** bodyweight. As noted above, there will be two separate cohorts of patients enrolled on the trial, Cohort 1: Patients who have not had an allogeneic hematopoietic stem cell transplant (allo-HSCT) and Cohort 2: Patients who have had a 9/10 or 10/10 HLA-matched sibling or an 9/10 or 10/10-matched unrelated

donor transplant. Each cohort will have a completely separate dose escalation, DLTs occurring in allo-HSCT recipients will not affect dose escalation on the cohort of patients who have not had an allo-HSCT. Likewise, the dose escalation of the allo-HSCT patients will not be affected by the no-allo-HSCT patient dose escalation. A separate dose MTD will be established for each cohort. In this trial, the first dose level for patients in Cohort 1 has been set at 0.3 x 10^6 cells/kg. Based on the favorable safety profile found at the 0.3 x 10^6 cells/kg and at the 1 x 10^6 cells/kg doses for patients in Cohort 1, this protocol was amended to allow 1 x 10^6 cells/kg to be the starting dose for patients in Cohort 2. The dose escalation for Cohort 1 is depicted in Table 2, and the dose escalation for Cohort 2 is depicted in ***Table 3***.

As of implementation amendment E (Protocol version: 08/03/2018), the cyclophosphamide dose in the conditioning chemotherapy regimen will be increased to 500 mg/m^2 daily. To allow a careful evaluation of this change, we will treat a minimum of 3 patients on cohort 1 dose level 3 with 500 mg/m^2 of cyclophosphamide daily before proceeding with the planned dose escalation, even though 3 patients have already been treated on cohort 1 dose level 3 with the prior cyclophosphamide dose of 300 mg/m^2 daily.

The following dose escalation plan refers to the **first** dose of CAR T cells received by patients on both cohorts of the protocol. CAR^+ T cells are defined as $CD3^+CAR^+$ cells as measured by flow cytometry according to Department of Transfusion Medicine (DTM) SOPs. The number of anti-CD30-CAR-expressing T cells transferred for each dose level will be as follows:

Table 2: ***First-dose Escalation Plan for Cohort 1***

First-dose Escalation Plan for Cohort 1: separate but identical dose escalation plans will be used for both Cohort 1 (patients who never had an allo-HSCT) and Cohort 2 (patients who have had an allo-HSCT). Doses +/- 10% from the listed doses are acceptable.	
Dose Level	Dose of anti-CD30 CAR T cells
Level -1	0.15x10^6 CAR+ T cells per kg of recipient bodyweight
Level 1	0.3x10^6 CAR+ T cells per kg of recipient bodyweight
Level 2	1.0x10^6 CAR+ T cells per kg of recipient bodyweight,
Level 3	3.0x10^6 CAR+ T cells per kg of recipient bodyweight with a maximum dose of 360 x10^6 CAR+ T cells

Level 4	$9.0x10^6$ CAR+ T cells per kg of recipient bodyweight with a maximum dose of 108 $x10^7$ CAR+ T cells
Level 5	$18x10^6$ CAR+ T cells per kg of recipient bodyweight with a maximum dose of 216 $x10^7$ CAR+ T cells

Table 3: ***First-dose Escalation Plan for Cohort 2***

First-dose Escalation Plan for Cohort 2: separate but identical dose escalation plans will be used for both Cohort 1 (patients who never had an allo-HSCT) and Cohort 2 (patients who have had an allo-HSCT). Doses +/- 10% from the listed doses are acceptable.	
Dose Level	Dose of anti-CD30 CAR T cells
Level -1	$0.3x10^6$ CAR+ T cells per kg of recipient bodyweight
Level 1	$1.0x10^6$ CAR+ T cells per kg of recipient bodyweight,
Level 2	$3.0x10^6$ CAR+ T cells per kg of recipient bodyweight with a maximum dose of 360 $x10^6$ CAR+ T cells
Level 3	$9.0x10^6$ CAR+ T cells per kg of recipient bodyweight with a maximum dose of 108 $x10^7$ CAR+ T cells
Level 4	$18x10^6$ CAR+ T cells per kg of recipient bodyweight with a maximum dose of 216 $x10^7$ CAR+ T cells

Each dose level will include a minimum of 3 patients. All cell doses will be cryopreserved cells thawed just before infusion. All infusions will be preceded by the fludarabine and cyclophosphamide conditioning regimen. The percentage of CAR^+ T cells and the number of total cells to infuse to obtain the indicated numbers of CAR^+ T cells will be determined prior to cryopreservation. There will be a minimum of 9 days between the CAR T-cell infusion of a patient and the start of the conditioning chemotherapy regimen for the next patient on the same cohort.

This will cause a 14 day delay between sequential CAR T-cell infusions on each cohort. Twenty-eight days must elapse between cell infusion to the last patient on a dose level and protocol enrollment of the first patient on the next highest dose level for each cohort.

Patients will be enrolled sequentially; therefore, enrollment will not proceed to a higher dose level until all patients have been treated in the prior cohort. If sufficient cells cannot be grown to meet the criteria for the first dose of cells for the assigned dose level, the treatment will be aborted. A second attempt will be made to prepare cells for the patient if the patient agrees and if the patient still meets all eligibility criteria.

Should none of the first 3 patients treated on a dose level experience a DLT enrollment can start on the next higher dose level. Should 1 of 3 patients experience a dose limiting toxicity on a particular dose level, three more patients would be treated at that dose level to confirm that no greater than 1/6 patients have a DLT prior to proceeding to the next higher level. If 1/6 patients have a DLT at a particular dose level, accrual can proceed to the next higher dose level. If a level with 2 or more DLTs in 3-6 patients has been identified, 3 additional patients will be accrued at the next-lowest dose for a total of 6, in order to further characterize the safety of the maximum tolerated dose. The maximum tolerated dose is the dose at which a maximum of 1 of 6 patients has a DLT. If 2 DLTs occur on dose level 1, accrual will proceed at dose level -1, as indicated in

***Table 2*: First-dose Escalation Plan for Cohort 1** and **Table 3.**

After a maximum tolerated dose is defined, additional patients can be treated on this trial. Up to 8 total additional recipients can be treated with the MTD after an MTD is established. If cell growth limitations preclude administration of the maximum tolerated dose, the patient will receive as many cells as possible up to the maximum tolerated dose. If it proves to be technically impossible or impractical to achieve the higher dose levels due to cell production constraints and a maximum tolerated dose has not been reached, the highest achievable dose level will be declared the maximum feasible dose.

Dose escalations will follow the rules outlined in the **Table 4** below.

Table 4:* *Dose Escalations Rules

Number of Patients with DLT at a Given Dose Level	**Escalation Decision Rule**
0 out of 3	Enter up to 3 patients at the next dose level
$\geq$ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Up to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter up to 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level.

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
	• If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Up to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is the MTD and is generally the recommended phase 2 dose.

3.2 DOSE MODIFICATIONS/DELAY

Other Toxicity:

- Patients may be removed from further treatment if they have active infections defined as infections causing fevers or infections requiring intravenous anti-microbial therapy and intravenous anti-microbial therapy has been initiated less than 72 hours before the start of chemotherapy. This means that patients are on-study but have not yet started treatment could have treatment cancelled or delayed; however such patients are eligible for treatment after the infection resolves.
- If a patient experiences a grade 3 or greater toxicity (with the exception of cytopenias or nausea) while on-study before the CAR T-cell infusion, the CAR T-cell infusion must be delayed until the toxicity improves to a grade 2 or less or cancelled.

3.3 STOPPING CRITERIA

- If no responses of PR or CR occur after 2 patients are treated on the highest dose level for both Cohort 1 and Cohort 2, the protocol will be stopped.
- Instructions for how to proceed when toxicity occurs will be as instructed by the dose escalation section of the protocol (**3.1.6**).
- A death on study not attributible to progressive malignancy within 30 days of a cell infusion for the initial 5 subjects in either cohort will be a cause for a pause to accrual to investigate the event followed by an amendment submission to the FDA and NIH Intramural IRB if required.
- If 3 or more of the first 9 patients on either cohort experience grade 4 toxicity possibly or probably attributable to CAR T cells within 30 days of cell infusion, this will be a cause for a pause to accrual to reassess the safety of the product followed by an amendment submission to the FDA and NIH Intramural IRB.

3.4 DRUG ADMINISTRATION

3.4.1 Leukapheresis

The patient will undergo a 15 liter leukapheresis (generally, 15 liters will be processed to target a yield of 6-10 x10^9 mononuclear cells) in the Department of Transfusion Medicine (DTM) Dowling Apheresis Clinic according to DTM standard operating procedures. The procedure requires dual venous access, and takes approximately 3-4 hours to complete. A central line will be placed if peripheral venous access is not sufficient.

3.4.2 Anti-CD30-CAR-expressing T-cell preparation

After cells are obtained by apheresis, further cell processing to generate anti-CD30 CAR-expressing T cells will occur in the DTM according to standard operating procedures (SOPs). Either freshly-collected cells or cryopreserved cells can be used to initiate the cell-preparation process. Peripheral blood mononuclear cells will be isolated. Sufficient cells for the initial cell production and 1 complete back-up cell production will be retained in the Department of Transfusion Medicine. The excess cells will be sent to the Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755 for cryopreservation at 200 to 300 million PBMC per vial. . The anti-CD3 monoclonal antibody OKT3 will be used to stimulate T-cell proliferation. The cells will be transduced by exposing them to replication-incompetent lentiviruses encoding the anti-CD30 CAR by using DTM SOPs. The cells will continue to proliferate in culture. Anti-CD30-CAR T cells will be cryopreserved between day 7 and day 9 of culture. CAR^+ T cells defined as cells staining for both CD3 and protein L in flow cytometry assays conducted in accordance with DTM SOPS will be quantitated by flow cytometry. Sufficient cells will be cryopreserved for a second dose that will be one dose-level higher (up to a maximum of 18x10^6 CAR^+ T cells/kg) than the first dose. Only for patients entering the study on the first 3 dose levels, sufficient cells will also be cryopreserved for a third dose that will be the same as the second dose or one dose level higher than the second dose (up to a maximum of 18x10^6 CAR^+ T cells/kg). For example for a patient receiving a first dose of 1x10^6 CAR^+ T cells/kg, sufficient cells need to be produced and cryopreserved for a second dose of 3x10^6 CAR^+ T cells/kg and a 3rd dose of 9x10^6 CAR^+ T cells/kg. Cryopreserved cells will be used for all infusions.

Ten vials of the infused cells will be cryopreserved for research use and stored in the Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755. Each vial will contain 10-20 million cells.

Before cryopreservation, the percentage of T cells expressing the CAR will be determined by flow cytometry, and this percentage of CAR^+ T cells will be used in calculating the total number of cells to be cryopreserved in a single-infusion bag to meet the dose requirements of the dose-escalation plan described in **Table 1**, Section **3.1**. As noted above, cells for potential 2nd doses will also be cryopreserved. For the first 2 dose levels, third infusions will also be cryopreserved at this time. In addition to flow cytometry, further testing of the cells will take place prior to infusion to evaluate for microbial contamination, replication-competent lentiviruses, and viability. Details of this testing can be found in the appropriate DTM SOPs. When a patient is no longer eligible on this protocol due to meeting any of the off-study criteria listed in section **3.8.2**, any remaining cryopreserved cells from this protocol will be coded-linked and used for research or discarded after approval of the Principal Investigator of this protocol.

Note: All cells sent to the Surgery Branch Cell Production facility will be transferred to NCI Frederick Biorepository.

3.4.3 Conditioning chemotherapy and anti-CD30 CAR T-cell administration

Table 5

Drug	Dose	Days
Cyclophosphamide	300 mg/m^2 IV infusion over 30 minutes (for the first 3 patients treated on Cohort 1 Dose Level 3, and for all patients on Cohort 1 Dose Level 1 and 2, and Cohort 2 Dose Level 1) 500 mg/m^2 IV infusion over 30 minutes (For all subsequent patients in either cohort)	Daily x 3 doses on days -5, -4, -3
Fludarabine	30 mg/m^2 IV infusion over 30 minutes administered immediately following the cyclophosphamide on day -5, -4, -3	Daily x 3 doses on days -5, -4, -3
Anti-CD30 CAR T cells	Variable.	Infuse on day 0

3.4.4 Overall summary of the treatment plan

3.4.4.1 Detailed treatment plan

Day -5, -4, and -3: Patients will receive pre-hydration with 1000 mL 0.9% sodium chloride I.V. over 1 to 3 hours.

Patients will receive anti-emetics following NIH Clinical Center guidelines, but **dexamethasone will not be administered**. One suggested regimen is ondansetron 16 to 24 mg orally on days -5, -4, and -3 1 hour before chemotherapy (I.V. ondansetron can be substituted). Patients should be provided with anti-emetics such as lorazepam and prochlorperazine to use at home.

Next, for all patients treated on cohort 1, dose levels 1 and 2, the first 3 patients treated on Cohort 1 Dose Level 3, and for all patients on Cohort 2 Dose Level 1, on days -5, -4, and -3, cyclophosphamide at a dose of 300 mg/m^2 I.V. will be diluted in 100 ml 5% dextrose solution and infused over 30 minutes. For all subsequent patients in either cohort, on days -5, -4, and -3, cyclophosphamide at a dose of 500 mg/m^2 I.V. will be diluted in 100 ml 5% dextrose solution and

infused over 30 minutes. After the cyclophosphamide on days -5, -4, and -3, all patients will receive 30 mg/m^2 I.V. fludarabine in 100 mL 0.9% sodium chloride over 30 minutes. **Note: in patients with a creatinine clearance calculated by the CKD-EPI equation less than 80 ml/minute/1.73 m^{2}of body surface area, the daily dose of fludarabine will be reduced by 20%.**

Following the fludarabine infusion, patients will receive 1000 mL 0.9% sodium chloride I.V. over 1-2 hours. Furosemide will be given if needed.

Days -2 and -1: No interventions except as needed for general supportive care such as anti-emetics. To minimize bladder toxicity, patients should increase normal oral fluid intake to at least 2 L/day.

Day 0: CAR T cells will be administered. Premedication for the cell infusion will be given approximately 30 to 45 minutes prior to the infusion. The premedications are acetaminophen 650 mg orally and diphenhydramine 12.5 mg IV. Cells will be delivered to the patient care unit from the Department of Transfusion Medicine. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), and identification of the product and documentation of administration are entered in the patient's chart as is done for blood banking protocols. **The cells are to be infused intravenously over 20-30 minutes. Details of the infusion procedure are included in section 14.5, Appendix E .**

Days 1 to 9: Mandatory hospitalization for observation and treatment as necessary. Note: hospitalization for CAR T-cell patients is routinely extended; extension of hospitalization beyond the required 9 days is anticipated for CAR T cell toxicity management. In addition, patients are required to stay within 60 minutes driving time from the Clinical Center until day 14 after the CAR T-cell infusion.

Guidelines for dealing with toxicities that often occur after CAR T cell infusions including hypotension, fever and tachycardia are given in **Appendix C**.

A CBC with differential will be obtained daily. **If the absolute neutrophil count becomes less than 500/microliter, filgrastim will be initiated** at a dose of 300 micrograms daily subcutaneously for patients under 70 kg in weight and at a dose of 480 micrograms daily for patients over 70 kg in weight. Filgrastim will be given daily and then discontinued as soon as the absolute neutrophil count recovers to 1500/microliter.

3.5 PROTOCOL EVALUATION

3.5.1 Baseline evaluations and interventions

The following tests must be completed within 14 days of the start of the conditioning chemotherapy regimen:

- Patients must have a central venous access before the time of cell infusion. This might require placement of a non-valved P.I.C.C line or other device. Non-valved PICC lines are greatly preferred over valved PICC lines for this protocol.
- Physical exam with vital signs and oxygen saturation
- CT scan of neck, chest, abdomen, and pelvis
- PET-CT of the torso
- Bone marrow aspirate and biopsy: specifically ask for CD30 immunohistochemistry staining of the bone marrow biopsy. Flow cytometry must be performed. The bone

marrow biopsy must take place at some time after the patient's most recent malignancy treatment. If a bone marrow biopsy was performed at the NIH as part of protocol screening within 4 weeks of the start of treatment, it does not have to be repeated unless necessary for staging.

- G-banding cytogenetics on the bone marrow if there is a suspicion of myelodysplastic syndrome.
- 250 microgram cosyntropin stimulation test if suspicious for adrenal insufficiency based on low serum sodium, high serum potassium, hypotension, low serum cortisol, a history of adrenal insufficiency, or other clinical indication
- Blood CMV PCR
- Blood will be collected for research purposes. Twelve CPT tubes (8 mL each) of blood will be collected within 3 days prior to initiation of the conditioning chemotherapy regimen. This is a total of 96 mL of blood. Some of this blood will be used for immunology assays and some will be used for RCL assays. This blood can be collected on different days as long as a total of 12 CPT tubes are collected prior to initiation of the chemotherapy. Send to the Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755.
- In addition to the CPT tubes, draw 16 mL of blood to obtain serum for research purposes (2 SST tubes, 8 mL per tube) within 3 days prior to the start of the chemotherapy. Send to the Figg lab; For sample pick-up, page 102-11964.

3.4.2 The following tests must be completed within 7 days of the start of the conditioning chemotherapy regimen:

- TBNK (T, B, and NK cell)
- Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid (to be repeated on the first day of the chemotherapy at the discretion of the PI)
- Serum quantitative immunoglobulins
- ABO typing
- CBC with differential and platelet count(to be repeated on the first day of the chemotherapy at the discretion of the PI)
- PT/PTT
- Urinalysis; if results are abnormal, send for urine culture
- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential
- C-reactive peptide (CRP)

3.5.2 Studies to be performed on Day 0 and during the mandatory 9-day inpatient admission after cell infusion

- Vital signs including pulse oximetry will be monitored q1h x 4 hours after completion of the CAR T cell infusion and then every 4 hours otherwise unless otherwise clinically indicated.
- Daily physical exam
- CBC twice daily from day 0 until day 9 with differential once daily. After day 9 do a CBC with differential daily until discharge. (Note: In the case of a later day infusion or early discharge, this may be only once a day)
- C-reactive peptide (CRP) within 7 days before the first dose of chemotherapy on day -5 and daily while hospitalized.
- TBNK(T, B, and NK cell) on the day of CAR T-cell infusion (day 0) and day 7 after infusion
- Chemistries twice daily starting from day 0 to day 9. After day 9, do chemistries once daily until discharge: Sodium (Na), Potassium (K), Chloride (Cl), Total CO_2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Uric Acid. (Note: In the case of a later day infusion or early discharge, this may be only once a day)
- PT/PTT and fibrinogen daily after infusion
- Other tests will be performed as clinically indicated.
- **Day 0 Research Blood:** 1 SST tube will be drawn on the morning of CAR cell infusion prior to infusion of CAR T cells. Send to the Figg lab; for sample pick-up, page 102-11964.
- **Post-infusion Research blood:** Every Monday, Wednesday, and Friday during hospitalization, starting on the first Monday, Wednesday, or Friday after the day of CAR T-cell infusion and lasting up to14 days after infusion, 56 mL of patient peripheral blood will be obtained (6 CPT tubes 8 mL each and 1 SST tube 8 mL). Send CPT tubes to the Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755. Send SST tubes to the Figg lab; for sample pick-up, page 102-11964.
- **Additional Post-infusion research blood:** 1 SST tube will be drawn on the first Sunday after CAR T-cell infusion. This tube will be stored refrigerated on the nursing unit and processed first thing Monday morning at the latest. Send to the Figg lab; For sample pick-up, page 102-11964.

3.5.3 Post-infusion outpatient evaluation

Patients will be seen at the NIH in follow-up to evaluate disease status and late problems related to CAR T-cell infusion at the following time-points: +14 (+/- 1 day), +30 (+/- 5 days), +60 (+/- 7 days), +90 (+/- 7 days), +120 (+/- 7 days) and at 180 (+/- 14 days), 270 (+/- 14 days), and 365 (+/- 30 days) after CAR T-cell infusion. After 12 months, the patient will be seen approximately every

6 months (+/- 30 days) up to three years post-infusion; subsequently, patients will be seen annually (+/- 30 days) up to 5 years post infusion. At all outpatient follow-up visits unless otherwise noted, patients will have the following tests performed to determine clinical response:

- 6 CPT tubes of Research Blood (48 mL) will be collected to obtain blood for immunological testing. Send to the Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755.
- 1 SST tube (8 mL) of Research Blood will be obtained for serum collection. Send to the Figg lab; For sample pick-up, page 102-11964
  Note: **after the first year of follow-up, research blood will be reduced to 4 CPT tubes (32 mL total) during required protocol visits**.
- CT scans and PET scans (as needed for disease assessment) at outpatient follow-up appointments starting 1 month after infusion as described in section **3.6**
- Patients may need 1 biopsy to confirm lymphoma progression. Patients may also undergo a maximum of 2 biopsy after CAR T-cell infusion to obtain material to study interactions between CAR T cell and the lymphoma microenvironment. These biopsies could occur either during the inpatient stay or during the outpatient phase. CT-guidance might be needed for these biopsies.
- Post-treatment bone marrow biopsies are needed only to document CR in patients in CR at all other sites and with pre-treatment bone marrow lymphoma involvement. Aspirate may be sent for flow cytometry to the lab of Dr. Maryalice Stetler-Stevenson. CD30 staining must be requested for the flow cytometry. CD30 immunohistochemistry should also be requested on the bone marrow biopsies.
- Post-CAR T-cell infusion research bone marrow samples will not be collected unless a sample is needed to investigate bone marrow toxicities such as prolonged cytopenia or second malignancies. In these cases, , send one tube of bone marrow aspirate for research purposes to Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755.
- Physical exam with vital signs and oxygen saturation
- Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid
- TBNK
- Blood for serum quantitative immunoglobulins
- CBC with differential
- Blood PCR for CMV, EBV, BK virus, and JC virus at all follow-up visits until six months have elapsed after the final cell infusion.
- Patients only need lumbar punctures if necessary to stage malignancy or to investigate toxicity.
- Gene-therapy-specific follow-up must be carried out as described in section **3.7**.

3.6 STUDY CALENDAR

Procedures [a]	Screening/ Baseline		Pre-cell infusion / Day 0	Day +7[b]	Day+14 (+/- 1 day) and Day+30 (+/- 5 days)	Day[q] +60 (+/- 7 days)	Day +90 (+/- 7 days)	Day +120 (+/- 7 days)	Day +180 (+/- 14 days)	Day +270 (+/- 14 days)	Day +365 (+/- 30 days)	Every 6 month after day 365 up to 3 years then annually(+/- 30 days)
Clinical Assessments												
History and PE	X		X	X	X	X	X	X	X	X	X	X
Vital signs, O2 saturation	X		X	X	X	X	X	X	X	X	X	X
Height, weight	X		X[j]									
Performance Score	X											
Laboratory Assessments												
CBC with differential	X		X	X	X	X	X	X	X	X	X	X
Sodium, Potassium Chloride, CO2, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Phosphorus, Alkaline Phosphatase, ALT, AST, T. Bilirubin, D. Bilirubin, LDH, Total Protein, CK, Uric Acid	X		X	X	X	X	X	X	X	X	X	X
Blood Beta HCG pregnancy test	X											
PT/PTT and Fibrinogen	X		X[b]	X[b]								
Thyroid Stimulating Hormone, T3	X											

Procedures [a]	Screening/ Baseline		Pre-cell infusion / Day 0	Day +7[b]	Day+14 (+/- 1 day) and Day+30 (+/- 5 days)	Day[q] +60 (+/- 7 days)	Day +90 (+/- 7 days)	Day +120 (+/- 7 days)	Day +180 (+/- 14 days)	Day +270 (+/- 14 days)	Day +365 (+/- 30 days)	Every 6 month after day 365 up to 3 years then annually(+/- 30 days)
Quantitative immunoglobulins	X				X	X	X	X	X	X	X	X
Antibody screen for Hepatitis B and C; CMV, HIV, HTLV-I/II, T. cruzi (Chagas agent), West Nile, and syphilis (RPR)	X[h]											
Blood PCR for CMV, EBV, BK virus and JC virus	X[c]				X	X	X	X	X			
TBNK	X		X	X	X	X	X	X	X	X	X	X
G6PD	X											
Urinalysis (culture prn)	X											
ABO typing	X											
CT neck, chest, abdomen, pelvis	X				X[l]	X	X	X	X	X	X	X
PET	X				X[l]	X	X	X	X	X	X	X
Brain MRI	X											
Other Specific Assessments												
Documentation of CD30 expression by malignancy	X											
Serum cortisol	X											
250 microgram cosyntropin test[e]	X[r]											

Procedures [a]	Screening/ Baseline		Pre-cell infusion / Day 0	Day +7[b]	Day+14 (+/- 1 day) and Day+30 (+/- 5 days)	Day[q] +60 (+/- 7 days)	Day +90 (+/- 7 days)	Day +120 (+/- 7 days)	Day +180 (+/- 14 days)	Day +270 (+/- 14 days)	Day +365 (+/- 30 days)	Every 6 month after day 365 up to 3 years then annually(+/- 30 days)
EKG, echocardiogram	X											
RCL (replication competent lentivirus)	X						X	X	X		X	X[i]
Central Venous catheter placement			X									
Donor Venous Assessment	X											
Bone marrow aspirate/biopsy with flow	X[f]				X[f]	X[f]	X[f]	X[f]	X[f]	X[f]	X[f]	X[f]
Research Blood	X[p]		X[m, n, o]	X[m, n, o]	X	X	X	X	X	X	X	X[k]
Adverse Event			X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X		X	X	X	X	X	X	X	X	X	X

[a] see section **2.2** and section **3.5** for details

[b] see section **3.4** or details of testing during hospitalization

[c] CMV PCR also performed at baseline, all others only during screening are required

[e] only if cortisol<18 mg/dL or clinical suspicion of adrenal insufficiency

[f] All patients need a pre-treatment bone marrow biopsies. Patients only need bone marrow biopsies post-treatment if the pre-treatment bone marrow was positive and the patient is in CR at all other sites.

[g] only at 2, 3, 4, 5 year post-infusion time points, see section **3.6** for details

[h] During screening only.

[i] Annually RCL blood collection will only continue after the 1 year time-point if a previous RCL test has been positive[J] Height and weight at admission is adequate even if not D0

[k]Until 5 years follow-up period

[l] CT and PET will be performed only at day 30

[m]Draw 1 SST tube on the first Sunday after CAR T-cell infusion. This SST tube should be stored refrigerated on the nursing unit until Monday morning when it should be processed first thing Monday morning at the latest.

[n]Draw 1 SST tube on the morning of CAR T-cell infusion (Day 0).

[o]In addition to the day 0 and 1st Sunday research blood mentioned above, research blood is drawn every Mon-Wed-Fri while the patient is inpatient.

[p]Baseline Only

[Q] Assessment may be performed remotely per PI discretion (outside labs will be accepted and symptoms assessment may be performed via telephone)

[r] required at baseline, only needed at screening if needed to evaluate disease.

3.7 GENE-THERAPY-SPECIFIC FOLLOW-UP

3.7.1 Clinical Evaluation

Long-term follow up of patients receiving gene transfer is required by the FDA and must continue even after the patient comes off the study. Long-term follow-up for patients who are no longer enrolled on this study will be done under a different protocol, 15-C-0141. Physical examinations will be performed and documented annually for 5 years following cell infusion to evaluate long-term safety. Physical exams can be performed by other physicians if clinic notes are obtained and retained in SB. A complete blood count should be done at these physician visits for the first 5 years after infusion. After 5 years, health status data will be obtained from surviving patients via telephone contact or mailed questionnaires for 10 additional years for a total of 15 years after cell infusion.

Testing for persistence of CAR transduced cells

Persistence of CAR transduced cells will be assessed by quantitative PCR and/or flow cytometry at 1, 2, 3, 6 and 12 months after cell infusion or until they are no longer detectable or until a stable or decreasing level of CAR T cells is present at least 3 years after infusion. If any patient shows a high level of persistence of CAR gene transduced cells or an increasing population of CAR gene transduced T cells at month 6 or later (by FACS staining or qPCR), the previously archived samples will be subjected to techniques that would allow the identification of predominant clonal populations of transduced cells.

3.7.2 Replication competent lentivirus (RCL) testing

Patients' blood samples will be obtained for analysis for detection of replication competent lentiviruses (RCL) by VSV-G-specific PCR prior to cell infusion and at 3, 6, and 12 months post cell administration. If all of these samples are negative for RCL, blood collection for RCL will be discontinued after the 12 month time-point.

In case of detection of replication-competent lentivirus, the following actions will be taken:

a. Immediately report the finding of RCL to the FDA, the NIH Intramural IRB, the NIH Institutional Biosafety Committee, the OSP, and the Indiana University Vector Production Facility (where the vector was made).

b. Repeat the culture-based RCL testing and RCL PCR on the infused cells for the patient in question. Repeat the RCL PCR on the sample that was found to be positive.

c. Have the patient come to the NIH for a clinic visit. Perform a complete history and physical exam. Draw blood for a complete blood count with differential, flow cytometry to assess T, B, and NK cell numbers in the blood, repeat RCL PCR, perform standard HIV screening, repeat PCR to assess for the presence of CAR-expressing T cells in the blood. Perform a bone marrow biopsy with flow cytometry, and assess the bone marrow for the presence of CAR-expressing T cells.

d. If no abnormalities requiring intervention are found after evaluating the patient, the patient should return monthly for a history, physical, CBC, and repeat RCL PCR tests on the blood.

3.8 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit:

- If no CAR-T cells administered at least 30 days after removal from study chemotherapy treatment.
- If CAR-T cells given, the first 60 days after the subject receives the last admistration of CAR-T-cells..

3.8.1 Criteria for removal from protocol therapy

Note that the treatment consists of a conditioning chemotherapy regimen followed by a T-cell infusion, so off-treatment criteria mainly apply to eligibility for cancellation of cell infusion for toxicity arising during the conditioning chemotherapy.

Patients will be taken off treatment for the following:

- The patient no longer meets the eligibility criteria for the protocol after enrolling but before start of the chemotherapy conditioning regimen. If the reason that the patient is not eligible can be rapidly resolved within 2 weeks, the patient can proceed on treatment for up to two weeks, otherwise the patient must come off treatment and then off study as appropriate. An exception to this is that a platelet count of 50,000 or more is considered adequate to start chemotherapy.The patient started chemotherapy but cannot complete the entire treatment (ending with completed cell infusion) for any reason specified in the protocol or PI discretion. If the reason that the patient is not able to complete treatment can be rapidly resolved, the patient can proceed on treatment, otherwise the patient must come off treatment and then off study as appropriate.
- The patient receives any other treatment for their malignancy (including corticosteroids at a dose higher than 5 mg/day of prednisone or equivalent) except the planned protocol treatment within 3 weeks of the start of the initial protocol treatment or if the patient receives any treatment for their malignancy at any time after their initial CAR T-cell infusion. If a patient receives corticosteroids in doses greater than 5 mg/day of prednisone or an equivalent dose of another corticosteroid within 2 weeks of the start of chemotherapy the treatment will need to be delayed or cancelled.
- General or specific changes in the patient's condition render the patient unacceptable for further treatment on this study in the judgment of the Principal Investigator.
- Participant requests to be withdrawn from active therapy
- Positive pregnancy test
- Investigator discretion

3.8.2 Off-study Criteria

- The patient completes the study
- The patient completes post treatment follow-up
- The patient voluntarily withdraws
- There is significant patient noncompliance
- PI discretion

- Death
- Development of progressive or relapsed malignancy after the CAR T cell infusion in patients not desiring or not eligible for re-treatment on this protocol.
- The patient receives any anti-malignancy therapy after the CAR T-cell infusion.
- Taking corticosteroids for any reason after CAR T-cell infusion at a dose higher than 5 mg/day of prednisone or equivalent dose of another corticosteroid (except to treat CAR T-cell toxicity).
- PI decision to end this study

Note:Patients must be followed until all adverse events possibly, probably or definitely attributable to protocol treatment have resolved to grade 2 or less with the exception of lymphopenia and alopecia. If an adverse event is not expected to resolve to grade 2 or less this will be noted in the patient medical record and the patient will be taken off study. In addition, all patients must be followed for the gene therapy specific follow up as outlined in section **3.7** even after being taken off-study. Patients taken off-study will be enrolled on a different protocol specifically to continue the FDA-required gene-therapy follow-up (NCI protocol 15-C-0141).

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 ANTIBIOTIC PROPHYLAXIS

- Patients with a CD4 T-cell count less than 200/μL will be maintained on pneumocystis prophylaxis with trimethoprim-sulfamethoxazole 1 double-strength tablet every Monday-Wednesday-Friday. If patients cannot tolerate trimethoprim sulfamethoxazole, an alternative pneumocystis prophylaxis will be used. PCP prophylaxis will be stopped after 2 consecutive CD4 counts greater than 200/μL are documented. If ganciclovir, valganciclovir, or foscarnet are started, val- acyclovir or acyclovir will generally be discontinued.
- Patients with a CD4 T-cell count less than 200/μL will be maintained on valacyclovir or acyclovir. This prophylaxis will be stopped after 2 consecutive CD4 counts greater than 200/μL are documented. If ganciclovir, valganciclovir, or foscarnet are started, valacyclovir or acyclovir will generally be discontinued**.**
- Patients with serum IgG level less than 400 mg/dL will receive intravenous immunoglobulin replacement as needed to maintain an IgG level above 400 mg/dL. An example of an intravenous immunoglobulin infusion to be used for this purpose would be Gammunex 500 mg/kg given as a single dose. Intravenous immunoglobulin infusions should be preceded by premedication with diphenhydramine and acetaminophen, and rate of infusion should be started at low rates and escalated in a step-wise manner.
- Neutropenic patients will start on broad spectrum antibiotics with a first fever of 38.3^0 C or greater or two fevers of 38.0 separated by at least 1 hour and concomitant ANC < 500/ml.
- Aminoglycosides will be avoided unless there is clear evidence of sepsis.
- A CBC with differential will be obtained daily. **If the absolute neutrophil count becomes less than 500/microliter, filgrastim will be initiated** at a dose of 300 micrograms daily subcutaneously for patients under 70 kg in weight and at a dose of 480 micrograms daily

for patients over 70 kg and over in weight. Filgrastim will be given daily and then discontinued as soon as the absolute neutrophil count recovers to 1500/microliter.

4.2 BLOOD PRODUCT SUPPORT

- Leukocyte filters will be utilized for all blood and platelet transfusions with the exception of the CAR-transduced T cell infusions to decrease sensitization to transfused WBC and decrease the risk of CMV infection.
- Patients who are seronegative for CMV should receive CMV-negative blood products whenever possible.
- Using daily CBC's as a guide, the patient will receive platelets and packed red blood cells (PRBC's) as needed. Attempts will be made to keep Hgb >8.0 gm/dl, and platelets >12,000/μL.
- All blood products with the exception of the CAR-transduced T cells will be irradiated.

4.3 ANTI-EMETICS

Anti-emetics will follow NIH Clinical Center Guidelines (except that corticosteroids will be avoided).

4.4 GRANULOCYTE COLONY-STIMULATING FACTOR

If the absolute neutrophil count becomes less than 500/microliter, filgrastim will be initiated at a dose of 300 micrograms daily for patients under 70 kg in weight and a dose of 480 micrograms daily for patients over 70 kg in weight. Filgrastim will be discontinued as soon as the absolute neutrophil count recovers to 1500/microliter.

4.5 AVOIDANCE OF CORTICOSTEROIDS

Patients should not take systemic corticosteroids including prednisone, dexamethasone or any other corticosteroid at any dose for any purpose without approval of the Principal Investigator.

4.6 GUIDELINES FOR MANAGEMENT OF COMMON ACUTE TOXICITIES THAT OCCUR AFTER CAR T CELL INFUSIONS

Please see section **14.3**, **Appendix C**. These are guidelines only. It is understood that treatment of these toxicities must be individualized for each patient. Not following the exact recommendations in Section **14.3** is not a protocol deviation.

4.7 EMERGENCY TREATMENT FOR PATIENTS NOT ELIGIBLE FOR THE PROTOCOL

It is allowed to give emergency standard of care anti-malignancy therapy to facilitate transfer of patients who become ineligible for the protocol treatment to another medical institution.

5 BIOSPECIMEN COLLECTION

Biospecimen collection on this protocol will consist of blood draws and acquisition of bone marrow aspirates and possible biopsies of tumors for research purposes. The amount of blood that may be drawn from adult patients for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

5.1 CORRELATIVE STUDIES FOR RESEARCH

5.1.1 Biospecimen collection before the start of the conditioning chemotherapy:

- Blood will be collected for research purposes. A total of 12 CPT tubes (8 mL each of blood) will be collected prior to initiation of the conditioning chemotherapy regimen. This is a total of 96 mL of blood. This blood can be collected on different days as long as a total of 12 CPT tubes are collected prior to the start of the chemotherapy and within 7 days of the start of the chemotherapy. Send to the Surgery Branch Cell Production Facility SB-CPF, Bldg. 10 3W-3808, Phone: 240-858-3755.
- 16 mL of blood will be drawn to obtain serum for research purposes (2 SST tubes, 8 mL per tube) within 14 days prior to the start of the chemotherapy. Send to the Figg lab.
- Specimens will be cryopreserved and assays will be performed retrospectively.

5.1.2 Biospecimen collection on Day 0 and after CAR T-cell infusion during the required hospitalization

Every Monday, Wednesday, and Friday during hospitalization, starting on the first Monday, Wednesday, or Friday after the CAR T-cell infusion and lasting up until 14 days after infusion of anti-CD30-CAR-transduced T cells, 56 mL of patient peripheral blood will be obtained (6 CPT tubes 8 mL each and 1 SST tube 8 mL). Also, 1 SST tube (8 mL) will be drawn on the morning of CAR cell infusion prior to infusion of CAR T cells. 1 SST tube (8 mL) will be drawn on the first Sunday after CAR T-cell infusion. The 1 SST tube will be sent to Figg lab. The 6 CPT tubes sent to Surgery Branch Cell Production Facility SB-CPF, Bldg. 10 3W-3808, Phone: 240-858-3755.

5.1.3 Biospecimen collection during outpatient follow-up

- Patients will return for outpatient follow-up clinic visits 2 weeks, 1 month, 2 months, 3 months, 4 months, 6 months, 9 months and 12 months after the CAR T-cell infusion. After the 12-month follow-up appointment patients will return for follow-up every 6 months up until 3 years after treatment. After 3 years, follow-up will be annual. The specimens listed below will be performed at each outpatient clinic visit during the first year of follow up.
  - 6 CPT tubes of Research Blood (48 mL) will be collected to obtain blood for immunological testing. Send CPT tubes to Surgery Branch Cell Production Facility SB-CPF, Bldg. 10 3W-3808, Phone: 240-858-3755.
  - 1 SST tube (8 mL) of Research Blood will be obtained for serum collection. The SST tubes will be sent to Figg lab.

NOTE: After 1 year, research blood collected will be reduced to 4 CPT tubes at each visit.

5.1.4 Immunological Testing

- T-cell assays: Direct immunological monitoring will consist of quantifying $CD3^+$ T cells that express the CAR by quantitative PCR, and/or by flow cytometry. These assays will be performed to measure the persistence and estimate the proliferation of the infused CAR^+ T cells. A quantitative PCR assay or a flow cytometry assay will be used to quantitate CAR^+ T cells at all post-infusion time-points up to at least 2 months after infusion, and CAR^+ T cell analysis will continue until the CAR^+ T cell level drops to undetectable levels unless a stable low level of CAR^+ T cells is present at more than 3 years after infusion.

The absolute number of CAR^+ PBMC will be estimated by multiplying the percentage of CAR^+ PBMC by the absolute number of lymphocytes plus monocytes per microliter of blood. Ex vivo immunological assays might be used to measure the antigen-specific functional activity of the CAR^+ T cells and will consist of assays such as enzyme-linked immunosorbent assays (ELISAs), intracellular cytokine staining, and anti-CD107a degranulation assays. Immunological assays will be standardized by the inclusion of pre-infusion recipient PBMC and in some cases an aliquot of the engineered T cells cryopreserved at the time of infusion.

- Serum cytokine levels will also be measured by enzyme-linked immunosorbent assays (ELISAs) or similar assays.
- Patients' blood samples will be obtained and saved for analysis for detection of replication competent lentiviruses (RCL) by PCR **at 3 months, 6 months, and 12 months after cell administration**. Infusion cells will be tested for RCL prior to infusion by PCR targeting the VSV-G gene. Blood collection for RCL monitoring will be discontinued if all patient samples have been negative for RCL at the 12 month time-point . If any post-treatment samples are positive, further analysis of the RCL and more extensive patient follow-up will be undertaken, in consultation with the FDA. RCL PCR assays are performed by the National Gene Vector Laboratory at Indiana University. The results of these tests are maintained by the National Gene Vector Laboratory at Indiana University and by the Surgery Branch (SB) research team.
- Due to nature of these studies, it is expected that expansion of specific T-cell clones will be observed as T-cell proliferate in response to the targeted antigen. Therefore, care will be taken to track T-cell persistence, but presence of an oligoclonal T cell population does not indicate an insertional mutagenesis event. If any patient shows an increasing population of CAR gene transduced T cells at month 6 or later (by FACS staining or qPCR), the previously archived samples will be subjected to techniques that would allow the identification of predominant clonal populations of transduced cells. Such techniques may include T cell cloning or LAM-PCR. If a predominant or monoclonal T cell clone derived from CAR gene transduced cells is identified during the follow-up, the integration site and sequence will be identified and subsequently analyzed against human genome database to determine whether the sequences are associated with any known human cancers. If a predominant integration site is observed, the T cell cloning or LAM-PCR test will be used at an interval of no more than three months after the first observation to see if the clone persists or is transient. In all instances where monoclonality is persistent and particularly in instances where there is expansion of the clone, regardless of whether or not the sequence is known to be associated with a known human cancer, the subject should be monitored closely for signs of malignancy, so that treatment, if available, may be initiated early.

5.1.5 **Additional biopsies and additional blood draws**

Patients might be asked to undergo biopsies (up to 3) or additional blood draws for research purposes. Additional blood draws might be necessary to investigate T cell responses and serum cytokine levels in cases of clinical events such as rapid regressions of malignancy or toxicity. Biopsies including open surgical biopsies, fine needle aspirations, and core needle biopsies could be used to investigate CAR T-cell persistence or function at tumor sites. Open surgical, fine needle, or core needle biopsies might also be needed in some but not all patients to confirm continued

antigen expression by tumor cells in order to meet protocol eligibility requirements for antigen expression on the tumor cells before any CAR T-cell infusion. For core needle biopsies 2 to 4 cores will be obtained. For fine needle aspirations, 1 to 3 aspirations will be performed. Standard techniques will be used for biopsies which may include CT and/or ultrasound guided biopsy. These research biopsies or blood draws are optional and patients can participate in this trial whether or not they agree to undergo biopsies for research purposes unless the biopsies are needed to prove that the target antigen is expressed on the tumor for protocol eligibility.. These biopsies will only be performed if minimal morbidity is expected based on the procedure performed and the granulocyte and platelet count. Biopsy tissue will be processed in the NCI Laboratory of Pathology. Studies will be performed to evaluate the antigen expression by the tumor and to evaluate the presence of transduced cells. Remainder material from any clinical biopsies obtained may also be utilized for research purposes.

5.1.6 Future studies

Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if prospective IRB approval is obtained or the project is determined to not be human subjects research. Patient PBMC not needed for future clinical use can be used for experiments aimed at developing new T-cell therapies not directly related to individual patients with permission of the PI. If new risks are associated with the research (e.g. analysis of germ line genetic mutations) a protocol amendment will be required and informed consent will be obtained from all research subjects to whom these new studies and risks pertain.

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

5.2.1 Samples Sent to Figg Lab

- Venous blood samples will be collected in either a 4-mL or an 8-mL SST tube to be processed for serum and stored for future research.
    - Record the date and exact time of draw on the tube. Blood tubes may be kept in the refrigerator until pick-up.
    - For sample pick-up, page 102-11964.
    - For immediate help, call 240-760-6180 (main Blood Processing Core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).
    - For questions regarding sample processing, contact the Blood Processing Core (BPC) at NCIBloodcore@mail.nih.gov or 240-760-6180.
    - The samples will be processed, barcoded, and stored in the Figg lab until requested by the investigator.

After delivery to the SB-CPF, peripheral blood mononuclear cell samples will be sent to the Head, Clinical Support Laboratory Clinical Services Program, Applied/Developmental

Directorate Frederick National Laboratory for Cancer Research for processing and cryopreservation. They will be stored long-term at the NCI Frederick Repository.

5.2.2 **Sample Storage, Tracking and Disposition**

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. All samples will be sent to Blood Processing Core (BPC) and/or SB-CPF for processing and/or and storage until they are distributed to the designated place of analysis as described in the protocol. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

5.2.2.1 Samples Managed by Dr. Figg's Blood Processing Core (BPC)

5.2.2.1.1 Sample Data Collection

All samples sent to the Blood Processing Core (BPC) (Dr. Figg's lab) will be barcoded, with data entered and stored in the Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.2.2.1.2 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB-approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed or returned to the patient, if so requested. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

Sample bar-codes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrixr. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.2.3 Sample Storage, Tracking, and Disposition for Surgery Branch

Samples received by the Surgery Branch research lab will be tracked using password protected web-based NCI database Labmatrix. All specimens will be tracked for date of receipt in the Surgery Branch lab, date analyzed, date returned to the originating hospital and/or date destroyed. Specimens will be stored in a locked laboratory cabinet or refrigerators in a locked research lab. All specimens will be entered into Labmatrix with identification and storage location. Access to the stored specimens will be restricted. Access to Labmatrix will be granted upon PI approval only. It is the responsibility of the NCI PI to ensure that the specimens are being used and stored in a manner consistent with IRB approval. All samples are stored in monitored freezers/refrigerators in 3NW NCI-SB laboratories at specified temperatures with alarm systems in place.

5.2.4 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol.

If the patient withdraws consent, the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

Data obtained on the study will be stored in real time into the Cancer Center Clinical Data System database (NCI C3D database) .It is expected that clinical data be entered into C3D no later than after 10 business days of the occurrence. The medical record will maintain complete records on each patient including any pertinent supplementary information obtained from outside laboratories, outside hospitals, radiology reports, laboratory reports, or other patient records. The medical record will serve as the primary source from which all research analyses will be performed. Data collection will include the eligibility criteria checklist, patient history, specialty forms for pathology, radiology, toxicity monitoring, and relapse data and an off-study summary sheet, including a final assessment by the treating physician. After patients are seen in clinic at each scheduled follow up, the database will be updated in real-time.

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

6.1.1 **Adverse event recording:**

- Grade 1 adverse events will not be captured in the database.
- All grade 2, 3, 4, and 5 adverse events will be recorded regardless of attribution.
- All adverse events are recorded in the medical record.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for adverse events for:

- If no CAR-T cells administered, document AEs from the first study intervention, Study Day 1, through the end of treatment. Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded
- If CAR-T cells given, document AEs from the first study intervention, Study Day 1, through the end of treatment. Beyond 60 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact

- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

_X_ Coded, linked data in an NIH-funded or approved public repository.

_X_ Coded, linked data in BTRIS

How and where will the data be shared?

Data will be shared through:

_X_ An NIH-funded or approved public repository. Insert name or names: clinical trials.gov.

_X_ BTRIS (automatic for activities in the Clinical Center)

_X_ Publication and/or public presentations.

When will the data be shared?

_X_ Before publication.

_X_ At the time of publication or shortly thereafter.

6.3 RESPONSE CRITERIA

6.3.1 Response Criteria for Lymphoma

Note: Do not evaluate for response until at least 4 weeks after cell infusion
(Cheson et al. Revised Response Criteria for Malignant Lymphoma, Journal of Clinical Oncology 2007 [182] and Recommendations for Initial Evaluation, Staging, and Response Assessment of Non-Hodgkin Lymphoma: The Lugano Classification Journal of Clinical Oncology, 2014[183])

6.3.1.1 Complete Remission (CR):

CR requires all of the following: Complete disappearance of all detectable clinical evidence of disease and disease-related symptoms if present before therapy. Regardless of FDG-avidity, if

all extranodal masses and lymph nodes are less than 1.0 cm or less in longest diameter, the patient is considered to be in CR.

1. In typically FDG-avid lymphoma (diffuse large B cell lymphoma, and T cell lymphomas): in patients with no pretreatment PET scan or when the PET scan was positive before therapy, a post-treatment residual mass of any size is permitted as long as it is PET negative.

2. Variably FDG-avid lymphomas/FDG avidity unknown: in patients without a pretreatment PET scan, or if a pretreatment PET scan was negative, all lymph nodes and nodal masses must have regressed to normal size (≤ 1.5 cm in greatest diameter if > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their long axis and more than 1 cm in their short axis before treatment must have decreased to ≤ 1.0 cm in their short axis after treatment.

3. The spleen and/or liver, if considered to be enlarged before therapy on basis of Physical exam or CT scan must be normal size on CT scan for FDG-negative lymphoma.If FDG-avid lesions were present in the spleen or liver before treatment, these FDG-avid Lesions must have resolved.

4. A bone marrow aspirate and biopsy is performed only when the patient had bone marrow involvement with lymphoma prior to therapy or if new abnormalities in the peripheral blood counts or blood smear cause clinical suspicion of bone marrow involvement with lymphoma after treatment. The bone marrow aspirate and biopsy must show no evidence of disease by morphology or if indeterminate by morphology it must be negative by immunohistochemistry.

6.3.1.2 Partial Remission (PR): PR requires all of the following:

1. ≥ 50% decrease in sum of the product of the diameters (SPD) of up to 6 of the largest dominant nodes or nodal masses. Dominant nodes or nodal masses should be clearly measurable in at least 2 perpendicular dimensions, should be from different regions of the body if possible and should include mediastinal and retroperitoneal nodes if possible.
2. No increase in size of nodes, liver or spleen and no new sites of disease.
3. If multiple splenic and hepatic nodules are present, they must regress by ≥ 50% in the SPD. There must be a ≥ 50% decrease in the greatest transverse diameter for single nodules.
4. Bone marrow is irrelevant for determination of a PR. If patient has persistent bone marrow involvement and otherwise meets criteria for CR the patient will be considered a PR.
5. Typically FDG-avid lymphoma: for patients with no pretreatment PET scan or if the PET scan was positive before therapy, the post-treatment PET scan should be positive in at least one previously involved site.

6.3.1.3 Progressive Disease (PD): Defined by at least one of the following:

1. ≥ 50% increase from nadir in the sum of the products of at least two lymph nodes, or if a single node is involved at least a 50% increase in the product of the diameters of this one node.
2. Appearance of a new lesion greater than 1.5 cm in any axis even if other lesions are decreasing in size
3. Greater than or equal to a 50% increase in size of splenic or hepatic nodules
4. At least a 50% increase in the longest diameter of any single previously identified node more than 1 cm in its short axis.
5. Lesions should be PET positive in typically FDG-avid lymphomas unless the lesion is too small to be detected by PET (<1.5 cm in its long axis by CT)
    - Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD. PET should be positive in typically FDG-avid lymphomas.
        1. Flow cytometric, molecular or cytogenetic studies will not be used to determine response.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting with the exception of cytokine release syndrome (CRS), which will be graded according to the CRS grading scale of Lee et al.[184]. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.3 NCI CLINICAL DIRECTOR (CD) REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of the study agent as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the study agent, but are not fatal or life-threatening, must be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form 3500a.

7.4.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information described below. Alternatively, the IRB continuing review report can be sent to IBC in lieu of a separate report. Please include the IBC protocol number on the report.

7.4.2.1 Clinical Trial Information

A brief summary of the status of the trial in progress or completed during the previous year. The summary is required to include the following information:

- the title and purpose of the trial
- clinical site
- the Principal Investigator
- clinical protocol identifiers
- participant population (such as disease indication and general age group, e.g., adult or pediatric);
- the total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons
- the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed,

- if the trial has been completed, a brief description of any study results.

7.4.2.2 Progress Report and Data Analysis

Information obtained during the previous year's clinical and non-clinical investigations, including:

- a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- a summary of all serious adverse events submitted during the past year
- a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death
- a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

7.5 NIH Required Data and Safety Monitoring Plan

7.5.1 Principal Investigator/Research Team

The clinical research team will meet weekly on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.5.2 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 SPONSOR PROTOCOL/ SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see section 8.1.3)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section 6.1. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor.

8.3 Reporting of Serious Adverse Events

Any AE that meets a protocol-defined serious criterion or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form.

All SAE reporting must include the elements described in section 8.2.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and

instructions can be found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=157942842

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 REPORTING PREGNANCY

8.4.1 Maternal exposure

If a patient becomes pregnant during the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy become known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section 8.1.2) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.4.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 4 months after the last dose of receiving protocol treatment.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until (*120 days)* after the last dose should, if possible, be followed up and documented.

8.5 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

9 CLINICAL MONITORING

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary and secondary study endpoints; adherence to the protocol, regulations, ICH E6, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation

- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

10 STATISTICAL CONSIDERATIONS

The primary endpoint of this trial is to determine the safety of administering CAR-expressing T cells to patients with malignancies. Secondary objectives of this trial are to measure any anti-malignancy effect that might occur, to assess the feasibility of administering CAR-expressing T cells, and to measure persistence and function of CAR-expressing T cells. The study will be conducted using a standard 3+3 approach as defined in section **3.1**.

Patients will be enrolled on a 2 different cohorts: **Cohort 1**: Patients who have not had an allogeneic hematopoietic stem cell transplant (allo HSCT) and **Cohort 2**: Patients who have had a 9/10 or 10/10 HLA-matched sibling or an 9/10 or 10/10-matched unrelated donor transplant. Each cohort will have a separate but identical dose-escalation scheme. The dose escalation schemes are separate because of the possibility of greater toxicity in the patients who have undergone prior alloHSCT. DLTs occurring on one cohort's dose escalation will not affect dose escalation of the other cohort.

The trial will be conducted as 2 different dose escalation schemes, one for Cohort 1 (non-alloHSCT) and Cohort 2 (alloHSCT recipients). For each cohort, a dose escalation scheme with up to 5 dose levels and up to 6 patients per dose level will be carried out. (There will be an additional 3 patients in Cohort 1 Dose Level 3 who received the lower chemotherapy dose before Amendment E, Protocol version: 08/03/2018) In addition, up to 8 additional patients can be treated at the MTD for each cohort to establish additional safety and toxicity data at that level. Thus, up to 79 patients may be enrolled onto the trial.

The fraction of post-allogeneic transplant patients experiencing Grade 3 or 4 toxicities will be compared to the fraction of patients who never had an allogeneic transplant experiencing Grade 3 or 4 toxicities by using a Fisher's exact test.

The degree of persistence of CAR-transduced T cells will be evaluated by a quantitative measure (flow cytometry or quantitative PCR) in all patients. Anti-malignancy effects will be measured by clinical response and categorized according to Section **6.3**. The clinical responses will be interpreted cautiously in the context of a pilot study which may be used to guide parameters for study in future protocols if warranted.

All other evaluations of secondary objectives will be performed using exploratory techniques. No formal adjustment for multiple comparisons will be used since the evaluations are being done to generate hypotheses.

It is anticipated that 5 years will be required to enroll up to a maximum of 79 patients onto this trial.

11 HUMAN SUBJECTS PROTECTIONS

11.1 RATIONALE FOR SUBJECT SELECTION

The patients to be entered in this protocol have advanced hematologic malignancies that are almost always incurable diseases. These patients have limited life expectancies. Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in disease response would be expected in one group compared to another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to gender or to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

11.2 PARTICIPATION/SELECTION RATIONALE

- The eligibility criteria for this protocol only allow enrollment of patients with advanced malignancies that are usually incurable despite recent advances in standard therapies.
- Patients with treatment options with proven efficacy and limited toxicity will not be enrolled.
- Improving the treatment of advanced hematologic malignancies is an important area of clinical research.
- In previous studies, anti-CD19 CAR T cells have demonstrated dramatic activity against B-cell lymphoma and B-cell leukemia. Many patients have obtained remissions lasting more than 2 years on multiple clinical trials of anti-CD19 CAR T cells. Some of these studies were clinical trials conducted by the Principal Investigator of this trial, and some patients treated on the Principal Investigator's prior trials have been in complete remission for over 3 years. The success of anti-CD19 CAR T cells against B-cell lymphomas provides a rationale for developing an anti-CD30 CAR for treating $CD30^+$ lymphomas.
- Because patients on previous trials of CAR T cells have experienced hypotension, tachycardia, prolonged fevers, neurological toxicities, and depressed myocardial function, participation in this trial clearly carries significant risk. In many patients on prior CAR trials, toxicities were severe enough to require intensive care unit admission. We will limit enrollment to patients 73 years of age or less because based on our admittedly limited experience with prior CAR-T cell clinical trials, younger patients tolerate and recover from these toxicities better than elderly patients.

11.3 PARTICIPATION OF CHILDREN

Children will not be enrolled on this study, since the efficacy of this experimental procedure is unknown, it does not seem reasonable to expose children to this risk without further evidence of benefit.

11.4 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **11.5**), all patients subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

11.5 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The experimental treatment has a chance to provide clinical benefit although it is quite possible that patients will obtain no clinical benefit. A goal of this study is to improve upon the number of patients who may benefit from adoptive cell therapy by using genetically-modified T-cells, specifically CAR T cells. The risks of the study fall into 6 general categories (see section **12** for details). First, chemotherapy that could cause cytopenias is part of the protocol. As with any chemotherapy that causes neutropenia and thrombocytopenia, this chemotherapy could cause toxicities such as infections and bleeding. The second category of toxicity is cytokine-release type toxicities such as high fevers, hypotension, and fever. A third area of toxicity is neurological toxicity such as delirium, obtundation, myoclonus, seizures, headache, and transient focal neurological toxicities including aphasia and focal paresis. Cytokine-release-type toxicities and neurological toxicities have appeared in other clinical trials of CAR T cells during the first 2 weeks after CAR T cells were infused.[122,158] The 4th main category of toxicity is direct damage to normal tissues by the CAR T cells. This could happen because of unexpected cross-reactivity of the anti-CD30 CAR with proteins other than CD30 in vivo. A 5th category of toxicity caused by anti-CD30 CAR T cells is impairment of normal immunity because CD30 is expressed on activated T cells and activated B cells. A 6th possible toxicity is gene-therapy-related toxicity caused by the lentiviral vector. The lentiviral vector used in this trial inserts into the T-cell DNA of patients, so in theory, insertional mutagenesis could occur, but insertional mutagenesis has not occurred in any of the hundreds of patients treated with mature T cells that were genetically modified by gammaretroviral or lentiviral vectors.[125-127]

The risks associated with biopsies are pain and bleeding at the biopsy site. In order to minimize pain, local anesthesia will be used. Rarely, there is a risk of infection at the sampling site.

The success of this clinical trial cannot be predicted at this time. Because all patients in this protocol have advanced hematologic malignancies and limited life expectancies, the potential benefit is thought to outweigh the potential risks. It is also anticipated that this study will provide scientific information relevant to tumor immunotherapy.

11.5.1 **Risks of exposure to Ionizing Radiation**

The procedures for performing the CT scans will follow clinical policies, no special procedures apply to these additional assessments for research purposes. In summary, subjects may receive additional radiation exposure from up to eight (8) CT neck +CAP , three (3) CT-Guided biopsies and eight (8)18FDG-PET/CT scans in the first year of the study.

The total additional radiation dose for research purposes will be approximately 22.4 rem in the first year of the study. This amount is more than would be expected from everyday background radiation. Being exposed to too much radiation can cause harmful side effects such as an increase in the risk of cancer. The risk of getting cancer from the radiation exposure in this study is 2.2 out of 100 (2.2%) and of getting a fatal cancer is 1.1 out of 100 (1.1%).

11.5.2 **Risks of Scans and Contrast**

If contrast dye is used, there is a small chance of developing an allergic reaction from the contrast material, which may cause symptoms ranging from mild itching or a rash to severe difficulty breathing, shock or rarely, death. The contrast material may also cause kidney problems. Common reactions include pain in the vein where the contrast was given, a metallic or bitter taste in the mouth , headache, nausea and a warm or flushing feeling that lasts from 1-3 minutes.

An IV line may need to be inserted for administration of the contrast agent or anesthetic, which may cause pain at the site where the IV is placed and there is a small risk of bruising or infection

11.5.3 **Risks of blood Sampling**

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

11.5.4 **Risks of Bone marrow aspiration and biopsy**

Side effect of bone marrow aspiration and biopsy may feel a pressure sensation when the needle is being inserted and a pulling sensation and brief pain as the marrow is withdrawn. Potential complications of this procedure are local bleeding, pain at the site, and infection. Both of these are very rare. Bleeding can be stopped by applying local pressure and an infection can be treated with antibiotics.

11.5.5 **Risks of Intravenous Catheter**

Side effect of placing some catheters include pain, bleeding, infection and rarely, collapsed lung. The long- term risks of the catheter rarely include infection and clotting of veins.

11.5.6 **Risks of Lumbar Puncture**

Side effect of lumbar puncture may cause pain at the site where the needle goes in and the spinal fluid is taken. There is a small risk of infection or bleeding. A third or fewer patients may experience some headache while the body replaces the fluid that is removed.

11.5.7 **Risks of Apheresis**

The risks of apheresis are similar to whole blood donation and include pain and bruising at the needle insertion site in the arms, lightheadedness, dizziness, nausea, and rarely fainting due to a rare reflex reaction to needle placement and to the temporary decrease in blood volume during apheresis. It may also feel tingling around your mouth or in your fingers caused by a blood thinner given during the procedure. The tingling may reduce by giving calcium containing

chewable antacid. All the symptoms usually go away within a few minutes of stopping the procedure.

11.6 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided to the participant or consent designee(s) (e.g., legally authorized representative [LAR] if participant is an adult unable to consent) for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent for the optional biopsies performed on this study will be obtained at the time of the procedure. If the patient refuses the optional biopsy at that time, the refusal will be documented in the medical record and in the research record

12 PHARMACEUTICAL INFORMATION

Note: The commercial drugs used in this study will not alter labelling of the FDA approved drugs and nor does the investigation involve a route of administration or dosage level in use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product.

12.1 LENTIVIRAL VECTOR CONTAINING THE ANTI-CD30 CAR GENE

12.1.1 Cells manufacturing

The lentiviral vector (LSIN-5F11-28Z) encoding a chimeric antigen receptor (CAR) directed against CD30 was prepared and preserved following cGMP conditions in the Indiana University Vector Production Facility. This self-inactivating 3rd generation lentiviral vector includes the murine stem cell virus promoter, and a truncated version of the woodchuck post-transcriptional regulatory element (WPRE) designated oPRE.[185] The anti-CD30 CAR protein encoded by this vector contains a signal peptide from human CD8-alpha, 5F11 fully-human antibody light chain variable region (5F11 VL), linker peptide, 5F11 fully-human antibody heavy chain variable region (5F11 VH), human CD28 hinge, transmembrane, and cytoplasmic regions and the CD3-zeta (cytoplasmic region) T-cell activation domain.

The vector will be stored at –80° C in the Dept. of Transfusion Medicine, Clinical Center NIH. Both storage facilities are equipped with around-the-clock temperature monitoring. Vector will be

used in *in vitro* transductions of T cells. There will be no re-use of the same unit of supernatant for different patients. Handling of the vector should follow the guidelines of Biosafety Level-2 (BSL-2). The specific guidelines for Biosafety Level-2 (BSL-2) can be viewed at http://bmbl.od.nih.gov/sect3bsl2.htm

12.1.2 **Toxicities**

Please refer to section 1.2.16

12.1.3 **Administration procedures:**

Please see section **3.4.4**

12.2 COMMERCIAL AGENTS:

Please refer to the US approved package insert for the full prescribing information here: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.DrugDetails

12.2.1 **Cyclophosphamide**

12.2.1.1 Source

Cyclophosphamide will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

12.2.1.2 Administration procedures

The cyclophosphamide used in this regimen will be given as Intravenous infusion over 60 minutes.

12.2.2 **FLUDARABINE**

12.2.2.1 Source

Fludarabine monophosphate will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

12.2.2.2 Administration procedures

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Fludarabine is administered as an IV infusion in 100 ml 0.9% sodium chloride, USP over 15 to 30 minutes. The doses will be based on body surface area (BSA).

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14 APPENDICES

14.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale*	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. 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).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

* As published in Am. J. Clin. Oncol.: Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

14.2 APPENDIX B: DATA COLLECTION ELEMENTS REQUIRED BY PROTOCOL

All of the following elements will be recorded in the C3D database:

A. Patient Enrollment

- Date of birth, age, gender, race, ethnicity
- Height
- Weight
- Performance Status
- Date of original diagnosis
- Stage at diagnosis

- Tumor Histology and date of confirmation
- CD30 expression by tumor type of tissue studied and date of confirmation
- Date of Informed Consent signature, consent version and date of registration
- Baseline History/Physical
- Baseline Symptoms
- Number of prior lines of therapy
- Findings of consultations done at screening

B. Study Drug administration and response for each course of therapy given

- Dates anti-CD30-CAR-transduced T cells given
- Dose level, actual dose, schedule and route given
- Height, weight, and body surface area at start of each course (a course is defined as chemotherapy followed by a CAR T-cell infusion)
- Response assessment for each restaging performed
- Concomitant medications will not be collected in C3D

C. Laboratory and Diagnostic Test Data

- All Clinical laboratory and diagnostic test results done at screening and until day 30 post infusion with the following exceptions:Diagnostic tests which are not specified in the protocol, and if the results are not needed to document the start or end of an adverse event that requires reporting. Serologies-CMV, HSV, EBV, toxoplasmosis, adenovirus (patient and donor)TTV data
- All staging studies including CT scan, PET scan results and bone marrow biopsy and peripheral blood flow cytometry results will be reported at the scheduled follow-up points at 1 months and 2 months after infusion; after 2 months only the overall malignancy status (CR, PR, stable disease, progression) will be reported.

D. Adverse Events

Please see section **6.1.1** Adverse Event Reporting

E. Tumor response and measurements

- Restaging studies performed at protocol specified time points and as clinically indicated.
- Any physical exam finding before 1 month after the infusion will not be collected in C3D, only labs and Adverse Events.
- Years 5-15 follow-up is only for survival.

F. Off study

- Date and reason for off study
- Date and cause of death
- Autopsy findings

14.3 APPENDIX C: GUIDELINES FOR MANAGEMENT OF COMMON TOXICITIES THAT OCCUR AFTER CAR T-CELL INFUSIONS

Infusions of CAR T cells are often complicated by significant acute toxicities in the first 2 to 3 weeks after the infusion. In many cases the toxicities correlate with serum inflammatory cytokine levels.[122]

The toxicities most often experienced by patients receiving infusions of CAR T cells include, but are not limited to, tumor lysis syndrome, fever, fatigue, hypotension, tachycardia, acute renal failure, and neurological toxicities such as aphasia, ataxia, headache, somnolence, and coma. Fever is usually the first toxicity to occur.

Note these are guidelines that might require modification based on clinical circumstances of each patient, and failure to exactly follow these guidelines is not a protocol deviation or violation.

Administration of corticosteroids should be avoided if at all possible to avoid killing or impairing the function of the CAR T cells.

General Supportive Care Guidelines:

1. All patients with significant malignancy burdens and without a contradiction such as allergy should be started on allopurinol at the time of the start of the chemotherapy conditioning regimen or 1 day before the CAR T cell infusion. The suggested allopurinol dose is 200 to 300 mg/day with a possible loading dose of 300 to 400 mg.
2. Vital signs should be checked a minimum of every 4 hours during hospitalization. Increasing the time interval between vital sign checks for patient convenience or other reasons should be avoided.
3. Strict ins and outs should be recorded on all patients.
4. As a minimum, keep hemoglobin greater than 8.0 g/dL and platelets greater than 20K/microliter.
5. Administer fresh frozen plasma (FFP) for a PTT 1.5-fold or more above the upper limit of normal.
6. For patients with an increased PTT, check the fibrinogen level and keep the fibrinogen level above 100 mg/dL with cryoprecipitate.
7. Fevers should be treated with acetaminophen and comfort measures. NSAIDs and corticosteroids should be avoided.
8. Patients with a heart rate persistently higher than 115/minute and fever should have vital signs checked every 2 hours.
9. Patients who are neutropenic and febrile should be receiving broad-spectrum antibiotics.
10. Avoid meperidine due to seizure risk.
11. Minimize benzodiazepine use to avoid aggravating delirium.
12. Patients on this protocol will be placed on strict fall precautions including instructions to get out of bed only with assistance under the following conditions:
    1) Any history of syncope or near-syncope within 1 month before CAR T-cell infusion or any time after CAR T-cell infusion.

2) Any blood pressure reading of less than 100 mm Hg systolic blood pressure after anti-CD19 CAR T-cell infusion if 100 mm Hg is lower than the patients baseline systolic blood pressure.
3) Heart rate greater than 100 beats per minute.

13. Any patient with syncope, near-syncope, or light-headedness will have orthostatic blood pressure and heart rate checked and receive intravenous fluids as appropriate. These patients will also receive an ECG.
14. A CBC will be obtained twice daily while the patient is inpatient. If the absolute neutrophil count becomes less than 500/microliter, Filgrastim will be initiated at a dose of 300 micrograms daily for patients under 70 kg in weight and a dose of 480 micrograms daily for patients over 70 kg in weight only in patients with absolute neutrophil counts less than 500/microliter. Filgrastim will be discontinued as soon as the absolute neutrophil count recovers to 2000/microliter.
15. Hypotension is a common toxicity requiring intensive care unit (ICU) admission. In general patients should be kept well-hydrated. Maintenance I.V. fluids (normal saline (NS) should be started on most patients with high fevers especially if oral intake is poor or the patient has tachycardia. I.V. fluids are not necessary for patients with good oral intake and mild fevers. For patients who are not having hypotension or tumor lysis syndrome, a generally even fluid balance should be strived for after allowing for insensible fluid losses in patients with high fevers. The baseline systolic blood pressure is defined for this protocol as the average of all systolic blood pressure readings obtained during the 24 hours prior to the CAR T-cell infusion. The first treatment for hypotension is administration of IV NS boluses.
    - **Patients with a systolic blood pressure that is less than 80% of their baseline blood pressure <u>and</u> less than 100 mm Hg should receive a 1 L NS bolus.**
    - **Patients with a systolic blood pressure less than 90 mm Hg should receive a 1 L NS bolus regardless of baseline blood pressure.**

    These I.V. fluid management suggestions may need to be modified based on the clinical characteristics of individual patients such as pulmonary status, cardiac function, edema and other factors.
16. Patients receiving more than 1 fluid bolus for hypotension should have a stat EKG and troponin, and a cardiac echocardiogram as soon as possible.

ICU Transfer

Patients should be transferred to the ICU under these circumstances. Patients not meeting these criteria could also require ICU admission at the discretion of the clinical team caring for the patient.

- Systolic blood pressure less than 75% the patient's baseline blood pressure and less than 100 mm Hg after administration of a 1L NS bolus.
- Anytime the systolic blood pressure is less than 90 mm Hg after a 1L NS bolus if 90 mm Hg is less than the patient's baseline systolic blood pressure.
- Continuous tachycardia with a heart rate higher than 125 beats per minute on at least 2 occasions separated by 2 hours.
- Oxygen requirement of more than a 4L standard nasal cannula

1. All patients transferred to the ICU for hypotension or tachycardia should have a stat EKG and a cardiac echocardiogram within 6 hours of the time of transfer.
2. Patients with hypotension not responding to IV fluid resuscitation should be started on norepinephrine at doses called for by standard ICU guidelines.
3. Patients should have a cardiac echocardiogram and an EKG within 6 hours of starting norepinephrine.
4. Patients in the ICU should get twice-daily labs (CBC with differential, acute care panel, mineral panel, hepatic panel, uric acid, LDH). Patients in the ICU should also get a daily troponin level.
5. Patients receiving vasopressors should have a cardiac echocardiogram at least every other day.

Immunosuppressive drug administration

In general, immunosuppressive drugs are administered in a stepwise escalation based on toxicity severity. The first immunosuppressive drug administered is usually tocilizumab. If toxicity does not improve after tocilizumab, treatment progresses to intermediate-dose or high-dose methylprednisolone. For certain severe toxicities listed below, high-dose methylprednisolone must be given immediately.

Tocilizumab administration

Tocilizumab should be administered under the following circumstances if the listed disorders are thought to be due to cytokine release from CAR T cells. Tocilizumab is administered at a dose of **8 mg/kg** infused IV over 1 hour (dose should not exceed 800 mg).

- Left ventricular ejection fraction less than 45% by echocardiogram
- Creatinine greater than 2-fold higher than the most recent level prior to CAR T-cell infusion
- Norepinephrine requirement at a dose greater than 3 micrograms/minute for 36 hours since the first administration of norepinephrine.
- Requirement of more than 5 mcg/minute of norepinephrine to maintain systolic blood pressure greater than 90 mm Hg
- Oxygen requirement 40% or greater fraction of inspired oxygen (FIO_2) for maintain oxygen saturation of >92%.
- Subjective significant dyspnea and respiratory rate greater than 25 for 2 hours or more.
- PTT or INR>2x upper limit of normal
- Bleeding possibly related to cytokine-release syndrome
- Creatine kinase greater than 5x upper limit of normal

Intermediate-dose methylprednisolone for toxicities not responsive to tocilizumab

1. Give methylprednisolone 50 mg every 6 hours for any of the toxicities under #19 above that don't improve after tocilizumab administration.

High-dose methylprednisolone should be given immediately under these circumstances:

1. Give methylprednisolone 200 mg every 6 hours for systolic blood pressure that is less than 90 mm Hg while the patient is on 15 mcg/minute or higher doses of norepinephrine. If the patient has not had tocilizumab, give 8 mg/kg of tocilizumab along with the methylprednisolone.
2. Give methylprednisolone 200 mg every 6 hours for hypotension requiring 15 mcg/minute or more of norepinephrine continuously for 8 hours or more. If the patient has not already had tocilizumab, administer 8 mg/kg tocilizumab in addition to methylprednisolone.
3. Give methylprednisolone 200 mg every 6 hours for any left ventricular ejection fraction 30% or less. If the patient has not already had tocilizumab, administer 8 mg/kg tocilizumab in addition to methylprednisolone.
4. Give methylprednisolone 200 mg every 6 hours for any situation in which pulmonary toxicity makes mechanical ventilation likely to be required within 4 hours. If the patient has not had tocilizumab, administer 8 mg/kg tocilizumab in addition to methylprednisolone.
5. In life-threating toxicity not improving after 200 mg of methylprednisolone, 1000 mg of methylprednisolone can be administered.

In general, stop corticosteroid use when toxicity improves to a tolerable level. For example, in patients with hypotension, stop methylprednisolone 6 to 12 hours after vasopressors are no longer needed.

Neurological toxicity

1. All patients with neurological toxicities other than somnolence and delirium should get a neurology consult.
2. All patients with significant neurological toxicity should get an MRI of the brain.
3. All patients with significant neurological toxicity should get a lumbar puncture after MRI if it is safe to perform a lumbar puncture.
4. The following patients should receive dexamethasone 10 mg intravenously every 6 hours until the toxicities improve. Note: for seizures administer standard seizure therapies in addition to dexamethasone. For patients already getting higher doses of corticosteroids for CAR-related toxicity, it is not necessary to add dexamethasone 10 mg every 6 hours. Stop dexamethasone as soon as toxicity improves to a tolerable level; the duration of dexamethasone use will need to be determined on a patient to patient basis. Tocilizumab is possibly not effective for neurological toxicity, so it should not be given when patients have isolated neurological toxicity.

    1. Inability of patient to follow simple commands such as “squeeze my fingers”.
    2. Any generalized seizure

3. Somnolence severe enough to potentially limit airway protection
4. Ataxia severe enough to preclude ambulation
5. Disorientation to person or place that persists longer than 48 hours
6. Neurologic toxicity lasting more than 2 hours that is severe enough to interfere with activities of daily living (ADLs)

14.4 APPENDIX D: CLINICAL STAGING AND TREATMENT OF ACUTE GVHD [108,109]

Stage	Skin	Liver	Gut
1	Rash < 25% BSA[a]	Total bilirubin 2.0-2.9 mg/dl[b]	Diarrhea >500-1000ml/day, or upper GI symptoms
2	Rash 25-50% BSA	Total bilirubin 3.0-6.0 mg/dl	Diarrhea 1001-1500ml/day
3	Rash >50% BSA	Total bilirubin 6.1-15.0 mg/dl	Diarrhea 1501 to 2000 ml/d
4	Bullae	Total bilirubin > 15.0 mg/dl	Diarrhea >2000 mL /day or severe abdominal pain or ileus

[a]BSA = body surface area; use "rule of nines" or burn chart to determine extent of rash.

[b]Range given as total bilirubin.

Note: Elevation of transaminase without elevation of bilirubin is not recognized as acute GVHD (A note should say "GVHD transaminitis" and mention if biopsy proven or not.)

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

The nominal stage is reduced by one if the organ is simultaneously and unequivocally affected by a complication other than GVHD.

Clinical Grading of Acute GVHD [108,109]

Grade[e]	Skin	Liver	Gut
0 (none)	None	None	None
I	Stage 1-2	None	None
II	Stage 3	Stage 1	Stage 1
III		Stage 2-3	Stage 2-4
IV[d]	Stage 4[e]	Stage 4	Stage 4

[e]Criteria for grading given as minimum degree of organ involvement required to confer that grade. The highest single organ stage determines the overall grade.

[d]Patients with Stage 4 gut GVHD are usually Grade IV. Stage 4 gut GVHD can only be a part of Grade III when it is not severe enough to cause a substantially impaired performance status. A substantially impaired performance status would be an ECOG performance status of 3 or 4.

[e]Designation of Grade III is appropriate for only scattered bullae.

The NIH Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-host Disease defines classic acute GVHD as maculopapular rash, nausea, vomiting, anorexia, profuse diarrhea, ileus, or cholestatic hepatitis occurring within 100 days after transplantation **or DLI** [107]. We will define acute GVHD in the same manner for this trial.

This schema is intended to serve as a guideline and to promote consistency in our clinical practice; it may be modified for individual patients as clinical circumstances warrant. Failure to follow these guidelines is not a protocol deviation.

Grade I GVHD:

1) Topical corticosteroids (usually 0.1% triamcinolone; 1% hydrocortisone to face) applied to rash BID.

Grade II-IV GVHD:

1) Methylprednisolone (MP) 1 mg/kg per dose IV, BID for 4 consecutive days.
2) If no response after 4 days, continue until response (7-day maximum trial); the dose may be doubled (4 mg/kg/day).
3) If response within 7 days, taper as follows:
    a) 0.75 mg/kg per dose IV BID for 2 days.
    b) 0.5 mg/kg per dose IV BID for 2 days.
    c) 0.375 mg/kg per dose IV BID for 2 days.
    d) If clinically appropriate, change MP to oral prednisone to equivalent of IV dose) daily for 2 days. MP may be converted to prednisone later in the taper at the investigators' discretion.
    e) After this, steroids will be reduced by 10% of starting oral dose each week until a dose of 10 mg/day is reached. Subsequent reductions will be made at the investigators' discretion.
    f) If GVHD worsens during taper, steroids should be increased to previous dose.
    g) During steroid taper, maintain cyclosporine at therapeutic levels.
4) If no response is observed within 7 days of MP treatment:
    a) Increase Methylprednisolone to 10 mg/kg per dose IV, BID for 2 days.
    b) If there is no improvement, consideration will be given to using second-line immunosuppressive therapy, e.g., tacrolimus, mycophenolic acid, monoclonal antibodies, or studies of investigational agents for acute GVHD, if they are available.
5) Antifungal prophylaxis with agents effective against mould will be started when it is anticipated that the patient will be receiving steroids at $\geq$ 1 mg/kg/day of methylprednisolone (or equivalent) for $\geq$ 2 weeks. Voriconazole, caspofungin, liposomal amphotericin B (Ambisome), posaconazole or amphotericin B lipid complex (Abelcet) are valid alternatives. During prophylaxis with any of the above agents, fluconazole should be discontinued. In patients with therapeutic cyclosporine levels at the initiation of voriconazole therapy, the

cyclosporine or Tacrolimus dose should be decreased by approximately 50%. In patients with therapeutic Sirolimus levels at the initiation of voriconazole therapy, the Sirolimus dose should be decreased by approximately 90%.

6) Determination of GVHD treatment response should be made within 96 hours of starting the treatment. The following are criteria to determine definitions of response to GVHD treatment:
   a) Complete response: Complete resolution of all clinical signs and symptoms of acute GVHD.
   b) Partial Response: 50% reduction in skin rash, stool volume or frequency, and/or total bilirubin. Maintenance of adequate performance status (Karnofsky Score $\geq$ 70%).
   c) Non-responder: < 50% reduction in skin rash, stool volume or frequency, and/or total bilirubin. Failure to maintain adequate performance status (Karnofsky Score $\leq$ 70%).

   Progressive disease: Further progression of signs and symptoms of acute GVHD, and/or decline in performance status after the initiation of therapy.

14.5 APPENDIX E: INFUSION INSTRUCTIONS

Equipment:

Primary IV tubing (2)

Secondary IV tubing (1)

NS (sodium chloride 0.9%) 250cc bags (2)

IV infusion pump

Gloves

Steps:	**Key Points:**
1. The RN will be informed of the approximate time of cell arrival at the bedside.	
2. Verify the physician orders: - to administer the cells - for the date of administration - for premedication orders - protocol number	a. Premeds are acetaminophen 650 mg PO and diphenhydramine 12.5 mg IV.
3. Verify that the protocol consent and DPA are signed.	
4. Ensure that emergency and monitoring equipment are available in the patient's room: - oxygen - suction - vital sign monitor with pulse oximeter and thermometer	
5. Provide patient education covering infusion procedure, potential complications and associated symptoms to report.	

7. Measure and record baseline vital signs, respiratory and circulatory assessments.	
8. Verify the patency of the patient's IV access.	A central venous access device such as a non-valved PICC line should be used.
9. Hang a primary line of 250cc NS at a kvo rate - **NEW** bag and **NEW** tubing. This **MUST** be ready and infusing **prior** to the cells being delivered to the unit. The patient's primary IV hydration can infuse via a separate lumen while the cells are infusing, but **NO MEDs** should be administered during this time. Have a second bag of 250cc NS and tubing ready as an emergency line.	This will be the dedicated NS line for infusing the cells. Under no circumstances are any other substances to be infused into the line. Cell death occurs quickly – the infusion must be initiated immediately. Do not infuse medication during the cell infusion. If emergency meds must be administered, use the hydration or emergency NS IV line. This will be the emergency IV solution and can be used for medication administration. **Do not use an inline filter for cells.**
10. The primary RN will be notified approximately 10 minutes before the cells arrive on the unit. The cells will be hand delivered to the bedside. It is critical to be at the bedside awaiting the arrival of the cells for infusion.	It is critical to be at the bedside awaiting the arrival of the cells for infusion; have baseline VS, assessment, and IV lines hooked up when the cells arrive. **Cell death occurs as soon as the cells are removed from the laboratory**. Initiate the infusion as quickly as possible.
12. Prior to spiking the cell bag, two RNs will perform the identification procedure. **Both** RNs must sign the tag on the cell bag.	
13. Infuse the cells by **INFUSION PUMP** or syringe over 20-30 minutes. a. Piggyback the cells into the dedicated NS line; use the backflush technique to prime the line. b. While the cells are infusing, **gently** agitate the bag of cells **every few minutes**. When the cell bag is	

empty, backflush NS to rinse the bag and infuse this at the same rate as the cells; rinse bag until NS runs clear. c. Note: in some cases cells will arrive from DTM in a syringe. In this case infuse the cells via syringe over 20-30 minutes in the dedicated NS line proximal port, see nursing cellular infusion SOP for further details.	This prevents the cells from clumping in the bag.
14. Measure and record VS before and after the cell infusion, q1h x 4, and then q4h after completion of the infusion. a. Assess and document the patient's respiratory and circulatory status post cell infusion.	
15. Documentation: a. After the cells have infused, remove the adhesive backed "cell therapy product" tag from the cell bag and place it on a progress note in the patient's chart. b. Document the cell infusion in CRIS using the appropriate screens.	