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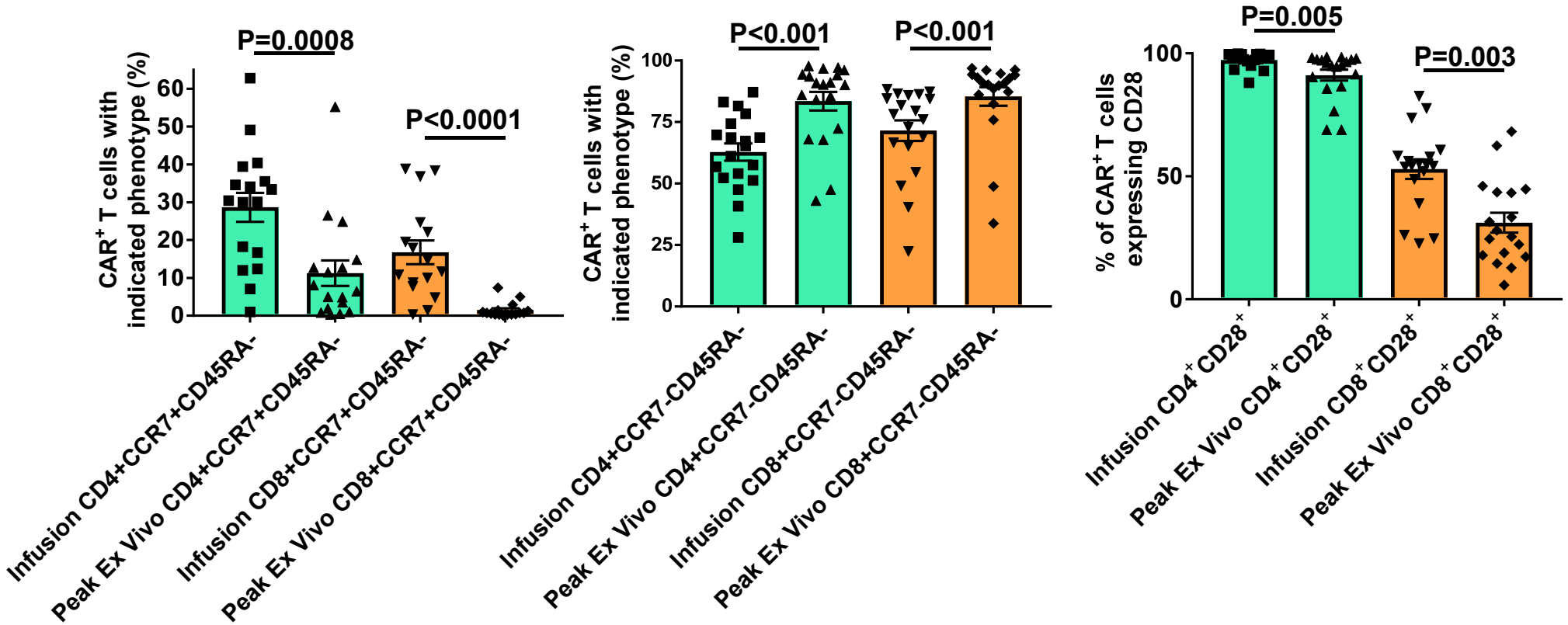
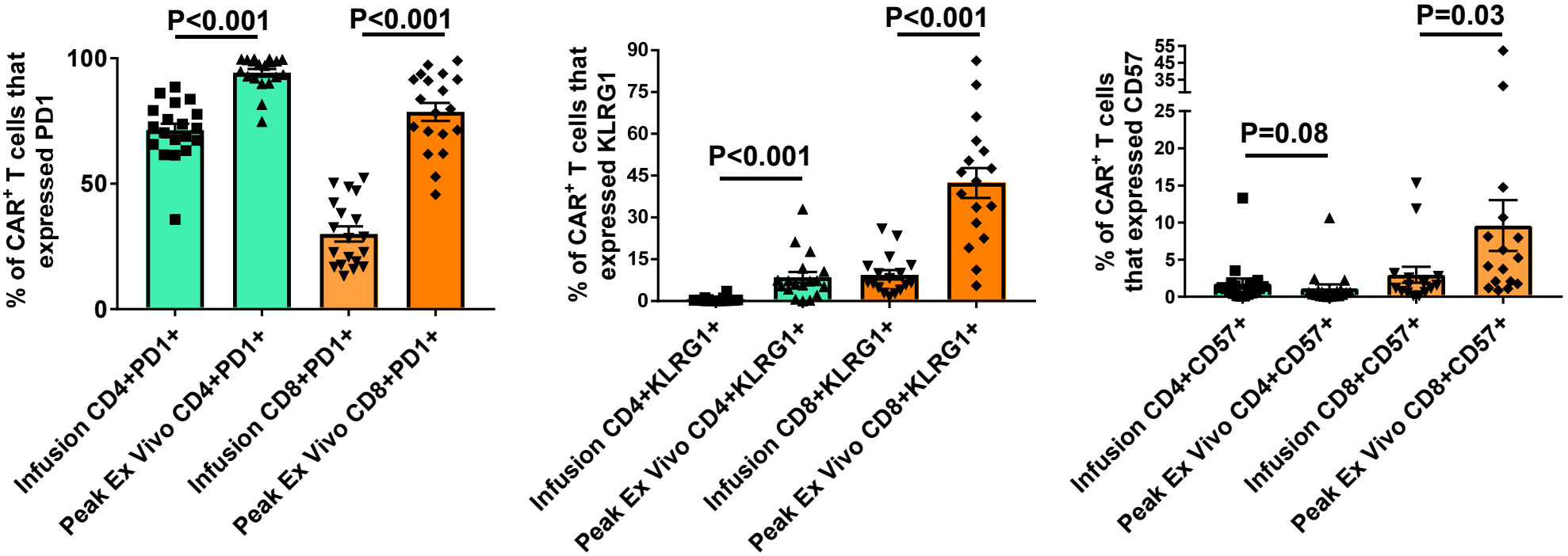
Supplementary Figure 1: central memory, effector memory, and CD28 expression



Hu19-CD828Z CAR T cells obtain a more differentiated phenotype after infusion. Central memory (CCR7$^+$CD45RA-negative), effector memory (CCR7-negative, CD45RA-negative), and CD28 expression was assessed on CAR$^+$ T cells at the time of infusion and on CAR$^+$ T cells from the blood of patients at the time of peak blood CAR$^+$ T-cell levels. CCR7, CD45RA, and CD28 levels were assessed on either CD4$^+$ or CD8$^+$ events that were also CAR$^+$, CD3$^+$, live lymphocytes. For central memory, n=17 unique patients for CD4$^+$ and n=16 unique patients for CD8$^+$. For effector memory, n=19 unique patients for CD4$^+$ and CD8$^+$. For CD28, n=19 unique patients for CD4$^+$ and n=18 unique patients for CD8$^+$. All samples with adequate event counts were included in these analyses. Statistical analyses were 2-tailed Wilcoxon matched-pairs signed-rank tests. Bars represent means +/- SEMs.

Supplementary Figure 2: Programmed cell death protein 1 (PD1), killer cell lectin-like receptor G1 (KLRG1), and CD57 expression



Hu19-CD828Z CAR T cells obtain a more differentiated phenotype after infusion. PD1, KLRG1, and CD57 expression was assessed on CAR$^+$ T cells at the time of infusion and on CAR$^+$ T cells from the blood of patients at the time of peak blood CAR$^+$ T-cell levels. PD1, KLRG1, and CD57 levels were assessed on either CD4$^+$ or CD8$^+$ events that were also CD3$^+$, live lymphocytes. For PD1, n=19 for CD4$^+$ and n=19 for CD8$^+$. For KLRG1, n=18 for CD4$^+$ and n=17 for CD8$^+$. For CD57, n=19 for CD4$^+$ and n=16 for CD8$^+$. Each “n” refers to a patient. All samples with adequate event counts were included in these analyses. Statistical analyses were 2-tailed Wilcoxon matched-pairs signed-rank tests. Bars represent means +/- SEMs.

Supplementary Table 1: Prior Treatment Regimens Patients Received Before Trial Participation

Patient number	Lymphoma type	Prior lines of therapy	Prior Treatment Regimens
1	DLBCL transformed from follicular lymphoma	6	-R-CHOP -BR -R-ICE -R-DHAP -Hyper-CVAD part B -radiation therapy
2	Follicular lymphoma	4	-R-CHOP -BR, bortezomib -ibrutinib -rituximab, idelalisib
3	Follicular lymphoma	9	-CHOP with consolidative radiation -rituximab -rituximab, second course -radiation with rituximab -BR -R-EPOCH -lenalidomide, rituximab -idelalisib -cyclophosphamide, dexamethasone

4	DLBCL	2	-R-CHOP -R-GemOx
5	DLBCL, double-hit	3	-R-CHOP -R-EPOCH -Auto HSCT
6	DLBCL	4	-R-CHOP -R-ICE, ibrutinib -radiation therapy -Auto HSCT
7	DLBCL, double-hit	2	-R-CHOP -R-GemOx
8	Mantle cell lymphoma	1	-EPOCH-RB
9	Burkitt lymphoma	2	-R-CODOX-M/R-IVAC -R-EPOCH
10	DLBCL transformed from follicular lymphoma	3	-R-CHOP -R-DHAP -Auto HSCT
11	DLBCL, triple hit	4	-R-EPOCH -R-DHAX -radiation therapy -bendamustine, obinutuzumab
12	DLBCL	6	-R-CHOP -R-DHAP -Auto HSCT -EPOCH-RIL -lenalidomide, ibrutinib

			-radiation therapy
13	Follicular lymphoma	6	-CHOP with consolidative radiation -radiation -R-CVP -R-ICE -Auto HSCT -R-GemOx
14	DLBCL	2	-R-CHOP -R-ICE
15	DLBCL transformed from CLL	4	-FR -rituximab, lenalidomide, ibrutinib -R-EPOCH -R-ICE
16	DLBCL, double hit	3	-R-CHOP -R-ICE -R-GemOx
17	DLBCL	5	-R-CHOP -R-EPOCH -R-ICE -Auto HSCT -R-GemOx
18	Mantle cell lymphoma	4	-R-CHOP -R-DHAP -Auto HSCT -BR

19	DLBCL, triple hit	4	-R-CHOP -R-DHAP -venetoclax -rituximab, cyclophosphamide, dexamethasone
20	DLBCL	2	-R-EPOCH -R-ICE

Auto HSCT: autologous hematopoietic stem cell transplant. BR: bendamustine, rituximab. CHOP: cyclophosphamide, doxorubicin, vincristine, prednisone. EPOCH-RB: etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, rituximab, bortezomib. EPOCH-RIL: etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, rituximab, ibrutinib, lenalidomide. FR: fludarabine, rituximab. R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone. R-CODOX-M/R-IVAC: rituximab, cyclophosphamide, doxorubicin, vincristine, methotrexate, leucovorin, intrathecal cytarabine, intrathecal methotrexate, followed by rituximab, ifosfamide, etoposide, cytarabine, intrathecal methotrexate, leucovorin. R-CVP: rituximab, cyclophosphamide, vincristine, prednisone. R-DHAP: rituximab, dexamethasone, high-dose cytarabine, cisplatin. R-DHAX: rituximab, dexamethasone, high-dose cytarabine, oxaliplatin. R-EPOCH: rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin. R-GemOx: rituximab, gemcitabine, oxaliplatin. R-Hyper-CVAD part B: rituximab, methotrexate, leucovorin, cytarabine, methylprednisolone. R-ICE: rituximab, ifosfamide, carboplatin, etoposide.

Supplementary Table 2: Adverse events within 30 days of Hu19-CD828Z CAR T-cell infusion*

Patient number	Grade 3	Grade 4
1	Anemia	White blood cell count decreased, neutrophil count decreased
2	Neutrophil count decreased	None
3	Hypotension, hypoxia, pleural effusion, anemia, tremor, electrocardiogram QT interval prolonged	Encephalopathy, white blood cell count decreased, neutrophil count decreased
4	Hypotension, anemia, AST increased, ALT increased, upper gastrointestinal hemorrhage	White blood cell count decreased, neutrophil count decreased
5	Fever, syncope, anemia, white blood cell count decreased, neutrophil count decreased, ALT increased, AST increased, hypophosphatemia	None
6	Fever, hypotension, hypoxia, anemia, pleural effusion, hiccups, syncope, white blood cell count decreased, neutrophil count decreased, hyponatremia, hypophosphatemia, electrocardiogram QT interval prolonged	None
7	Fever, hypotension, anemia, hypophosphatemia	White blood cell count decreased, neutrophil count decreased
8	Fever, hypoxia, platelet count decreased, fibrinogen decreased, hyponatremia, hypophosphatemia, hyperglycemia	Hypotension
9	Anemia, white blood cell count decreased, neutrophil count decreased, hypophosphatemia	None
10	None	White blood cell count decreased, neutrophil count decreased
11	Fever, *Bacteroides fragilis* bacteremia, anemia, white blood cell count decreased, hypophosphatemia, hypokalemia	None
12	Anemia, white blood cell count decreased, neutrophil count decreased	None
13	Fever, hypotension, hypophosphatemia, white blood cell count decreased	Cardiac arrest (unresponsiveness with lack of a palpable pulse; recovery to awake and alert in approximately 5 minutes)
14	Hypertension, anemia, hypophosphatemia	White blood cell count decreased, neutrophil count decreased

15	Fever, diarrhea, anemia, platelet count decreased, hyponatremia, hypophosphatemia	None
16	Fever, platelet count decreased, hypophosphatemia	White blood cell count decreased, neutrophil count decreased
17	Anemia, syncope	White blood cell count decreased, neutrophil count decreased
18	Fever, retroperitoneal hematoma, anemia, hyponatremia	Neutrophil count decreased
19	Anemia, hypokalemia	White blood cell count decreased, neutrophil count decreased, platelet count decreased
20	Anemia, platelet count decreased, hyponatremia, hypophosphatemia	White blood cell count decreased, neutrophil count decreased

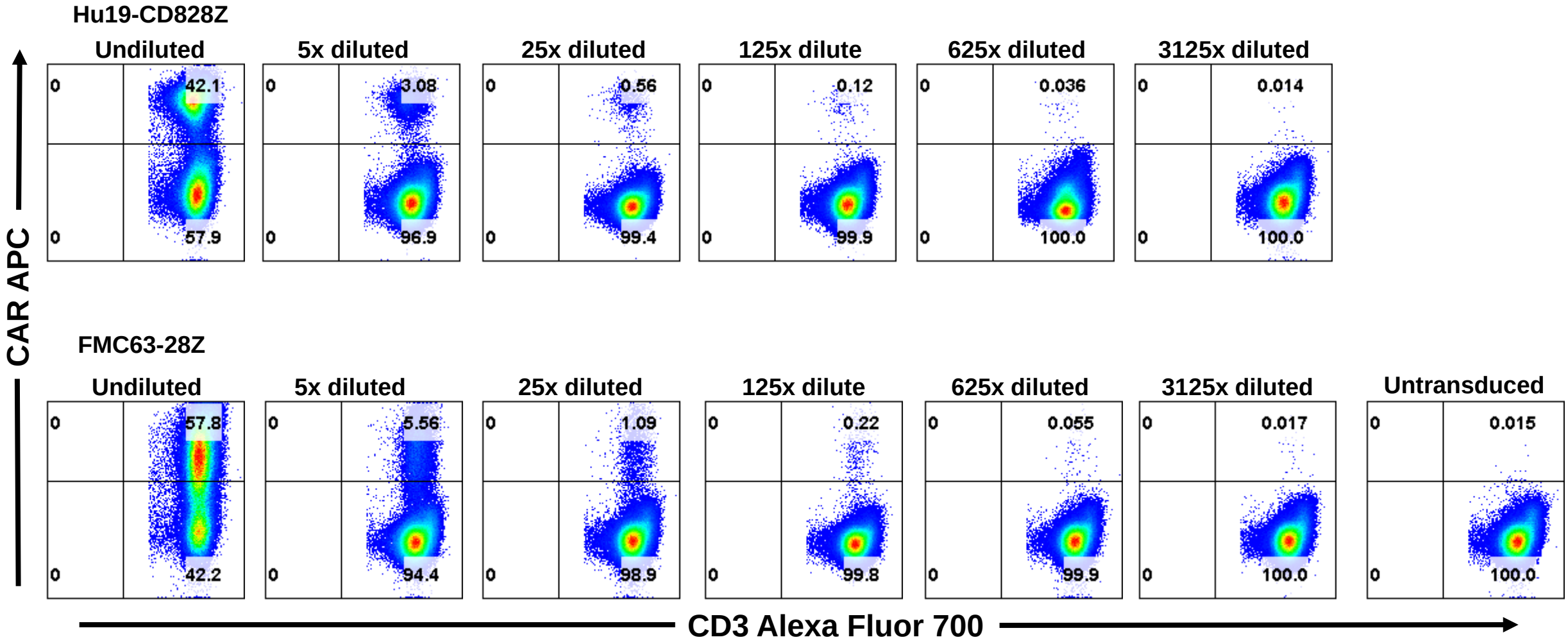
*All Grade 3-4 adverse events regardless of attribution are reported, except lymphocyte count decreased. All patients experienced Grade 4 absolute lymphocyte count decreased following cyclophosphamide and fludarabine chemotherapy. The highest-grade adverse event is reported. For example, if an adverse event occurred for a patient at both Grade 3 and Grade 4, only grade 4 is listed. Adverse events are graded according to the "National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4." ALT: alanine aminotransferase. AST: aspartate aminotransferase.

Supplementary Table 3: Comparison of clinical characteristics and outcomes Hu19-CD828Z versus FMC63-28Z

	Hu19-CD828Z clinical trial (2016-2018)	FMC63-28Z clinical trial (2013-2015)
T-cell culture process	Whole PBMC* stimulated with anti-CD3, cultured for 7 to 9 days	Whole PBMC stimulated with anti-CD3, cultured for 6 to 10 days
Conditioning regimen	Low-dose cyclophosphamide+fludarabine	Low-dose cyclophosphamide+fludarabine
Lymphoma types treated	DLBCL**, Burkitt, follicular, and mantle cell lymphoma; 40% chemo-refractory	DLBCL, follicular, and mantle cell lymphoma; 50% chemo-refractory
Lymphoma responses	Overall remission rate 70%, complete remission 55% (n=20)	Overall remission rate 73%, complete remission rate 55% (n=22)
Immunosuppressive drug administration	Tocilizumab: 2 patients Corticosteroids: 2 patients	Tocilizumab: 2 patients Corticosteroids: 1 patient
Grade 3 or 4 neurologic toxicity	1 of 20 (5%) of patients	11 of 22 (50%) of patients

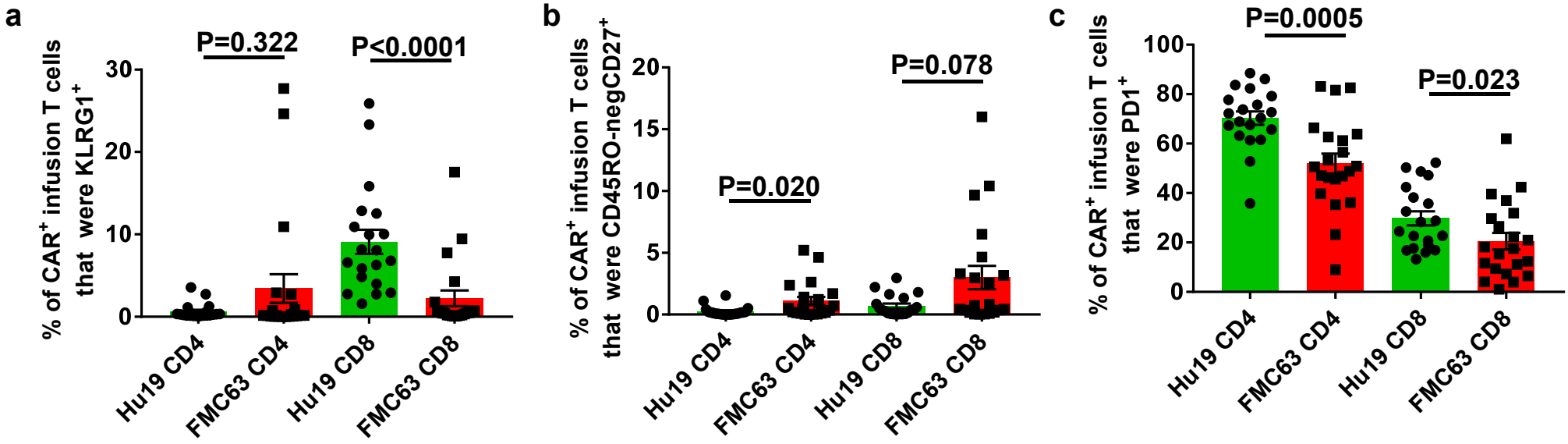
*PBMC, peripheral blood mononuclear cells. **DLBCL, diffuse large B-cell lymphoma

Supplementary Figure 3: Comparison of anti-CAR antibody binding to Hu19-CD828Z and FMC63-28Z



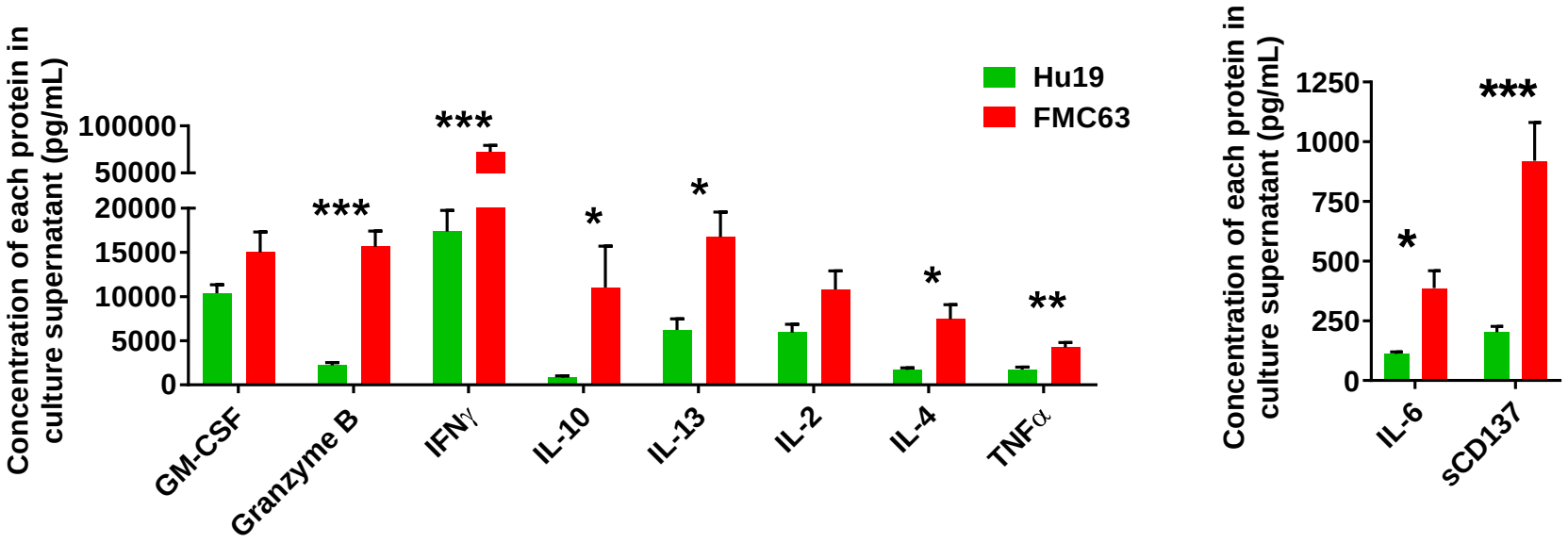
The anti-CAR antibody has similar sensitivity in detecting Hu19-CD828Z and FMC63-28Z T cells. T cells expressing either Hu19-CD828Z or FMC63-28Z were serially diluted in 5-fold steps by adding untransduced T cells to the transduced cells. As the percentage of CAR$^+$ T cells decreased, the percentage of CAR$^+$ T cells staining with anti-CAR decreased in a similar manner for both Hu19-CD828Z and FMC63-28Z. Experiment conducted once with multiple dilutions as shown with cells from 1 donor. Plots are gated on CD3$^+$ live cells.

Supplementary Figure 4: KLRG1, CD45RO-negCD27^{+}, and PD1 comparison of Hu19-CD828Z T cells and FMC63-28Z infusion T cells



Killer cell lectin-like receptor subfamily G member 1 (KLRG1) and CD45RO-negative, CD27^{+}, and programmed cell death protein 1 (PD1) expression on samples of infusion CAR^{+} T cells. Flow cytometry with anti-CAR antibody, anti-CD3, anti-CD4, anti-CD8 and antibodies against KLRG1 or CD45RO plus CD27 or PD1 was performed on samples of infusion cells from patients receiving either Hu19-CD828Z (Hu19) T cells or FMC63-28Z (FMC63) T cells. (a) percentage of CD4^{+} or CD8^{+} CAR^{+} T cells that were KLRG1^{+}, (b) percentage of CD4^{+} or CD8^{+} CAR^{+} T cells that were CD45RO-negative, CD27^{+}, (c) percentage of CD4^{+} or CD8^{+} CAR^{+} T cells that were PD1^{+}. Tops of the colored bars represent the means; error bars show +/- SEM. The lines above the bars end over the bars being compared. Each symbol represents one patient; comparisons are by 2-tailed Mann-Whitney test; n=20 patients for Hu19; n=21 patients for FMC63.

Supplementary Figure 5: CD19-specific protein release by Hu19-CD828Z versus FMC63-28Z T cells not normalized for CAR expression



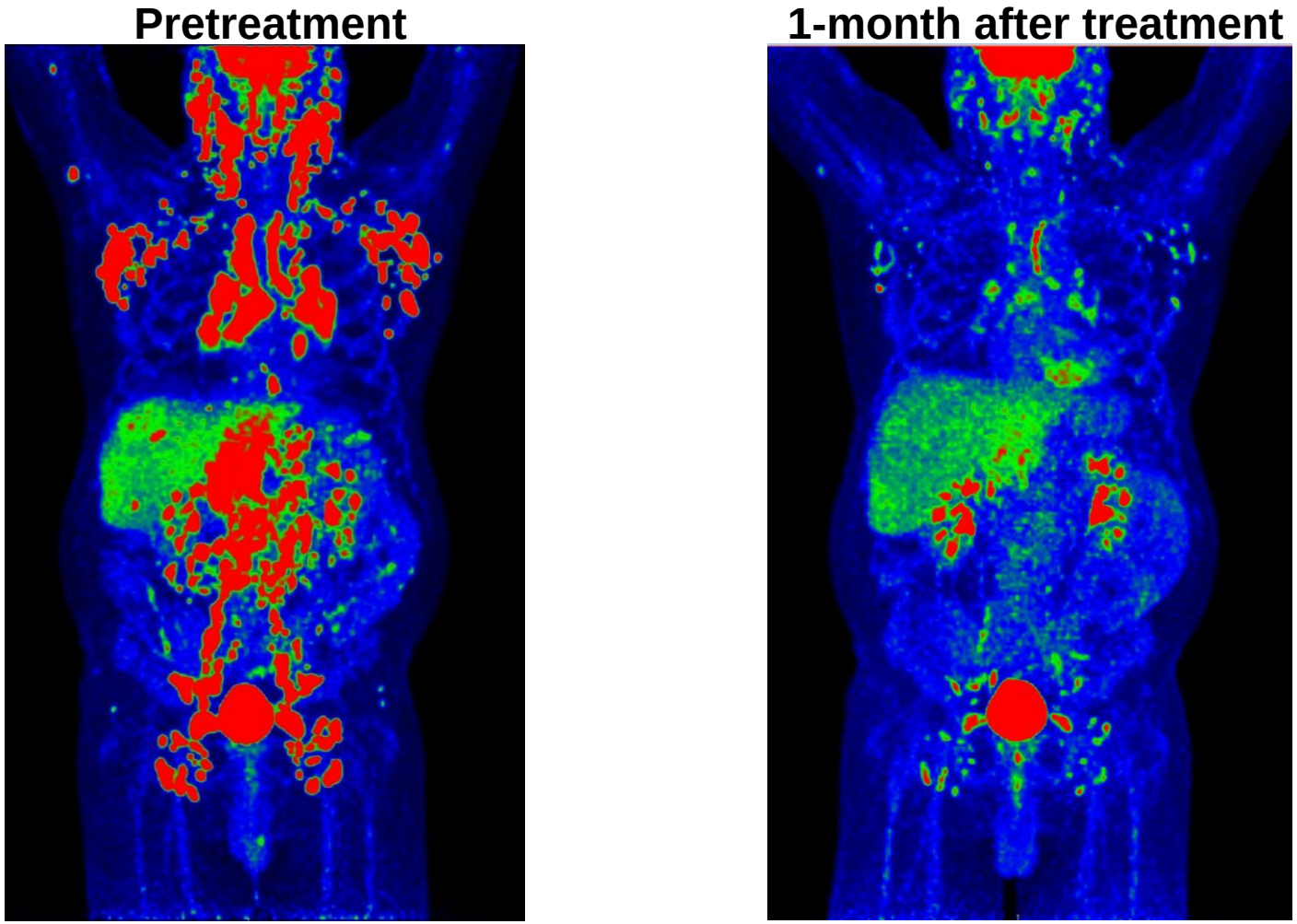
T cells expressing Hu19-CD828Z released lower levels of immunological proteins compared with T cells expressing FMC63-28Z. Samples of infusion CAR T cells from patients treated with either Hu19-CD828Z (Hu19) T cells or FMC63-28Z (FMC63) T cells were cultured overnight with CD19-K562 or NGFR-K562 cells. Supernatant was collected and tested in Luminex® assays. Background release of each protein after overnight culture of CAR T cells with CD19-negative NGFR-K562 cells was subtracted from the protein release when CAR T cells were cultured with CD19-K562 cells. P<0.0001 is indicated by ***; P<0.001 is indicated by **; P<0.01 is indicated by *; comparisons without an asterisk above the bars had P>0.05. Statistical comparisons were by 2-tailed Mann-Whitney tests. N=18 patients for Hu19 and n=22 patients for FMC63. Bars represent means +/- SEMs.

Supplementary Table 4: Cytokine-release syndrome grades of patients receiving FMC63-28Z T cells

Patient	Grade
22	2
23	1
24	2
25	2
26	1
27	1
28	2
29	1
30	1
31	2
32	2
33	2
34	2
35	3
36	4
37	1
38	1
39	1
40	3
41	2
42	3
43	1

Cytokine-release syndrome (CRS) toxicity grading is by Lee et al. Biology of Blood and Marrow Transplantation 2019; 25: 625-638 (Reference 39 of manuscript)

Supplementary Figure 6: Positron emission tomography images of mantle cell lymphoma of Patient 8



Patient 8 had extensive cervical, axillary, abdominal, and inguinal mantle cell lymphoma as shown in red in the pretreatment image. One month later, almost all lymphoma resolved. Note that the brain, kidneys, and bladder are normally red as shown in the 1-month after treatment image. Patient 8 experienced Grade 4 CRS. He eventually obtained complete remission; at the time of the 1-month after treatment PET scan, his response assessment was partial remission.

Supplementary Table 5: Summary of enrollment of treated patients

Phase of protocol	Explanation of patients treated
Dose Level 1: 0.66x10^6 CAR$^+$ T cells/kg	6 total patients had to be treated on Dose Level 1 because Patient 3 had a dose-limiting toxicity (DLT). Note that Patient 3 was the 3rd patient on this dose level, and her DLT was delayed; it occurred 27 days after CAR T-cell infusion, so Patient 4 had already been treated on Dose Level 2 at the time of Patient 3's DLT. We had to treat 3 more patients on Dose Level 1 after Patient 3's DLT and the treatment of Patient 4 on Dose Level 2. Patients treated on Dose Level 1 were Patients 1, 2, 3, 5, 6, 7.
Dose Level 2: 2x10^6 CAR$^+$ T cells/kg	6 total patients were treated on Dose Level 2. There were 2 DLTs on this Dose Level. Patient 8 had a DLT of hypotension. Patient 9 had delayed Grade 4 neutropenia that had onset 12 weeks after CAR T-cell infusion. This was strictly a DLT, but the regulatory bodies governing the trial allowed the protocol to progress to Dose Level 3 because this DLT in Patient 9 was: 1) very delayed from CAR T-cell infusion, 2) very rapidly resolved with a few days of filgrastim treatment 3) might have been due to either rituximab administered prior to protocol enrollment or to trimethoprim-sulfamethoxazole that was being administered at the time of neutropenia onset. In summary, patients treated on Dose Level 2 were Patients 4, 8, 9, 10, 11, 12.
Dose Level 3: 6x10^6 CAR$^+$ T cells/kg	6 total patients had to be treated on Dose Level 6 because of a DLT in Patient 13. For 2 reasons, we decided to declare 6x10^6 CAR$^+$ T cells/kg the optimal dose. Most importantly, we were running out of lentiviral vector. Secondarily, we saw no difference in efficacy on the first 3 dose levels tested. Patients treated on Dose Level 3 were Patients 13, 14, 15, 16, 17, 18.
Expansion: 6x10^6 CAR$^+$ T cells/kg	We only treated 2 patients on the Expansion phase because we ran out of lentiviral vector. The protocol was closed to accrual at this point. Patients treated on the Expansion phase were Patients 19 and 20.

Supplementary Table 6: Product-release criteria for FMC63-28Z CAR T cells

Test	Method	Limits
Cell viability	trypan blue exclusion	>70%
%CAR+ T cells	Flow cytometry	>30%
Microbiology	aerobic culture	no growth
	fungal culture	no growth
	anaerobic culture	no growth
	mycoplasma test	negative
Endotoxin	limulus assay	<5 E.U./kg
Replication competent retrovirus (RCR)	RCR PCR*	negative

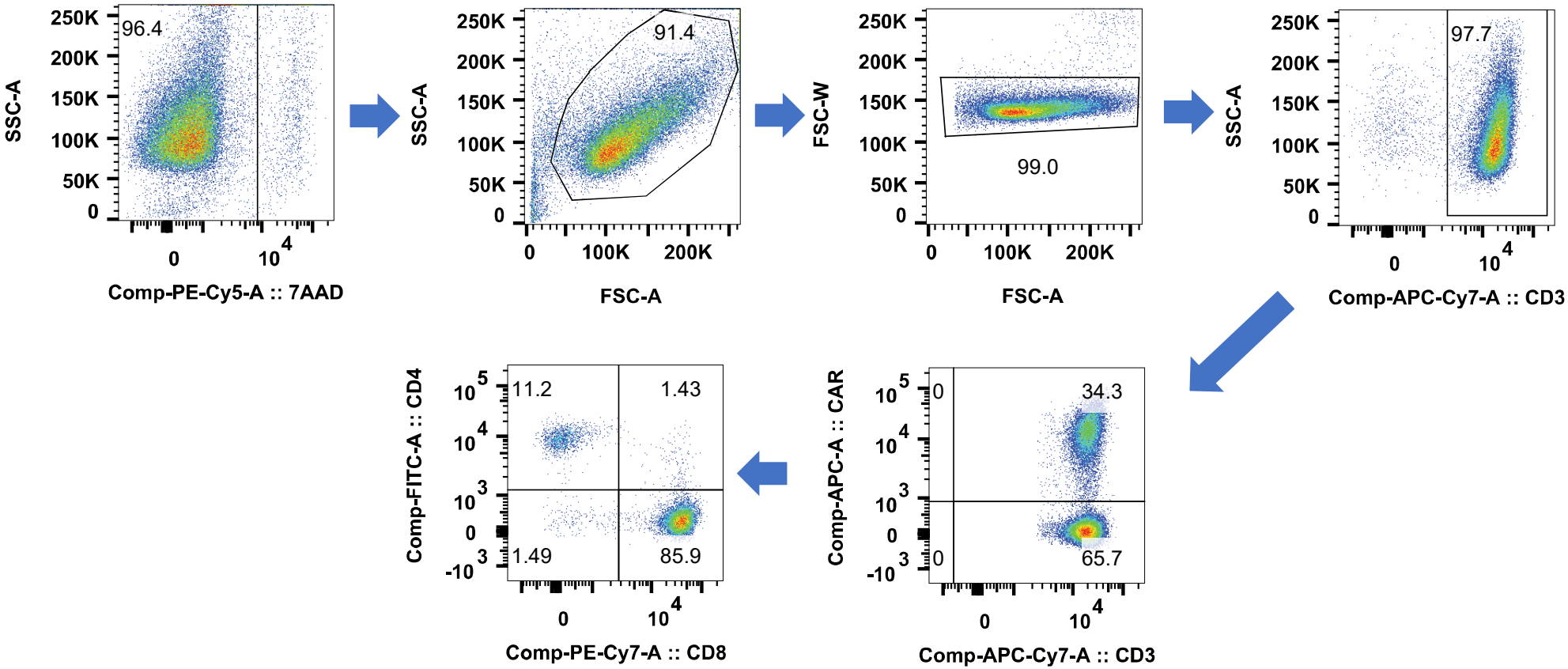
*Culture-based RCR assay conducted after CAR T-cell infusion

Supplementary Table 7 ELISPOT peptides

Domains	Peptide bins for FMC63-28Z construct	Number of peptides per FMC63-28Z bin	Peptide bins for Hu19-CD828Z construct	Number of peptides per Hu19-CD828Z bin
Signal sequence, linker, CD28 hinge or CD8α hinge	mBin A	13 peptides	hBin A	4 peptides
scFv light chain	mBin B	29 peptides	hBin B	14 peptides
scFv heavy chain	mBin C	33 peptides	hBin C	15 peptides
TM and IC domains	mBin D	46 peptides	hBin D*	32 peptides

*hBinD peptide pool lacks coverage of 5AA (DFACD) in the CD8α domain. TM transmembrane. IC, intracellular.

Supplementary Figure 7: Gating strategy



Gating strategy used in flow cytometry experiments. Dead cell exclusion with 7-aad, forward scatter versus side scatter gating, gating on singlets, and gating on CD3 was performed prior to assessing CAR versus CD3. A CD4 versus CD8 plot was gated on $CAR^{+}CD3^{+}$ cells.

Abbreviated Title: Anti-CD19-CAR T cells

CC Protocol #: *16-C-0054*

OSP#: 1509-1459

IBC#: RD-15-X-06

Amendment: H

Version Date: February 5, 2018

NCT Number: NCT02659943

Title: T Cells Expressing a fully-human anti- CD19 Chimeric Antigen Receptor for treating B-cell malignancies

Principal Investigator: James N Kochenderfer, M.D.

Experimental Transplantation & Immunology Branch

Center for Cancer Research,

National Cancer Institute, National Institutes of Health

10 Center Drive, Rm. 3-3132, MSC 1203

Telephone: 240-760-6062

Email: kochendj@mail.nih.gov**Investigational Agents:**

Drug Name:	Anti-CD19-CAR T cells
IND Number:	16682
Sponsor:	Center for Cancer Research

Commercial Agents: Cyclophosphamide, Fludarabine, OKT3

PRÉCIS

Background:

- Improved treatments for a variety of treatment-resistant B-cell malignancies including B-cell lymphomas, and chronic lymphocytic leukemia (CLL) are needed.
- A particular need is development of new treatments for chemotherapy-refractory B-cell malignancies.
- 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 leukemia or lymphoma. These results demonstrate that anti-CD19 CAR-expressing T cells have anti-malignancy activity in humans.
- The vast majority of B-cell malignancies express CD19.
- CD19 is not expressed by normal cells except for B cells.
- We have constructed a novel fully-human anti-CD19 CAR that can specifically recognize CD19-expressing target cells in vitro and eradicate CD19-expressing tumors in mice.
- This fully-human CAR targeting CD19 has not been tested in humans before.
- Possible toxicities include cytokine-associated toxicities such as fever, hypotension, and neurological toxicities. Elimination of normal B cells is probable, and unknown toxicities are also possible.

Objectives:

Primary

- Determine the safety and feasibility of administering T cells expressing a novel fully-human anti-CD19 CAR to patients with advanced B-cell malignancies.

Secondary

- Evaluate the in vivo persistence and peak blood levels of anti-CD19 CAR T cells after initial and repeated CAR T-cell infusions. CAR T-cell blood levels will be compared retrospectively to results with an anti-CD19 CAR containing an antigen-recognition moiety derived from a murine antibody.
- Assess for evidence of anti-malignancy activity by anti-CD19 CAR T cells
- Assess the impact of repeated CAR T-cell infusions on residual malignancy after an initial CAR T-cell infusion.
- Assess the immunogenicity of the CAR used in this protocol.

Eligibility:

- Patients must have any B-cell lymphoma, or CLL/SLL. Lower grade lymphomas transformed to DLBCL are potentially eligible as is primary mediastinal B-cell lymphoma and all other subtypes of DLBCL.
- Patients must have malignancy that is measurable on a CT scan or by flow cytometry of bone marrow or blood.
- Patients must have a creatinine of 1.4 mg/dL or less and a normal cardiac ejection fraction.
- An ECOG performance status of 0-1 is required.
- No active infections are allowed including any history of hepatitis B or hepatitis C.
- Absolute neutrophil count≥1000/μL, platelet count≥45,000/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) and initiation of protocol enrollment.
- The patient's malignancy will need to be assessed for CD19 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 CD19 expression by immunohistochemistry; otherwise, patients will need to come to the NIH for a biopsy to determine CD19 expression. The sample for CD19 expression can come from a biopsy obtained at any time before enrollment.
- Patients who have never had an allogeneic hematopoietic stem cell transplant are potentially eligible.

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-CD19 CAR
- Patients will receive a lymphocyte-depleting chemotherapy conditioning regimen with the intent of enhancing the activity of the infused anti-CD19-CAR-expressing T cells.
- The chemotherapy conditioning regimen is cyclophosphamide 300 mg/m^2 daily for 3 days and fludarabine 30 mg/m^2 daily for 3 days. Fludarabine will be given on the same days as the cyclophosphamide.
- Two days after the chemotherapy ends, patients will receive an infusion of anti-CD19-CAR-expressing T cells.
- The initial dose level of this dose-escalation trial will be 0.66×10^6 CAR$^+$ T cells/kg of recipient bodyweight.

- 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, 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.
- Repeat treatments consisting of the conditioning chemotherapy followed by a CAR T-cell infusion are planned 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.

TABLE OF ABBREVIATIONS

alloSCT - allogeneic stem cell transplantation
alloHSCT - allogeneic hematopoietic stem cell transplant
AML - acute myeloid leukemia
BSL – Biosafety level
CAR - chimeric antigen receptor
CFSE - carboxyfluorescein diacetate,succinimidyl ester
CLL - Chronic lymphocytic leukemia
CML - chronic myeloid leukemia
CR – Complete remission
CRO – Central Registration Office
CRP - C-reactive peptide
CTCAE - Common Terminology Criteria for Adverse Events
DLBCL - Diffuse large B-cell lymphoma
DCI - Donor Cell Infusion
DTM - Department of Transfusion Medicine
ELISA - enzyme-linked immunosorbent assay
GVHD - Graft-versus-host disease
GVM - graft-versus-malignancy
HLA - human leukocyte antigen
IBC - Institutional Biosafety Committee
OSP - Office of Science Policy
PBMC - peripheral blood mononuclear cells
PD – Progressive disease
PET - positron emission tomography
PR – Partial remission
RCL - replication competent lentiviruses
SD – Stable disease
SMC - Safety Monitoring Committee
scFv - single chain variable fragment
TBNK – T cell, B cell, and NK cell blood test
TCR –T cell receptor
URD – Unrelated donor

WPRE - woodchuck post-transcriptional regulatory element

TABLE OF CONTENTS

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-CD19 chimeric antigen receptor (CAR) to patients with advanced B-cell malignancies.

1.1.2 **Secondary Objectives**

- Evaluate the in vivo persistence and peak blood levels of anti-CD19 CAR T cells after initial and repeated CAR T-cell infusions. CAR T-cell blood levels will be compared retrospectively to persistence results with an anti-CD19 CAR containing an antigen-recognition moiety derived from a murine antibody.
- Assess for evidence of anti-malignancy activity by anti-CD19 CAR T cells
- Assess the impact of repeated CAR T-cell infusions on residual malignancy after an initial CAR T-cell infusion.
- 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-CD19 CAR, and we have demonstrated that T cells expressing this CAR have CD19-specific activity in vitro and in vivo. Anti-CD19-CAR-expressing T cells expressing this CAR can eradicate tumors in mice. We propose to conduct a phase I clinical trial of anti-CD19-CAR-expressing T cells. This clinical trial will enroll patients with advanced B-cell malignancies of all types. 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-CD19-CAR T cells. The T cell dose will escalate with sequential cohorts of patients until a maximum tolerated dose is determined. Sequential chemotherapy plus CAR T cell treatments with intra-patient dose escalation of the CAR T-cell dose will be an integral part of the protocol so that the effect of repeated CAR T-cell infusions on residual malignancy after an initial CAR T-cell infusion can be assessed.

1.2.2 B-cell malignancies: epidemiology and standard treatment

Annually in the United States, approximately twenty-three thousand people die of B cell malignancies [4]. B cell malignancies have quite heterogeneous clinical manifestations and prognoses. Chronic lymphocytic leukemia (CLL) is a common disease that is incurable by chemotherapy [5]. Recently, the new tyrosine-kinase inhibitor drug ibrutinib has revolutionized the treatment of CLL, so most patients will be treated with ibrutinib or another signal transduction inhibitor before participating in a phase I clinical trial. The tyrosine-kinase

inhibitors have very high response rates, and progression-free survivals of greater than 2 years have been reported[6], but follow-up is short, and some patients receiving these agents do develop progressive CLL that in some cases has transformed to a higher grade[6]. A B-cell malignancy for which new therapies are urgently needed is mantle cell lymphoma. This disease is almost always incurable by chemotherapy and has an aggressive course that is characterized by short responses to chemotherapy [7]. The tyrosine kinase inhibitor ibrutinib has been shown to have significant activity against mantle cell lymphoma, but in most cases it is not curative, so new therapies for mantle cell lymphoma are a great need[8]. Follicular lymphoma is a common lymphoma with an extremely variable course [9]. Patients with follicular lymphoma have a median survival of about 4.5 years after first relapse [10,11]. Follicular lymphoma is susceptible to a variety of therapies, but many of the therapies are toxic, and patients are seldom cured of follicular lymphoma, so improved therapies are needed for follicular lymphoma [12]. Diffuse large B-cell lymphoma (DLBCL) is the most common B-cell malignancy. DLBCL is often curable by standard chemotherapy, and autologous stem cell transplants are sometimes able to cure patients with relapsed large cell lymphoma [13]. However, patients with DLBCL that is refractory to 1st salvage chemotherapy have response rates to 2nd salvage therapy of only 14 to 43% [14-18]. Patients with DLBCL refractory to 2nd-line chemotherapy have very poor prognoses with a reported median survival of only 4 months and a 1-year survival rate of only 4% [18]. Allogeneic stem cell transplantation is a potentially curative treatment option for patients with advanced B cell malignancies, but allogeneic stem cell transplantation generally has a treatment-related mortality rate of 10-30% [19,20]. All in all, improved therapies for B cell malignancies are clearly needed.

1.2.3 Allogeneic Stem Cell Transplantation for B-cell Malignancies

In some patients with persistent or relapsed B-cell malignancies after extensive treatment with chemotherapy and monoclonal antibodies, allogeneic stem cell transplantation (alloSCT) can lead to long-term progression-free survival [21-26]. Khouri and coworkers reported the results of their experience treating patients with chemotherapy-sensitive follicular lymphoma with alloHSCT[22]. These investigators used a conditioning regimen that consisted of cyclophosphamide and fludarabine in nonmyeloablative doses plus high-dose rituximab. The five-year progression-free survival of patients treated with this regimen was 83%. There was evidence for a plateau in relapses because no relapses occurred more than 20 months after transplant [22]. Durable remissions can also be achieved with nonmyeloablative alloHSCT in patients with advanced chronic lymphocytic leukemia (CLL) [24,27], and nonmyeloablative alloHSCT is a promising treatment for relapsed mantle cell lymphoma [21,28]. AlloHSCT is the only curative therapy for adults with relapsed acute lymphoblastic leukemia (ALL) [25].

Although alloHSCT can be an effective therapy for B-cell malignancies, many patients develop persistent or relapsed malignancy after alloHSCT [24,25,28-30]. Evidence for an immunologic graft-versus-malignancy effect exists for most B-cell malignancies [20,21,23,30-32] ; therefore, when patients that are receiving immunosuppressive drugs to prevent GVHD after alloHSCT develop persistent or relapsed malignancy, immunosuppressive drugs are usually discontinued. In patients with persistent malignancy and without significant GVHD after immunosuppressive drugs are discontinued, relapsed B-cell malignancies after alloHSCT are usually treated with (donor lymphocyte infusion) DCI from the original transplant donor. DCI are infusions of unmanipulated lymphocytes from the original transplant donor.

1.2.4 Donor Cell Infusion (DCI)

DCI can induce remissions in 70-80% of patients with chronic myeloid leukemia (CML) that relapses into chronic phase after alloHSCT [33-35]. Susceptibility of B-cell malignancies to DCI after alloHSCT varies depending on disease histology [23]. Multiple reports of complete remission (CR) following DCI for persistent or relapsed follicular lymphoma after alloHSCT provide clear evidence of a GVM effect against this malignancy [20,31,36]. DCI administered without any other therapy have been reported to induce remissions in a small number of patients with progressive CLL after alloHSCT [20,30,37] . While these reports of CR after DCI for CLL prove the existence of a GVM effect against CLL, DCI is probably not as effective against CLL as follicular lymphoma because some studies have shown a complete lack of CRs after DCI for CLL in a substantial number of patients [31,38]. There is some evidence of efficacy of DCI alone against relapsed mantle cell lymphoma after alloHSCT [21,39] , and four patients with mantle cell lymphoma achieved CR after DCI combined with chemotherapy [20]. Although diffuse large B-cell lymphoma (DLBCL) has been thought to be resistant to the GVM effect, the responses that sometimes occur after withdraw of immunosuppressive drugs and after DCI demonstrate that DLBCL does have some susceptibility to immunologic interventions [32]. Acute lymphoblastic leukemia (ALL) is resistant toDCI. The percentage of adult ALL patient that obtain a CR after DCI for ALL ranges from 0% to 18% in multiple clinical trials [34,35,40,41]. In the studies discussed in this paragraph, most of the DCIs that induced complete remissions of B-cell malignancies contained $1x10^7$-$5x10^7$ T cells/kg of recipient bodyweight [20,31,36].

The most important toxicity of DCI is GVHD. In three studies cited in the previous paragraph, grade II-IV acute GVHD occurred in 28% to 44% of patients [20,31,36]. A recent review of DCI found that grade II-IV acute GVHD occurred after DCI in 34% to 48% of patients receiving DCI for various diseases and grade III-IV acute GVHD occurred after DCI in 20-35% of patients [40]. Chronic GVHD occurs commonly afterDCI with an incidence of 33% to 83% and highly variable severity [20,31,36,40]. When DCI is used to treat CML, the incidence of GVHD increases as the number of T cells contained in the DCI increases [42,43]. For CML, DCIs with higher numbers of T cells had an equivalent anti-leukemia effect as DCIs containing lower numbers of T cells [42]. In another study, DCIs 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 DCIs containing lower doses of T cells [44]. Miller and coworkers induced profound lymphodepletion with fludarabine and cyclophosphamide chemotherapy before DCI in an attempt to increase GVM [45] This therapy was associated with high levels of severe acute GVHD [45]. Forty-seven percent of patients treated with fludarabine and cyclophosphamide prior to a DCI 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 [45]. Because many patients with relapsed malignancy after alloHSCT require urgent treatment for rapidly progressive disease, many patients receive chemotherapy prior to DCI [20,31,32]. The chemotherapy regimens that are administered to patients for disease control prior to DCI 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 DCI. These patients received DCIs 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 [46]. The anti-leukemia activity of chemotherapy plus DCI appeared to be greater than the anti-leukemia activity achieved in historical control patients that were treated with DCI alone [46]. Porter and coworkers treated patients with a conventional DCI 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 [37]. 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 DCI alone [37].

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

The prolonged remissions of advanced B-cell malignancies that have often occurred after nonmyeloablative alloHSCT demonstrate that there is an immunologic GVM effect against these diseases [21,22,24,26]. The GVM effect against B-cell malignancies is clearly demonstrated by the remissions that have sometimes occurred when these diseases were treated with DCI in the absence of other therapies after alloHSCT [20,31,33-35,47]. 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 [48] [49]. Comparisons of relapse rates of T cell-depleted alloHSCT and T-replete alloHSCT indicated that transplantation of donor T cells prevented relapse after alloHSCT for ALL, AML and CML [48,50]. 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 [48], 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 [51]. However, this study only assessed patients that were treated with myeloablative transplants between the years 1985 and 1998. Nonmyeloablative transplants were excluded [51]. 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 hisotcompatibility antigens [52]. Most importantly, when T cell-replete alloHSCT and T cell-depleted alloHSCT were compared, there was an increased relapse risk in ALL, AML and CML patients that received T cell-depleted alloHSCT [48,50]. In addition, a patient with CML that had relapsed after standard DCI was treated with T cells that specifically recognized the patient's CML cells [53]. 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 [53]. 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 [54]. 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 [54].

CML is clearly very susceptible to an immunologic GVM effect [33-35,47]. The results obtained when acute leukemia and lymphoma are treated with DCI demonstrate that a weaker GVM effect is active against these diseases [33-35,20,31,55]. The strong GVM effect that is present when CML is treated with T-cell-replete alloHSCT 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 alloHSCT for B-cell malignancies might improve the outcomes of patients with these diseases. One possible way to augment the GVM effect against B-cell malignancies is to genetically engineer donor T cells to express receptors that specifically recognize antigens expressed by malignant B cells.

1.2.6 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 [56]. For GVHD to occur, there must be antigenic differences between the recipient and the allograft donor [56]. 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 cholesteric liver disease[57]. Chronic GVHD can manifest with a wide variety of signs and symptoms[57]. Formal definitions of four subsets of GVHD have published[57].

1. Classic acute GVHD: GVHD occurring less than or equal to 100 days after transplantation or DCI 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 DCI 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 **13.4**) [58,59]. 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[60] . ENREF_57 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.

1.2.7 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[61]. T cells can be prepared for adoptive

transfer by genetically modifying the T cells to express receptors that specifically recognize tumor-associated antigens.[61-68] 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.[61,69-72] Genetically modified antigen-specific T cells can be generated from peripheral blood mononuclear cells (PBMC) in sufficient numbers for clinical treatment within 10 days.[70] Genetically modifying T cells with gammaretroviruses consistently causes high and sustained levels of expression of introduced genes without in vitro selection[69,72-74], and genetic modification of mature T cells with gammaretroviruses has a long history of safety in humans.[75-77] 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).[61,63,65,67] CARs are fusion proteins incorporating antigen recognition moieties and T cell activation domains.[66,78-80] The antigen-binding domains of most CARs currently undergoing clinical and preclinical development are antibody variable regions.[63,66,78,80] 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[61,63,65,78], 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.[61,63,81-83]

1.2.8 Chimeric antigen receptors preclinical background

Preclinical experiments evaluating CAR-expressing T cells as cancer therapy were initiated in 1993.[84,85] 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.[86] 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 gamma retroviruses[69,72-75,87-89], lentiviruses[70,90-93], or transposon systems[94,95] 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.[70,87,96] 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.[78,87,91,97-99]

Much of the preclinical work evaluating CARs has been performed with CARs targeting the B-cell antigen CD19.[87-89,94,100-102] 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.[89,98] These studies showed that adding CD28 moieties to CARs enhanced antigen-specific cytokine production and proliferation by anti-CD19 CAR T cells.[98,103,104] 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.[103,104] Subsequently, CARs incorporating other signaling domains from costimulatory molecules such as 4-1BB (CD137) were developed.[90] 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.[91,97] Similar to CD28, including 4-1BB signaling moieties in CARs led to increased CD19-specific proliferation and enhanced in vivo persistence.[91] 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.[91,97]

1.2.9 CD19

CD19 is commonly used as the target of CAR T cells because it is expressed on most malignant B cells [105,106], but the only normal cells that express CD19 are B cells and perhaps follicular dendritic cells [106,107]. Importantly, CD19 is not expressed on pluripotent hematopoietic stem cells [108]. While destruction of normal B cells is a drawback to targeting CD19, several factors indicate that destruction of normal B cells is tolerable. When patients receive the anti-CD20 monoclonal antibody rituximab, the number of normal B cells is severely depressed for several months [109], yet patients that receive chemotherapy plus rituximab do not have an increased rate of common infections when compared to patients who receive chemotherapy alone [110]. Finally, patients can be treated with intravenous infusions of IgG if necessary to increase IgG levels[111].

1.2.10 Clinical results with anti-CD19 CAR T cells

Results from several clinical trials of anti-CD19 CAR T cells have been reported to date in peer-reviewed papers.[69,70,72-74,112-116] 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.[73] This clinical trial utilized a gamma retroviral vector to introduce an anti-CD19 CAR containing the signaling domains of the CD28 and CD3ζ molecules.[73] 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.[73] 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.[73] The bone marrow B-cell eradication was confirmed by flow cytometry, and it persisted for over 36 weeks.[73] B cells were also completely absent from the blood during this time, while T cells and other blood cells recovered rapidly.[73] 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.[72]

Seven more patients were subsequently treated with the same regimen of chemotherapy, anti-CD19 CAR T cells, and high-dose IL-2.[72] 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.[72,73] 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.[73] 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.[72] Six of the seven evaluable patients had remissions of their malignancies. Two of the remissions were complete remissions (CRs) of CLL.[72] Both of these CRs were confirmed by multicolor flow cytometry of bone marrow cells.[72] One of these CRs lasted 24 months, and the other is ongoing at 48 months.[72] 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.[72] All of these toxicities peaked within 10 days after the cell infusion and resolved less than 3 weeks after the cell infusion.[72] 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.[72] 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[117]. In summary this paper reported treatment of 15 patients with advanced B-cell malignancies. Nine patients had diffused 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[92,114-116,118] Multiple groups have recently demonstrated the 70% to 90% complete remission rates when anti-CD19 CAR T cells are used to treat ALL[114,116,118] 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[114,116,118] . 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[116,118] .

This clinical trial will also enroll patients who have had allogeneic hematopoietic stem cell transplants alloHSCT in the past. The cells for producing anti-CD19 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 [114,119,120] . 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 [119]). 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 [114,118-120].

1.2.11 Anti-CD19 CAR development and preclinical testing

We designed a CAR that incorporated a single chain variable fragment (scFv) from the fully-human anti-CD19 antibody 47G4[121]. The 47G4 antibodies was derived by immunizing mice transgenic for human immunoglobulin genes. This CAR also contained the hinge and transmembrane regions of the human CD8-alpha molecule, the cytoplasmic part of the CD28 molecule, and the cytoplasmic region of the CD3-zeta molecule. The CAR gene is encoded by a self-inactivating lentiviral vector[122]. The CAR was designated LSIN-47G4-CD828Z. LSIN stands for self-inactivating lentivirus. A diagram of the CAR is shown in Figure 3. After transductions, we found high levels of cell surface expression of the anti-CD19 CAR on the transduced T cells (Figure 4).

Figure 3

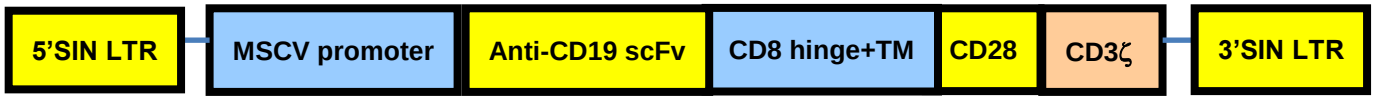


Figure 4

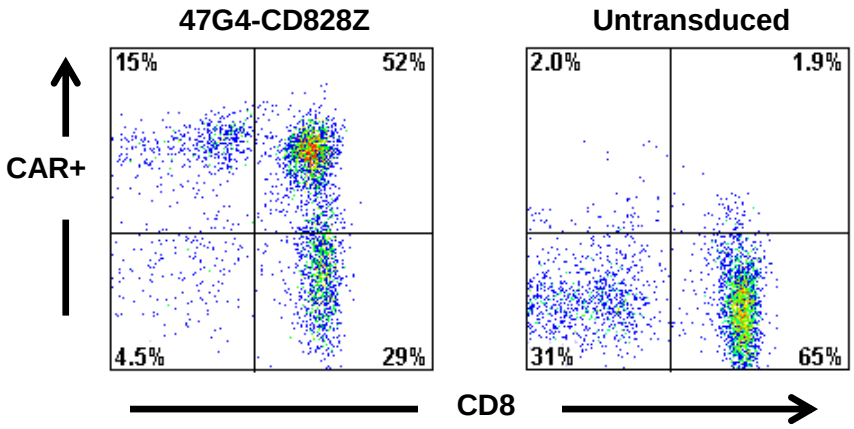


Figure 4 shows anti-CD19 CAR expression on T cells from Donor 1 five days after transduction with lentiviruses encoding the LSIN-47G4-CD828Z CAR. Transductions were carried out 2

days 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-CD19-CAR-expressing T cells, and we found that CD19-CAR-expressing T cells exhibit CD19-specific activities including CD107a upregulation and cytokine production in vitro. These experiments showed that anti-CD19-CAR-expressing T cells are activated in an antigen-specific manner.

Figure5

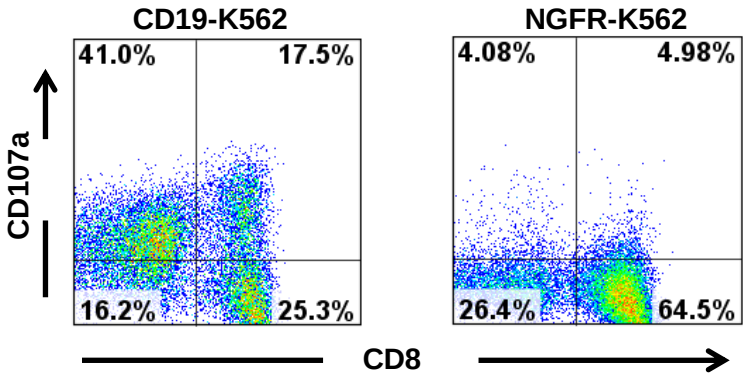


Figure 5 shows upregulation of CD107a, which indicates degranulation and correlates with cytotoxicity[123], when anti-CD19 CAR-expressing T cells from Donor 1 were cultured with the CD19-expressing cell line CD19-K562. CD107a was not upregulated when anti-CD19-CAR-expressing T cells from Donor 1 were cultured for 4 hours with the negative control cell line NGFR-K562, which does not express CD19. Untransduced T cells from Donor 1 did not upregulate CD107a when cultured with either CD19-K562 or NGFR-K562. The plots are gated on live $CD3^+$ lymphocytes. The T cells depicted in Figure 5 are from the same cultures as the cells shown in Figure 4 and were used in this experiment on the same day of culture as the cells shown in Figure 4.

It is critical to test any new CAR for specificity. To test for specificity, we cutured 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-CD19 CAR T cells should only react with $CD19^+$ target cells. We performed ELISA assays on culture supernatant from overnight co-cultures of T cells plus either CD19+ or CD19-negative target cells. T cells transduced with the anti-CD19 CAR produced large amounts of IFNγ when they were cultured overnight with the CD19-expressing cell lines but only small amounts of IFNγ when cultured with CD19-negative cell lines or CD19-negative primary human cells (Table 1). In all of the experiments reported in Table 1, effector T cells from a patient were either transduced with 47G4-CD828Z or left untransduced (UT). All numbers in the table are IFNγ levels in picograms/mL. The CD19+ target cells used in these experiments were, primary chronic lymphocytic leukemia cells (CLL), the CD19+ lymphoma cell line SU-DHL-4, the NALM6 ALL cell line, and the Toledo lymphoma cell line. These

CD19-negative cell lines were used: melanoma cell line 624, the leukemia cell line NGFR-K562, the T-cell leukemia cell line CEM, Saos-2 (a bone sarcoma cell line); A549 (a lung carcinoma cell line); MDA-MB231 (a breast cancer cell line), 293T (a human embryonic kidney cell line), TC71 (a Ewings sarcoma cell line), COLO205 (a colon carcinoma cell line), U251 (a glioblastoma cell line). Panc10.05 (a pancreatic carcinoma cell line), and A431-H9 (an epidermoid (skin) carcinoma cell line that was transduced with the gene for mesothelin),. Fibroblasts were primary human skin fibroblasts; endothelial cells were primary human endothelial cells. The primary microvascular endothelial cells and primary hepatocytes were from Lonza. The primary human cardiac myocytes were from Dr. Yongshun Lin, NHLBI, NIH In each experiment, the result for effector T cell cultured alone was also given.

Table 1

Specificity of 47G4-CD828Z-transduced T cells

Experiment 1

Effector T cells	CLL	SU-DHL4	624	NGFR-K562	CEM	T cells alone	% CAR expressing
47G4-CD828Z	1625	1238	31	37	43	19	32.4
Untransduced	17	23	20	14	<12	<12	_

Experiment 2

Effector T cells	CD19-K562	CLL	A549	MDA-MB231	293T	TC71	CEM	T cells alone	% CAR expressing
47G4-CD828Z	29198	3922	<12	<12	26	26	171	101	36
Untransduced	431	123	<12	<12	143	28	21	<12	-

Experiment 3

Effector T cells	NALM6	CD19-K562	COLO205	U251	Fibroblasts	Endothelial cells	CEM	T cells alone	% CAR expressing
47G4-CD828Z	2784	6985	35	33	73	36	74	53	49.2

Untransduced	**48**	**98**	**18**	**15**	**36**	**16**	**ND**	**ND**	

Experiment 4

Effector T cells	**NALM6**	**Toledo**	**Panc10.05**	**A431-H9**	**Saos-2**	**CEM**	**T cells alone**	**% CAR expressing**
47G4-CD828Z	**2219**	**1782**	**31**	**28**	**23**	**22**	**17**	**65.6**
Untransduced	**202**	**159**	**76**	**65**	**41**	**75**	**31**	**-**

Experiment 5

Effector T cells	**NALM6**	**Primary cardiac microvascular endothelial cells**	**Primary cardiac myocytes**	**Primary hepatocytes**	**HH**	**CEM**	**T cells alone**
47G4-CD828Z	**13746**	**28**	**21**	**26**	**96**	**62**	**27**
Untransduced	**136**	**12**	**12**	**12**	**26**	**14**	**<12**

LSIN-47G4-CD828Z-transduced T cells also proliferated in a CD19-specific manner.

Figure 6 shows a carboxyfluorescein diacetate,succinimidyl ester (CFSE) proliferation assay in which anti-CD19-CAR-transduced T cells were cultured for 4 days with either CD19-K562 cells or CD19-negative NGFR-K562 cells. CFSE was diluted to a greater degree, indicating more proliferation, when the T cells were cultured with CD19-K562 target cells (solid histogram in Figure 6) compared to when anti-CD19-CAR T cells were cultured with CD19-negative NGFR-K562 target cells. The assay was conducted as described previously.[124]

Figure 6

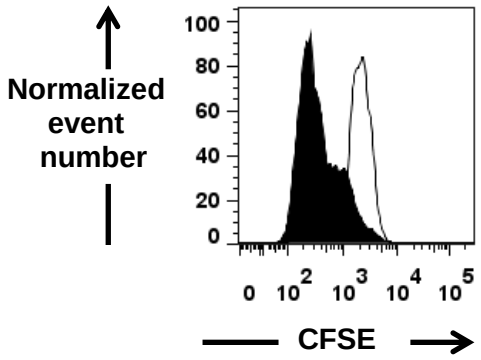
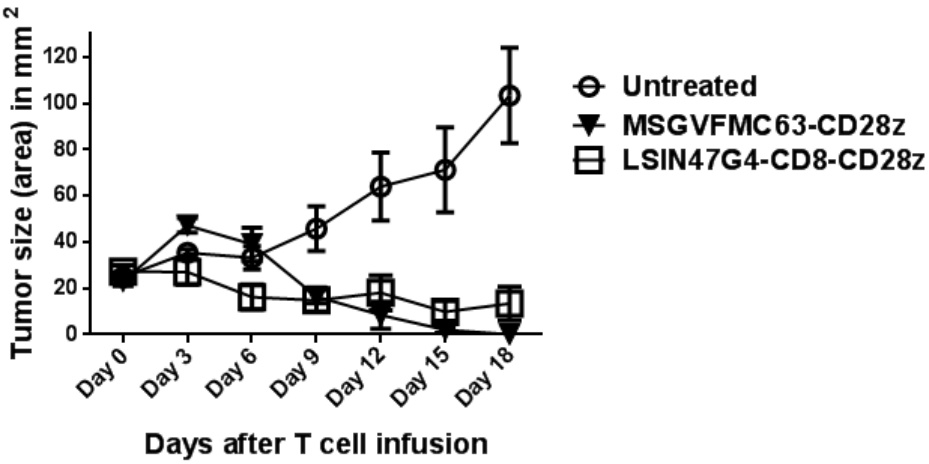


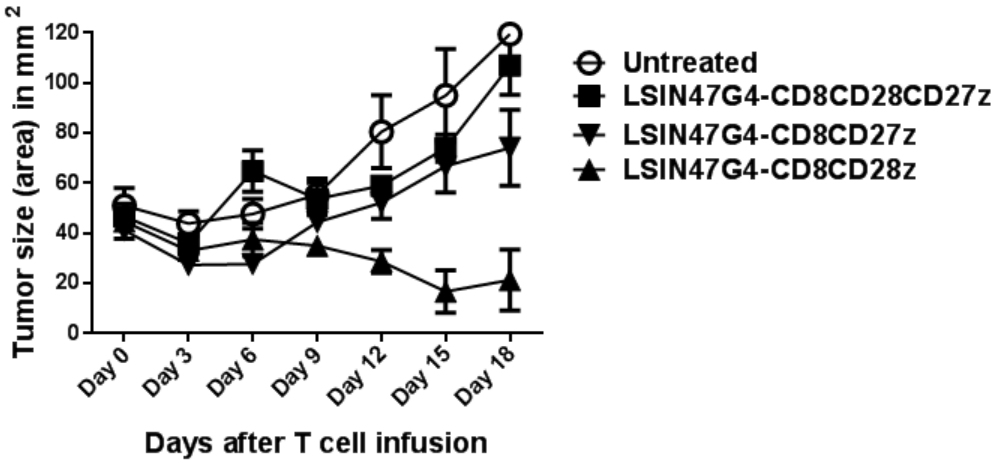
Figure 7 shows a comparison between LSIN-47G4-CD828Z and the CAR currently being used in NCI anti-CD19 CAR clinical trials, MSGV-FMC63-28Z. In the experiment depicted in Figure 7, immunocompromised NSG mice were engrafted with NALM6 leukemia cells in a manner to form a solid mass. Mice were then treated with a single infusion of T cells that were transduced with either LSIN-47G4-CD828Z or MSGV-FMC63-28Z. T cells expressing either CAR 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 7



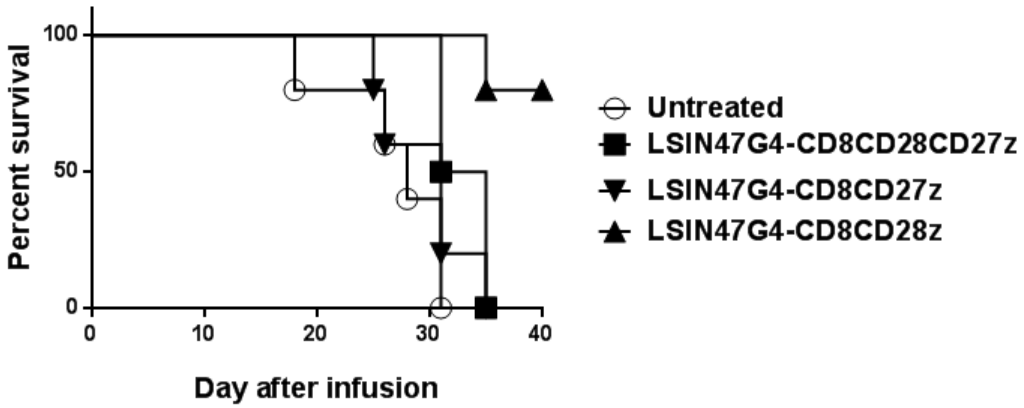
We constructed 2 CARs that had the same 47G4 scFv as LSIN-47G4-CD828Z. Each of these CARs also contained the hinge and transmembrane regions of the human CD8-alpha molecule and the cytoplasmic region of the CD3-zeta molecule. These CARs were encoded by the same lentiviral vector as LSIN-47G4-CD828Z. These CARs had different costimulatory domains than LSIN-47G4-CD828Z, either CD27 or both CD27 and CD28. We established NALM6 human $CD19^{+}$ acute lymphoblastic leukemia cell line tumors in immunodeficient mice. In this model, the NALM6 cells formed a solid subcutaneous mass; in addition, systemic spread of the tumor cells to the blood and spleen also took place. We allowed sizable tumors to develop over 6 days then we treated the mice with a single intravenous infusion of anti-CD19-CAR-transduced human T cells. After infusion of LSIN-47G4-CD828Z CAR T cells, regressions of tumors occurred between day 6 and day 15 after the T cell infusion, and a sizable fraction of mice receiving anti-CD19-CAR T cells were cured (Figures 8 and 9) [124]. In contrast, tumors continued to increase in size in untreated mice and in all mice receiving infusions of T cells expressing CARs containing CD27 moieties even when the CD27 moiety was combined with a CD28 moiety. This inability of CD27-containing CARs to eradicate established NALM6 tumors is unexplained, but it was repeated in multiple experiments. All 3 CARs used in this experiment functioned well in vitro. The mice receiving infusions of anti-CD19-CAR-transduced T cells had no signs of toxicity during this experiment.

Figure 8



In the experiments depicted in Figures 8 and 9, anti-CD19-CAR T cells were infused on Day 0, and no other treatments were administered. In the experiments reported in Figure 9, there were 5 mice in each group except the LSIN-47G4-CD82827z group, which had 4 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.

Figure 9



1.2.12 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 anti-CD19-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.[1,2,125] 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.[1-3,61,65,125] 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.[1-3,126] 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.[127] Myeloid suppressor cells have been shown to inhibit anti-tumor responses.[126] 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.[102] 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.[102]

Strong suggestive evidence of enhancement of the activity of adoptively-transferred T cells has been generated in humans.[69,128,129] 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.[69,74] 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.[70,72,73,92] 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.[70-73,128,129] Both cyclophosphamide and fludarabine are highly effective at depleting lymphocytes.[128,129] 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.[130] Multiple cycles of this regimen can be tolerated by heavily pretreated leukemia and lymphoma patients.[130]

1.2.13 Rationale for dose-escalation and repeated chemotherapy plus CAR T-cell treatments

The clinical trial described in this protocol is planned as a dose escalation in which the number of anti-CD19-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.[131-133] Second, in the setting of allogeneic transplantation, relapsed malignancy is often treated with infusions of allogeneic donor lymphocytes (DCIs).[134,135] 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 DCIs increases'.[134,135]

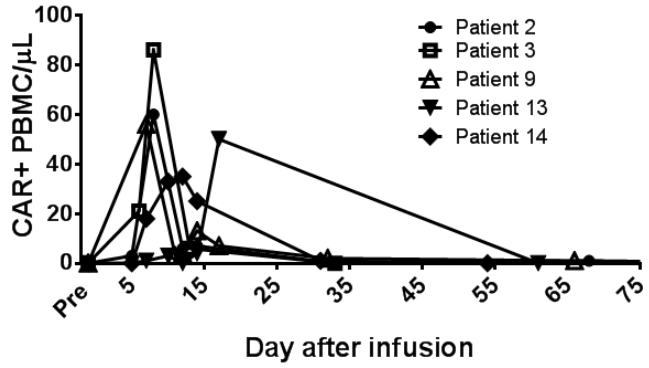
In addition to sequentially escalating the dose of T cells administered with the first treatment received by patients on sequential dose levels, second and third treatments will be a planned part of the protocol. The doses of cells received with the second treatment will be 3-fold higher than the dose received with the first treatment, and the dose of CAR T cells received with the 3rd treatment will be 3-fold higher than the dose received with the second treatment. Administration of second and third treatments will involve both the same chemotherapy conditioning regimen given with the first treatment as well as an infusion of CAR T cells. Second and third treatments will only be administered if the patient has measurable residual malignancy 2 months after the most recent prior treatment, and the patient did not experience dose-limiting toxicity with the most recent prior treatment. The rationale for administering second and third treatments comes from the allogeneic transplant field where intra-patient dose escalation of donor lymphocyte infusions is a standard practice [44]. The planned dose escalation will allow for a more intensive treatment in only those patients who clearly still require additional treatment of some type because of their measurable residual malignancy. Intra-patient dose escalation adds to the safety of the treatment because the response of each patient to prior treatments can be used to judge the risk of subsequent treatments. Only those patients not experiencing DLTs with prior treatment(s) will receive a subsequent treatment. Intra-patient dose escalation is especially promising for the CAR T-cell field because the toxicity experienced by individual patients varies widely. Starting with a low or moderate dose of T cells is prudent, and intra-patient dose escalation will allow a higher dose of T cells for those patients needing a more intensive treatment to eradicate persisting malignancy. The intra-patient dose-escalation strategy will be made feasible by the use of cryopreserved cell products.

1.2.14 Rationale for a fully-human CAR

Immune responses against genetically-modified T cells have previously been reported [113,136,137]. Because treatment with anti-CD19 CAR T cells is expected to eliminate recipient B cells, the most concerning type of immune response against anti-CD19 CAR T cells is a cytotoxic T-cell response directed against foreign components of the CAR. Such cytotoxic T-cell responses against suicide-gene-modified T cells have previously been documented [136]. 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. 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 [113,137]. 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 (Figure 10)[117], we hope that the fully-human anti-CD19 CAR will have longer persistence. The graphs shown in Figure 10 are absolute numbers of CAR T cells in the blood of 5 patients as determined by a quantitative PCR assay that is specific for the CAR. These results show the usual pattern of T-cell persistence with the current anti-CD19 CAR used in ETIB CAR trials. The CAR is called FMC63-28Z, and it contains a murine scFv. After infusion, CAR T cells rapidly rise to a peak. The cells do not persist in the blood long-term. Cells were undetectable in the blood by approximately 2 months after infusion.

Figure 10



1.2.15 Use of T cells from patients with malignancy versus healthy donor T cells to produce anti-CD19 CAR T cells

While most clinical trials have assessed anti-CD19 CAR therapy in patients who had not undergone alloHSCT, some trials have addressed this question. The trials fall into 2 main categories, those in which the CAR T cells were from the patient with malignancy (autologous)[118,137] and those in which the CAR T cells were from normal healthy donors (allogeneic) [119,120]. Success has been seen with both approaches, and sufficient data has not been accrued to determine which approach is best. The main advantage of use of autologous cells from the patient with malignancy is convenience as it avoids the need for a donor. Potential advantages to use of use of allogeneic healthy donor-derived T cells are numerous. First, many patients with advanced malignancy post-transplant have low lymphocyte counts, so use of healthy donor-derived cells allows collection of sufficient cells for CAR T-cell production. Second, healthy donor-derived cells, in contrast to malignancy patient-derived cells, will not have been exposed to multiple cycles of lymphocyte-depleting chemotherapy, so the healthy donor-derived T cells might be less differentiated and contain a higher fraction of naïve and central memory T cells. Naïve and central memory T cells have greater proliferative capacity than more differentiated T cells[138], and might be more effective for treating malignancy[139]. Another potential advantage of healthy donor T cells is that they are not tolerized against antigens on the malignant cells.

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 B-cell malignancies, which are often incurable diseases [16,18,140]. Only patients with B-cell malignancies persisting despite at least 2 prior lines of therapy will be enrolled. The risks of the study fall into 4 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.[72,115] 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. 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-CD19 CAR with proteins other than CD19 in vivo. This trial will be the first of a CAR containing the fully-human 47G4 variable regions, so cross-reactivity with normal proteins is not inconceivable. We have performed extensive testing of 47G4 CARs by culturing 47G4-CD28Z CAR T cells with a variety of human cell lines, and we have not seen recognition of cell lines that did not express CD19. A fifth category of toxicity caused by anti-CD19 CAR T cells is hypogammaglobulinemia due to depletion of all B cells and some plasma

cells. Hypogammaglobulinemia has been a complication for many patients on clinical trials of anti-CD19 CAR-expressing T cells.[72,73] Hypogammaglobulinemia in these patients was routinely treated with infusions of intravenous immunoglobulins.[72]

The potential benefits to subjects enrolling on this trial include the possibility that the anti-CD19-CAR T cells can cause a significant anti-malignancy effect. Many patients enrolled on earlier trials of anti-CD19 CAR T cells obtained prolonged complete remissions of advanced malignancies[70,72,141], so there is a chance that recipients of the anti-CD19 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, so further research aimed at improving anti-CD19 CAR T-cell therapies is needed. 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.

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 with the following malignancies are potentially eligible: any B-cell lymphoma, and chronic lymphocytic leukemia (CLL). Patients with indolent malignancies that have transformed to diffuse large B-cell lymphoma are eligible.
- Clear CD19 expression must be uniformly detected on 75% or more of malignant cells from either bone marrow or a leukemia or lymphoma mass by flow cytometry or immunohistochemistry. These assays must be performed at the National Institutes of Health. It is preferable but not required that the specimen used for CD19 determination comes from a sample that was obtained after the patient's most recent treatment. If paraffin embedded unstained samples of bone marrow involved with malignancy or a lymphoma mass are available, these can be shipped to the NIH for CD19 staining; otherwise, new biopsies will need to be performed for determination of CD19 expression.
- DLBCL patients must have received at least two prior chemotherapy-containing regimens at least one of which must have contained doxorubicin and a monoclonal antibody. Follicular lymphoma patients must have received at least 2 prior regimens including at least 1 regimen with chemotherapy. All other lymphoma and leukemia patients must have had at least 1 prior chemotherapy-containing regimen. All patients with CLL or small lymphocytic lymphoma must have had prior treatment with ibrutinib or another signal transduction inhibitor.

- Patients must have measurable malignancy as defined by at least one of the criteria below.
  - Lymphoma or leukemia masses that are measurable (minimum 1.5 cm in largest diameter) by CT scan is required for all diagnoses except CLL. All masses must be less than 10 cm in the largest diameter.
  - 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 CLL and 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 in lymphoma and CLL .

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 0-1
- Room air oxygen saturation of 92% or greater
- Patients of both genders must be willing to practice birth control from the time of enrollment on this study and for four months after receiving the preparative regimen.
- Women of child bearing potential must have a negative pregnancy test because of the potentially dangerous effects of the preparative chemotherapy on the fetus. 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.
- 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.)
- Patients with a known history of hepatitis B or hepatitis C are not eligible due to the risk of re-activation of hepatitis after prolonged B-cell depletion due to anti-CD19 CAR T cells.
- Seronegative for hepatitis B antigen, positive hepatitis B tests can be further evaluated by confirmatory tests, and if confirmatory tests are negative, the patient can be enrolled. Patients with a known history of hepatitis B are not eligible.
- 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. Patients with a known history of hepatitis C are not eligible.

- 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 45,000/mm^3 without transfusion support
- Hemoglobin greater than 8.0 g/dl.
- 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 1.4 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 any dose). 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 of any dose are not allowed within 14 days prior to the required leukapheresis. NOTE: Because of the long half-life and potential to affect CAR T cells, 60 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 55% 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 including prednisone, dexamethasone or any other corticosteroid for 14 days before apheresis and CAR T-cell infusion. Patients must also not take corticosteroids at doses higher than 5 mg/day of prednisone or equivalent at any time after the CAR T cell infusion.
- Patients who have been treated on other protocols of genetically-modified T cells <u>at the NIH only</u> 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).

2.1.2 Exclusion criteria:

- Patients that require urgent therapy due to tumor mass effects or spinal cord compression.

- Patients that have active hemolytic anemia.
- Patients with second malignancies in addition to their B-cell malignancy 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.
- Active uncontrolled systemic infections (defined as infections causing fevers and infections requiring intravenous antibiotics when 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 ventricular tachycardia or ventricular fibrillation, active cardiac arrhythmias (active atrial fibrillation is not allowed, resolved atrial fibrillation not requiring current treatment is allowed (anticoagulants count as current treatment)), active obstructive or restrictive pulmonary disease, 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).
- Systemic corticosteroid steroid therapy of any dose 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).

2.2 SCREENING EVALUATION

The following assessments must be completed within **30 days** 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 a B-cell malignancy by the NCI Laboratory of Pathology and confirmation of clear CD19 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 CD19 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 $CD19^{+}$ malignancy for enrollment purposes. If not necessary for enrollment, the bone marrow biopsy can be delayed until the baseline evaluation.
- Lumbar Puncture is required for patients with acute lymphoblastic lymphoma, and Burkitt's lymphoma only.
- Donor venous assessment (will be performed within 6 months before apheresis. Does not need to be repeated if no further apheresis needs to take place.)
- Antibody screen for Hepatitis B and C; HIV, HTLV-I/II, T. cruzi (Chagas agent), West Nile, and syphilis (RPR)
- Cardiac echocardiogram
- (Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic 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 panel
- Serum cortisol
- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential

2.3 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any

investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

2.4 TREATMENT ASSIGNMENT PROCEDURES:

Cohort:

Number	Name	Description
1	Cohort 1	Phase I dose escalation cohort who never had an alloHSCT

Arms:

Number	Name	Description
1	CAR T-cells + Immuno therapy	All patients will be receiving starting dose: $0.66x10^6$ anti-CD19 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 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.

3 STUDY IMPLEMENTATION

Note: Cohort 2 (Patients who have had an HLA-matched sibling or an 8/8-matched unrelated donor transplant): Per amendment B this cohort has been closed to Accrual until further notice.

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-CD19 CAR. The cell dose will be escalated unless occurrence of dose-limiting toxicities limits further escalation or until the maximum planed dose level is reached. The protocol will enroll patients who have never had an allogeneic hematopoietic stem cell transplant (alloHSCT) and patients who have had alloHSCT.

The protocol will enroll patients with B-cell 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 CD19 expression will be an important part of the

eligibility screening. Patients enrolled on the study will undergo leukapheresis, and anti-CD19-CAR-expressing T cells will be generated by transducing the patient's T cells with a lentivirus encoding the anti-CD19 CAR. Patients will receive a conditioning chemotherapy regimen of cyclophosphamide 300 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. Two days after the end of the conditioning chemotherapy, patients will receive a single infusion of anti-CD19-CAR-expressing T cells. A minimum 9-day hospitalization will be required after the cell infusion to monitor closely for acute toxicities. 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, 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-CD19 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

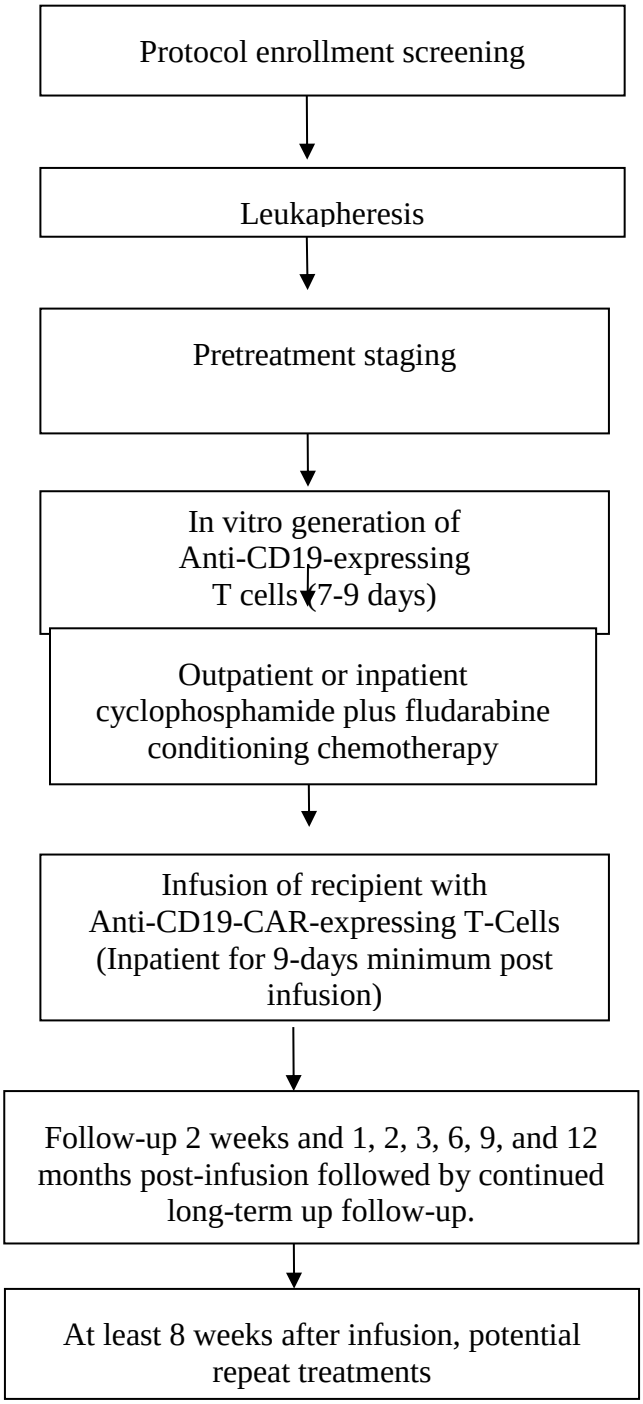
An integral part of the protocol is a planned second treatment for patients obtaining all responses except continuing complete remission (CR) or progressive disease at the 1-month follow-up. This second treatment will include chemotherapy and a CAR T-cell infusion and will be identical to the first except that the Principal Investigator (PI) has the option of giving either the same cell dose as the first CAR T-cell infusion or a cell dose one dose level higher than the first cell infusion (up to a maximum dose of 18x10^6 CAR+ cells/kg). A third treatment with a cell dose either the same as the second infusion or one dose level higher than the second cell infusion (up to a maximum dose of 18x10^6 CAR+ cells/kg) is also possible if the malignancy response is any response except continuing CR or progressive malignancy 1 months after the second treatment. Patients experiencing a dose-limiting toxicity (DLT) at any time will not be eligible for retreatment, and patients must continue to meet the same eligibility requirements that were required for original protocol enrollment to receive second or third treatments. The eligibility screening (Section **2.2**) will need to be repeated for each treatment except for the infectious disease serology. The maximum number of treatments that a patient can receive on this protocol is 3.

3.1.3 Inclusion of patients who have had allogeneic hematopoietic stem cell transplantation (alloHSCT)

This trial will enroll patients with a history of alloHSCT. Because patients who have had an alloHSCT 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, alloHSCT patients will be enrolled on a separate cohort than patients who have not had an alloHSCT. **Cohort 1** will enroll patients who have not had an allogeneic hematopoietic stem cell transplant, and **Cohort 2 (**closed as of Amendment B) will

enroll patients who have had an HLA-(human leukocyte antigen)-matched sibling or an 8/8-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 that are possibly, probably, or definitely attributable to protocol interventions and occurring within 60 days of CAR T-cell infusion.

- Grade 3 toxicities possibly or probably related to either the anti-CD19 CAR T cells or the fludarabine and cyclophosphamide chemotherapy and lasting more than 7 days
- Grade 4 toxicities possibly or probably related to the study interventions with the exceptions listed below

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

- Neutropenia (ANC<500/μL) lasting continuously 9 days or less
- Anemia (Hgb<8 g/dL) lasting continuously 9 days or less
- Grade 3 or 4 thrombocytopenia lasting 21 days or less
- 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 120 hours or less. The 120 hours is measured from the first institution of vasopressors even if vasopressors are temporarily discontinued and then re-started.
- Fever
- Hypophosphatemia
- Grade 4 elevation in alanine aminotransferase, 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

For past recipients of allogeneic hematopoietic stem cell transplants only, the following will also be DLTs (Allogeneic Cohort 2 closed as of Amendment B)

- Grade IV acute GVHD by 60 days after infusion of anti-CD19 T cells (see Section **13.4**)
- Grade III acute GVHD by 60 days after infusion of anti-CD19 T cells that does not resolve to a Grade 0-I with corticosteroid therapy within 30 days of onset (see Section **12.4**)

3.1.6 Dose Escalation

The trial will be a dose-escalation with 4 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 (alloHSCT) and Cohort 2: Patients who have had an HLA-matched sibling or an 8/8-matched unrelated donor transplant (closed as of amendment B). Each cohort will have a completely separate dose escalation, DLTs occurring in alloHSCT recipients will not affect dose escalation on the cohort of patients who have not had an alloHSCT. Likewise, the dose escalation of the alloHSCT patients will not be affected by the no-alloHSCT patient dose escalation. A separate dose MTD will be established for each cohort.

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-CD19-CAR-expressing T cells transferred for each dose level will be as follows:

Table 2

First-dose Escalation Plan: separate but identical dose escalation plans will be used for both Cohort 1 (patients who never had an alloHSCT) and Cohort 2 (patients who have had an alloHSCT- closed as of amendment B) Doses +/- 10% from the listed doses are acceptable.	
Dose Level	Dose of antri-CD19 CAR T cells
Level -1	$0.3x10^6$ CAR+ T cells per kg of recipient bodyweight
Level 1	$0.66x10^6$ CAR+ T cells per kg of recipient bodyweight
Level 2	$2.0x10^6$ CAR+ T cells per kg of recipient bodyweight
Level 3	$6.0x10^6$ CAR+ T cells per kg of recipient bodyweight
Level 4	$18.0x10^6$ CAR+ T cells per kg of recipient bodyweight

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 cell 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. To be clear, a patient on Cohort 1 could receive an infusion of anti-CD19 CAR T cells less than 2 weeks after the most recent infusion on Cohort 2 or vice versa. There will be a 4-week wait from the time of cell infusion to the last patient on a dose level of a cohort until treatment start (which means starting the chemotherapy) of the first patient on the next dose level of that same cohort.

Following each patient's first infusion, second and third infusions might be administered. The dose of cells administered with the second dose of cells will either be the same as or 3-fold higher than the first dose (up to a maximum dose of $18x10^6$ CAR+ T cells/kg). A patient's potential third dose will either be the same or 3-fold higher than the second dose up to a maximum dose of $18x10^6$ CAR+ T cells/kg). At least 8 weeks (56 days) must elapse between sequential cell infusions.

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.

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.

3.1.6.1 Effect of repeat doses on the dose escalation plans

Note that repeated dosing of chemotherapy plus CAR T cells for each patient is a planned part of the protocol for both patients who have not had allogeneic hematopoietic stem cell transplants (alloHSCT) and for patients who have not had alloHSCT.

Repeat doses in which the same patient receives 2 or 3 infusions of CAR T cells will not count toward escalating the dose to the next dose level on the dose escalation plans for first cell infusions. In other words, repeat doses of CAR T cells cannot be used to accelerate the protocol dose

escalation plan for initial T-cell infusions. In contrast, DLTs that occur with repeat doses at a given dose level will preclude dose escalation of the first dose of CAR T cells administered at the same dose level if more than 1 of the first 3 or more than 2 of the first 6 total patients (patients receiving first doses+repeat doses) to receive a particular dose of CAR T cells experiences a DLT. If more than 1 of the first 3 or more than 2 of the first 6 total patients on one of the cohorts to receive a particular dose of CAR T cells experiences a DLT, this dose will be declared the maximum administered dose for that cohort, and the First-dose Escalation Plan described above (Table 1) will be abandoned. All subsequent doses administered to patients on that cohort (both repeat doses and first doses) will be one dose level lower than the maximum administered dose; if at least 6 patients receive this dose with no more than 1 DLT, this dose will be declared the maximum tolerated dose. Note that toxicities occurring with repeat doses on Cohort 1 (no-alloHSCT) will not affect the dose escalation of Cohort 2 (allo HSCT) and vice versa.

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

Table 3

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. • 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.
$\leq$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 of 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**).

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-CD19-CAR-expressing T-cell preparation

After cells are obtained by apheresis, further cell processing to generate anti-CD19 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 ETIB Preclinical Core for cryopreservation at 200 to 300 million PBMC per vial. Contacts in the ETIB preclinical core are Fran Hakim and Jeremy Rose (301-594-5339). 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-CD19 CAR by using DTM SOPs. The cells will continue to proliferate in culture. Anti-CD19-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 2 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 $2x10^6$ CAR^+ T cells/kg, sufficient cells need to be produced and cryopreserved for a second dose of $6x10^6$ CAR^+ T cells/kg and a 3rd dose of $18x10^6$ CAR^+ T cells/kg. Cryopreserved cells will be used for all infusions unless poor cell growth precludes obtaining enough cells rapidly enough for cryopreservation on day 7 of culture in which case, cells can be infused without cryopreservation.

Ten vials of the infused cells will be cryopreserved for research use and stored in the ETIB preclinical core. Each vial will contain 20 million cells.

Before cryopreservation, the percentage of T cells expressing the anti-CD19 CAR will be determined by flow cytometry, and this percentage of anti-CD19 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 for retreatment 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 de-identified and used for research or discarded after approval of the Principal Investigator of this protocol.

3.4.3 Conditioning chemotherapy and anti-CD19 CAR T-cell administration-this can be either inpatient or outpatient

3.4.3.1 **Overall summary of the treatment plan**

Table 4

Drug	**Dose**	**Days**
Cyclophosphamide	300 mg/m^2 IV infusion over 30 minutes	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-CD19 CAR T cells	Variable.	Infuse on day 0

3.4.3.2 **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, 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. After the cyclophosphamide on days -5, -4, and -3, 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 are, 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: Anti-CD19 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 **13.5**, Appendix E.

Days 1 to 9: Mandatory hospitalization for observation and treatment as necessary. 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 12.3, 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 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.

3.4.4 Potential repeat treatment

- The chemotherapy component of repeat treatments will be initiated a minimum of 8 weeks after the most recent prior CAR T-cell infusion. There is no maximum time interval between treatments.
- Patients with any response except progressive disease or continuing complete remission after an initial or second CAR T-cell infusion are potentially eligible for a repeat treatments consisting of conditioning chemotherapy followed by an infusion of CAR T cells.

- Patients experiencing a DLT with any prior treatment on this protocol will not be eligible for re-treatment.
- To be eligible for repeat treatments, patients must meet the same eligibility requirements listed in Section **2.1**.
    - Uniform CD19 expression needs to be documented on the malignant cells after previous anti-CD19 CAR T-cell infusions in patients undergoing a subsequent CAR T-cell infusion unless a biopsy is not technically possible or is unsafe by interventional radiology methods. In all patients with palpable adenopathy or bone marrow involvement, a biopsy to evaluate for CD19 expression will be performed prior to 2^{nd} and prior to 3^{rd} treatments
    - The patients must undergo screening evaluation as listed in Section **2.2** except infectious disease serology is not required to be repeated unless clinically indicated. Follow-up testing for retreatment will be the same as for the first treatment. A maximum of 3 total treatments can be administered to any one patient, and at least 8 weeks must elapse between each cell infusion.

- The dose of anti-CD19 CAR T cells administered during repeat treatments:
    - The second dose of CAR T cells will be either the same or one dose-level higher (up to a maximum of $18x10^6$ CAR+ T cells/kg) than the first dose. Whether the second dose will be the same as the first dose or one doses level higher than the first dose will be determined by the PI of the protocol based on toxicity and the anti-malignancy response experienced by the patient after the first dose.
    - A potential third dose will be either the same or one dose-level higher (up to a maximum of $18x10^6$ CAR+ T cells/kg) than the second dose. Whether the third dose will be the same as the second dose or one doses level higher than the second dose will be determined by the PI of the protocol based on toxicity and anti-malignancy response experienced by the patient after the second dose.
    - If a maximum tolerated dose has been determined as stated under 3.1.4, the dose of cells for repeat doses will be the maximum tolerated dose.
    - It is possible that a patient might receive only 1 dose of CAR T cells even if the patient is eligible for repeat doses if this is the preference of either the patient or the PI of the protocol.
    - A maximum of 3 treatments will be administered

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 for all patients except patients with CLL
- Bone marrow aspirate and biopsy: specifically ask for CD19 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. Collect one tube of bone marrow aspirate to be sent to Fran Hakim's lab for research purposes. Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594 5339. 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.
- Peripheral blood flow cytometry for patients with CLL
- Neurology Consult if clinically indicated
- 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 or high serum potassium or hypotension or a low serum cortisol or a history of adrenal insufficiency or other clinical indication
- Anti CMV antibody titer, HSV serology, and EBV panel, T cruzi serology, toxoplasmosis serology (Note: patients who are known to be positive for any of the above do not need to be retested; may be performed within 3 months of chemotherapy start date)
- 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 Dr. Fran Hakim's Pre-Clinical Core lab; Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.
- 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 Dr. Fran Hakim's, Pre-Clinical Core lab; Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.

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), Inorganic 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 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.
- C-reactive peptide (CRP) within 7 days before the first dose of chemotherapy on day -5 and daily while hospitalized.
- TBNK 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), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Uric Acid)
- PT/PTT 4 days and 7 days after cell infusion
- Other tests will be performed, as clinically indicated.
- 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 to 14 days after infusion of 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). Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.

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), +21 (+/- 1 day), +30 (+/- 5 days), +60 (+/- 7 days), +90 (+/- 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; subsequently, patients will be seen annually (+/- 30 days). 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. Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339. **EXCEPT**: **Day 30 after CAR T-cell infusion (+- 7days), a 5 liter research apheresis** will be conducted to obtain cells for research purposes instead of 6 CPT tubes of blood. Only if the research apheresis cannot be done, 30 days after anti-CD19-CAR-transduced T cell infusion, 6 CPT tubes of blood will be collected to obtain blood for immunological testing.
- 1 SST tube (8 mL) of Research Blood will be obtained for serum collection. Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.

  Note: **after the first year of follow-up, research blood will be reduced to 4 CPT tubes (32 mL total) during required protocol visits**. Research blood will not be collected after the 5 year follow-up.
- CT scan of neck, chest, abdomen, and pelvis and/or PET scan at outpatient follow-up appointments starting 1 month after infusion (as necessary to stage malignancy only). All lymphoma patients should get a PET scan at the 1 month, 2 month, and 3 month and 6 month follow-up visits and anytime progression or relapse is suspected on the CT scan or anytime a PET scan is clinically indicated. CLL patients should not get PET scans.
- For CLL, obtain peripheral blood flow cytometry at all outpatient follow-up visits.
- For lymphoma, 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. For CLL, post-treatment bone marrow biopsies will only be done to document CR if the patient is in CR at all other sites. Aspirate must be sent for flow cytometry to the lab of Dr. Maryalice Stetler-Stevenson. CD19 staining must be requested for the flow cytometry. CD19 immunohistochemistry should also be requested on the bone marrow biopsies. For each bone marrow aspirate performed, send one tube of bone marrow aspirate to Fan Hakim's lab for research purposes. Attention Jeremy Rose, Bldg 10, room 12C316 contact phone: 301-594 5339.
- 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), Inorganic 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. **NOTE: Patients must have an additional CBC with differential performed at 6 weeks (+/- 5 days) and at 10 weeks (+/- 5 days) after the CAR T-cell infusion.**
- Gene-therapy-specific follow-up must be carried out as described in section **3.7**

3.6 **STUDY CALENDAR (TABLE 5)**

Procedures [a]	Screening/ Baseline	Pre-cell infusion/ Day 0	Day +7[b]	Day+14 and Day+21 (+/- 1 day) and Day+30 (+/- 5 days)	Day +60 (+/- 7 days)	Day +90 (+/- 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
Vital signs, O2 saturation	X	X	X	X	X	X	X	X	X	X
Height, weight	X	X								
Performance Score	X	X								
Laboratory Assessments										
CBC with differential	X	X	X	X[P]	X[P]	X	X	X	X	X
Sodium, Potassium Chloride, CO2, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Inorganic 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
PT/PTT	X		X							

Procedures [a]	Screening/ Baseline	Pre-cell infusion/ Day 0	Day +7[b]	Day+14 and Day+21 (+/- 1 day) and Day+30 (+/- 5 days)	Day +60 (+/- 7 days)	Day +90 (+/- 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)
			(also on day 4)							
Thyroid panel	X									
Quantitative immunoglobulins	X			X	X	X	X	X	X	X
Anti-CMV, HSV, EBV, t.cruzi,toxoplasma	X[h]									
Antibody screen for Hepatitis B and C; HIV, HTLV-I/II, T. cruzi (Chagas agent), West Nile, and syphilis (RPR)	X[h]									
TBNK	X	X	X	X	X	X	X	X	X	X
G6PD	X									
Urinalysis (culture prn)	X									
ABO typing	X									
CT chest, abdomen, pelvis [c]	X			X[n]	X	X	X	X	X	X
PET [d]	X			X[n]	X	X	X	X[e]	X[e]	X[e]
Brain MRI	X									

Procedures [a]	Screening/ Baseline	Pre-cell infusion/ Day 0	Day +7[b]	Day+14 and Day+21 (+/- 1 day) and Day+30 (+/- 5 days)	Day +60 (+/- 7 days)	Day +90 (+/- 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)
Lumbar Puncture	X[k](ALLymph and Brk only)									
Neurology Consult[e]	X[j]									
Peripheral blood flow cytometry	X[j] (CLL only)			X (CLL only)	X (CLL only)	X (CLL only)	X (CLL only)	X (CLL only)	X (CLL only)	X (CLL only)
Other Specific Assessments										
Documentation of CD19 expression by malignancy	X									
Serum cortisol	X									
250 microgram cosyntropin test[e]	X									
EKG, echocardiogram	X									
RCL (replication competent lentivirus)	X					X	X		X	X[i]
Central Venous catheter placement		X								
Donor Venous Assessment	X									

Procedures [a]	Screening/ Baseline	Pre-cell infusion/ Day 0	Day +7[b]	Day+14 and Day+21 (+/- 1 day) and Day+30 (+/- 5 days)	Day +60 (+/- 7 days)	Day +90 (+/- 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)
Bone marrow aspirate/biopsy with flow [f]	X									
Research Blood		X	X	X[o]	X	X	X	X	X	X[l]
Research Apheresis				X[o]						
Adverse Event		X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X

[a] see section **2.2** and section **3.4** for details

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

[c] CT scan for lymphoma and CLL patients at the indicated time-points including within 14 days of chemo start.

[d] PET-CT at indicated time-points including within 14 days of chemo start for all lymphoma patients. No PET-CT scans should be done for CLL patients unless CLL is transformed to DLBCL.

[e] only if clinically indicated or if needed to document relapse suspected on CT

[f] All patients need a pre-treatment bone marrow biopsy. Post-treatment bone marrow biopsies are only done if useful for staging. CLL patients only need post-treatment bone marrow biopsies if in CR at all other sites. Lymphoma 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] Patients who are known to be positive for any of these tests do not need to be retested; may be performed within 2 months of chemotherapy start date

[i]blood must be collected for RCL annually during years 2 through 5 after infusion

[j] Only at Baseline

[k] Only at Screening. Lumbar punctures will also need to be repeated after treatment if clinically indicated.

[l] until 5 years after infusion

[m]Only if positive before protocol treatment or if clinically indicated

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

[o]For Day 30 only, a 5L apheresis will be collected instead of 6CPT tubes of blood. If the apheresis is unable to be performed, only then will 6CPT tubes of blood be collected. The 1SST of blood will be collected as normal.

[p]An additional CBC with diff will be performed at 6 weeks (+- 5days) and 10 weeks (+- 5days) between the regular time point visits.

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 for other follow-up. 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 ETIB. 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.

3.7.2 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.

3.7.3 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. Blood samples will also be collected and cryopreserved annually 2 years through 5 years after CAR T-cell infusion in case the samples are needed for future gene-therapy-specific tests.

- 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 NCI IRB, the NIH Institutional Biosafety Committee, the OSP, and the Indiana University Vector Production Facility (where the vector was made).

  b. Repeat the S+L- 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 documenting removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

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 potential repeat treatments and cancellation of cell infusion for toxicity arising during the conditioning chemotherapy.

Patients will be taken off treatment for the following:

- Occurrence of a dose-limiting toxicity makes a patient ineligible for repeat treatments.
- 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 treatment can proceed if not, the patient will be off-study.
- 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 protocol treatment, 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. Patient will be taken off study after the 30-day safety visit if they have received any part of the treatment.
- Participant requests to be withdrawn from active therapy
- Investigator discretion
- Positive pregnancy test
-

3.8.2 Off-study Criteria

- The patient voluntarily withdraws
- There is significant patient noncompliance
- 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 except for repeat treatments on this protocol.

- 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.
- PI decision to end this study

Note: Patients must be followed until all adverse events that are at least possibly, probably or definitely attributable to protocol treatment will be followed until return to baseline or stabilization of event. If an adverse event is not expected to resolve to baseline 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 (NCI 15-C-0141), which has been IRB approved, specifically to continue the FDA-required gene-therapy follow-up.

3.8.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the web site **(**http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 ROUTINE SUPPORTIVE CARE

- Patients with a CD4 T-cell count less than 200/μL will be maintained on pneumocystis prophylaxis Because of the potential to cause cytopenias, trimethoprim sulfamethoxazole will not be used for infection prophylaxis, 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.
- 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. **During inpatient hospitalization within 10 days after cell infusion, 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 70 kg and over in weight. During inpatient hospitalization within 10 days of cell infusion, filgrastim will be given daily and then discontinued as soon as the absolute neutrophil count recovers to 1500/microliter.

  If an outpatient develops an absolute neutrophil count less than 1000/microliter, the patient will be required to stay in the Bethesda, MD area for close follow-up at the NIH Clinical Center until the absolute neutrophil count is above 1000/microliter for at least 2 days. Any outpatient with an absolute neutrophil count less than 800/microliter will immediately be started on filgrastim, and filgrastim will be continued until the absolute neutrophil count is at least 2000/microliter.
- Outpatients with absolute neutrophil counts less than 1000/mL will be advised to check their temperatures twice daily and to report to a local health care facility immediately for any temperature of 100.5 degrees or higher.
- Any patient who develops a temperature of 100.5 degrees or higher during the first year after CAR T-cell infusion should be seen immediately at a local health care facility for a complete blood count with differential to detect possible neutropenia.
- Patients with a neutrophil count less than 1000/microliter 1 month or more after CAR T-cell infusion will get a blood quantitative PCR for CMV.

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

During the planned inpatient admission after CAR T-cell infusion, 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 **13.3**, 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 **13.3** is not a protocol deviation.

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.

5.1 CORRELATIVE STUDIES FOR RESEARCH

5.1.1 Biospecimen collection before the start of the conditioning chemotherapy:

- One heparinized syringe containing 5 to 8 mL of bone marrow aspirate to be sent to Fran Hakim's lab. Attention Jeremy Rose, Bldg 10, room 12C316 contact phone: 301-594 5339.
- 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 Dr. Fran Hakim's, Pre-Clinical Core lab; Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.
- 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 Dr. Fran Hakim's, Pre-Clinical Core lab; Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594 5339.
- Specimens will be cryopreserved and assays will be performed retrospectively.

5.1.2 Biospecimen collection 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-CD19-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). Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594 5339.

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, 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. Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594 5339.
  - 1 SST tube (8 mL) of Research Blood will be obtained for serum collection. Attention Jeremy Rose, Bldg 10, room 12C316 contact phone: 301-594 5339.

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

- When bone marrow aspirate and biopsy is performed, part of the aspirate will be sent to the ETIB Preclinical Core and will be cryopreserved.

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 monoctyes 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.
- Gene expression studies will be performed on patient lymphoma cells and on the infusion CAR T cells of each patient. Methods used will be either Nanostring and/or RNAseq (RNA sequencing).

- Patients' blood samples will be obtained and saved for possible future 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 samples will be archived annually thereafter if all previous testing has been negative with a brief clinical history. If a patient dies of any cause that might be due to gene-therapy toxicity or develops new neoplasms during this trial, efforts will be made to assay a biopsy sample for RCL. 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 ETIB 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 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 NIH Department of Pathology. Studies will be performed to evaluate the antigen expression by the tumor and to evaluate the presence of transduced cells.

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 the risks of the new questions were covered in the consent document. 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 Storage/Tracking in the Preclinical Development and Clinical Monitoring Facility (PDCMF)

- 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. Samples will not be sent outside NIH without IRB notification and an executed MTA.
- Patient blood and tissue samples, collected for the purpose of research under IRB approved protocols of the Experimental Transplantation and Immunology Branch, may be archived by the ETIB Preclinical Service. All data associated with archived clinical research samples is entered into the ETIB Preclinical Service's Microsoft Excel databases on frozen cells and serum. These databases are stored on the NCI group drive in the ETIB Preclinical Service folder. Access to this folder is limited to ETIB clinical staff, requiring individual login and password. All staff in the Preclinical Service laboratory has received annually updated NIH/CIT training and maintains standards of computer security.
- The data recorded for each sample includes the patient ID, trial name/protocol number, date drawn, treatment cycle/post-transplant time point, cell source (e.g. peripheral blood, marrow,) as well as box and freezer location. Patient demographics that correlate treatment outcomes and therapies with the samples can be obtained only through the NCI/ETIB clinical records. As of January 2007, all newly received samples will receive a unique bar code number, which will be added to the sample Preclinical Service database. Only this bar code will be recorded on the sample vial and the vials will not be traceable back to patients without authorized access to the Preclinical Service database. All non-coded samples previously archived will be stripped of identifiers prior to distribution for any use other than as a primary objective of the protocol under which they were collected.

- Samples are stored in freezers at -80°C (sera and plasma) or under liquid nitrogen (cells), according to stability requirements. These freezers are located onsite at the Preclinical Service laboratory (12C216). Access to samples from a protocol for research purposes will be by permission of the Principal Investigator of that protocol and either an OHSRP exemption form indicating the research is exempt from IRB-review or IRB approval of this new research activity. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with objectives of the original protocol for which the samples were collected, or (using only unlinked or coded samples) for an IRB approved or exempted research activity, and that any unused samples must be returned to the Preclinical Service laboratory.

5.2.2 Protocol Completion/Sample Destruction

- Once primary research objectives for the protocol are achieved, researchers can request access to remaining samples, providing they have both approval of the Principal Investigator of the original protocol under which the samples or data were collected and either an IRB approved protocol and patient consent or an OHSRP exemption indicating that the activity is exempt from IRB review.
- Samples, and associated data, can only be permanently archived if the subject has provided informed consent for future use. If researchers have samples remaining once they have completed all studies associated with the protocol, they must be returned to the Preclinical Service laboratory.
- The Preclinical Service staff will report to the Principal Investigators any destroyed samples, if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container), lost in transit between facilities or misplaced by a researcher.
- The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

5.2.3 Samples for Genetic/Genomic Analysis

Samples used for gene expression analysis will be RNAseq and/or Nanostring™. These studies will be used to determine gene expression in lymphoma cells and infusion CAR T cells. The purpose of these studies is to assess gene expression at the RNA level not to study germline mutations.

5.2.3.1 Description of the scope of genetic/genomic analysis

RNAseq and/or Nanostring™ will be used to determine gene expression in lymphoma cells and infusion CAR T cells. The purpose of these studies is to assess gene expression at the RNcrisA

level not to study germline mutations. One purpose of these studies is to determine if different levels of gene expression in malignant cells are associated with response to CAR T-cell therapy. Another purpose of these studies is to determine if different levels of gene expression in infusion CAR T cells are associated with anti-malignancy responses caused by CAR T cells or persistence of CAR T cells.

5.2.3.2 Certificate of Confidentiality

As part of study efforts to provide confidentiality of subject information, this study has obtained a Certificate of Confidentiality, which helps to protect personally identifiable research information. The Certificate of Confidentiality allows investigators on this trial to refuse to disclose identifying information related to the research participants, should such disclosure have adverse consequences for subjects or damage their financial standing, employability, insurability or reputation. The informed consent includes the appropriate coverage and restrictions of the Certificate of Confidentiality.

5.2.3.3 Management of Results

The analyses that we perform in our laboratory are for research purposes only; they are not nearly as sensitive as the tests that are performed in a laboratory that is certified to perform genetic testing. Changes that we observe unrelated to our research may or may not be valid. Therefore, we do not plan to inform participants of the results of testing on the tissue and blood that is performed in our research lab. However, subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists) Subjects who still remain on the study will be contacted at this time with a request to provide a blood sample to be sent to a CLIA certified laboratory. The CLIA testing may be funded by the PI or the CCR. If the research findings are verified in the CLIA certified lab, the subject will be referred to the NCI Genetics Branch for genetic counseling on the implications of the results. Subjects that do not wish to return to the NCI will be referred to a local genetics health care provider (at their expense).

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

Note: Subjects must remain on the study in order to maintain up to date contact information and to have results returned. Subjects opting for voluntary withdrawal from the study are to be informed at the time that results cannot be returned once this has been done.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

Data will be prospectively collected and entered in real time into the Cancer Center Clinical Data System database (NCI C3D database) (information at http://ccrtrials.nci.nih.gov). It is expected

that clinical data be entered into C3D no later than after 10 business days of the occurrence. The NCI PI and research nurse will have access to these data via web access.

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 NCI C3D 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.

All data will be kept secure. 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.

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 at least 30 days after removal from study treatment or until off study, whichever comes first.

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 regulations 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, the IRB will be notified.

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.

6.2 GENOMIC DATA SHARING PLAN

Unlinked genomic data will be deposited in the database of genotypes and phenotypes (dbGaP) in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

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 [142] and Recommendations for Initial Evaluation, Staging, and Response Assessment of Hodgkin and Non-Hodgkin Lymphoma: The Lugano Classification Journal of Clinical Oncology, 2014[143])

- Complete Remission (CR):
  1. 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.
  2. Typically FDG-avid lymphoma (large cell, mantle cell and follicular lymphomas are all typically FDG-avid): 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.
  3. 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.
  4. 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.
  5. A bone marrow aspirate and biopsy is performed only when the patient had bone marrow involvement with lymphoma prior to therapy (all patients must have a pre-treatment bone marrow biopsy) 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.

- 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. Note: in patients with follicular lymphoma or mantle-cell lymphoma, a PET scan is only indicated in patients with one or at most two residual masses that have regressed by 50% on CT scan.

- 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.

Response criteria for CLL:

(Hallek et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: A report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines [144])

- Complete Remission: The designation of a complete response of CLL for this protocol requires all of the following as assessed at least 2 months after anti-CD19-CAR-transduced T cell infusion:
  1. Absence of lymphadenopathy (physical exam, relevant CT scans)
  2. No hepatomegaly or splenomegaly (physical exam, relevant CT scans)
  3. Absence of constitutional symptoms
  4. Normal CBC as exhibited by PMN's ≥ 1500/ul, platelets >100,000/ul, hemoglobin > 11.0 g/dl (untransfused), and
  5. Blood B lymphocyte count<4,000/ul
  6. Bone marrow aspirate and biopsy should be performed only after requirements a-d are first met. Bone marrow aspirate and biopsy that is normocellular for age with <30% of the nucleated cells being B cells (CD19 or CD 20) positive. Lymphoid nodules must be absent. If the marrow is hypocellular, a repeat determination should be performed in one month.
  7. Patients who fulfill all of the criteria for CR except for having bone marrow lymphoid nodules will be considered to be in a PR.

- Partial Remission: The designation of partial response requires at least one of the following: a ≥ 50% decrease in peripheral B lymphocyte count from pre-treatment value, a ≥ 50% reduction in lymphadenopathy, or a ≥ 50% reduction in splenomegaly/hepatomegaly for a period of at least 8 weeks based on physical exam and relevant CT scans. No increase in any lymph node or appearance on newly enlarged nodes is allowed. Additionally, designation of PR requires at least one of the following:
  1. PMN's ≥ 1,500/ul, or a 50% improvement from pre-treatment value
  2. Platelets > 100,000/ul or 50% improvement from pre-treatment value
  3. Hemoglobin > 11.0 g/dl (untransfused) or 50% improvement from pre-treatment value

- Progressive Disease: The designation of progressive disease is characterized by any one of the following:
  1. A ≥ 50% increase in the greatest diameter of any lymph node that was enlarged pre-treatment.
  2. The appearance of new palpable lymph nodes
  3. A ≥ 50% increase in the absolute number of circulating B lymphocytes (value must exceed 5,000/ul)
  4. ≥50% increase in the size of the liver and/or spleen as determined by measurement below the respective costal margin or by CT scan; appearance of palpable hepatomegaly or splenomegaly, which was not previously present.
  5. Transformation to a more aggressive histology
  6. Patients not fulfilling the above criteria for progressive disease but demonstrating a decrease in hemoglobin value of > 2 gm/dl from baseline or a decrease of > 50% in platelet or granulocyte count will not be considered as evidence of progressive disease, because these changes may occur as both a consequence of many therapies or of underlying CLL/SLL; in such cases, a repeat bone marrow biopsy is recommended.

- Stable Disease: Patients who do not fulfill the criteria for complete or partial response and do not fulfill the criteria for progressive disease will be considered as having stable disease.

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. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

7.1.2 Suspected adverse reaction

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

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

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
- Inpatient hospitalization or prolongation of existing hospitalization
- 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.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-threatening adverse event

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

7.1.9 Non-compliance (NIH Definition)

Failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB AND CLINICAL DIRECTOR REPORTING

7.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:

- All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research up to 3 months after CAR T-cell infusion;
- All Grade 3 and 4 events that are possibly, probably or definitely related to the research up to 1 year after CAR T-cell infusion
- All Grade 5 events regardless of attribution;
- All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported.

7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

7.3 IND SPONSOR REPORTING CRITERIA

During the first 30 days after the subject receives investigational agent/intervention, the investigator must **immediately** report to the sponsor, using the mandatory MedWatch form 3500a or equivalent, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

Required timing for reporting per the above guideline:

- Deaths (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- Other serious adverse events as well as deaths due to progressive disease must be reported within one business day

In addition to the reporting criteria described above, the Investigator will report all cytokine release syndrome (CRS) events ≥ grade 3 to the Sponsor expeditiously with the exception of grade 3 fevers with no other signs or symptoms of CRS and will include in these reports the results of all echocardiograms performed on subjects suspected of having CRS. The Investigator will also report all grade 4 neurological toxicities in an expedited manner.

Events will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov and to the CCR PI and study coordinator.

7.3.1 Reporting Pregnancy

7.3.1.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agents (s) should be documented in box B5 of the MedWatch form "Describe Event or Problem".

Pregnancy itself is not regarded as an SAE. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)" under the Pregnancy, puerperium and perinatal conditions SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within 1 day, i.e., immediately, but no later than 24 hours of when he or she becomes aware of it.

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

7.3.1.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study treatment, and for 4 months after finish the last cell infusion

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 after last cell infusion should, if possible, be followed up and documented.

7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify the 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 the 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 DATA AND SAFETY MONITORING PLAN

7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a regular biweekly 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. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS and to the Sponsor.

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 Sponsor Monitoring Plan

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 study endpoints; adherence to the protocol, regulations, 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 an NCI 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.

7.5.3 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 NCI-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 STATISTICAL CONSIDERATIONS

Note: Cohort 2 (Patients who have had an HLA-matched sibling or an 8/8-matched unrelated donor transplant): Per amendment B this cohort has been closed to Accrual until further notice.

The primary endpoint of this trial is to determine the safety of administering CAR-expressing T cells to patients with relapsed or persistent B-cell 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, to assess repeated CAR T-cell treatments, 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 (alloHSCT) and **Cohort 2** (closed as of amendment B): Patients who have had an HLA-matched sibling or a 8/8-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. Both cohorts can undergo repeat treatments following an identical re-treatment plan.

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 4 doses and up to 6 patients per dose level will be carried out. 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 64 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 anti-CD19-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.

. As of approval of Amendment C, we will treat a maximum of 14 more patients on this protocol (6 on Dose Level 3 plus an expansion cohort of 14 patients).

Despite 2/6 patients experiencing strictly-defined DLTs on dose level 2 of the study, the second DLT on this dose level was actually not definitively related to CAR T cells and likely unrelated to dose, so the dose escalation is allowed to proceed to dose level 3 starting with the 13th patient to receive CAR T cells on this trial. If more than 1 of the first 3 patients experience a DLT on dose level 3, enrollment will continue on dose level 2. In other words, if more than 1 of the first 3 patients treated on dose level 3 has a DLT, Dose Level 2 will be declared the MTD despite the fact that 2 DLTs have occurred on Dose Level 2. If Dose Level 2 is declared the MTD, an 8 patient expansion cohort will be treated at this dose level. If 1 of the first 3 patients on dose level 3 experiences a DLT, 3 more patients will be enrolled on dose level 3.

9 COLLABORATIVE AGREEMENTS

There is a Collaborative Research and Development Agreement (CRADA# 03019) in place between Kite Pharma, Inc. and the National Cancer Institute for this protocol.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

The patients to be entered in this protocol have advanced B-cell 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.

10.2 PARTICIPATION/SELECTION RATIONALE

- The eligibility criteria for this protocol only allow enrollment of patients with advanced B-cell 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 B-cell 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. Despite these impressive results, improvements in anti-CD19 CAR T cells to increase efficacy and to decrease toxicity are still needed. This trial aims to improve upon prior results by evaluating several important changes in the administered CAR T cells including: use of a fully-human CAR, use of a new lentiviral vector, use of a new CAR design, and use of planned second and third CAR T-cell treatments.

- 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.

10.3 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.

10.4 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 **10.4**), all 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 for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 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.

10.5 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 5 general categories. 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.[72,115] 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-CD19 CAR with proteins other than CD19 in vivo. A 5th possible toxicity is hypogammaglobulinemia. Hypogammaglobulinemia has been a complication of many patients on clinical trials of anti-CD19 CAR-expressing T cells.[72,73] Hypogammaglobulinemia in these patients was routinely treated with infusions of intravenous immunoglobulins.[72]

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.[75-77]

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. CT guidance may be used in obtaining biopsies. If so, there will also be a risk of exposure to radiation from up to 3 CT scans. This radiation exposure is not required for medical care and is for research purposes only. The amount of radiation received in this study is 0.43 rem which is below the guideline of 5 rem per year allowed for research subjects by the NIH Radiation Safety Committee.

The success of this clinical trial cannot be predicted at this time. Because all patients in this protocol have advanced B-cell 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.

10.6 CONSENT AND ASSENT PROCESS AND DOCUMENTATION

The patient, along with family members or friends, will be presented with a detailed description of the protocol treatment. The specific requirements, objectives, and potential advantages and disadvantages will be presented. The Informed Consent document is given to the patient who is requested to review it and to ask questions prior to agreeing to participate in the treatment portion of this protocol. The patient will be reassured that participation on trial is entirely voluntary and that he/she can withdraw or decide against treatment at any time without adverse consequences. The research nurse, Principal Investigator or his designee is responsible for obtaining written informed consent from the patient.

10.6.1 Telephone re-consent procedure

Reconsent on this study may be obtained via telephone according to the following procedure: the informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records. The informed consent process will be documented on a progress note by the consenting investigator.

10.6.2 Short form consent process for non-English speaking patients

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2) and 21 CFR 50.27 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation. Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

11 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.

11.1 LENTIVIRAL VECTOR CONTAINING THE ANTI-CD19 CAR GENE

The lentiviral vector (LSIN-47G4-CD828Z) encoding a chimeric antigen receptor (CAR) directed against CD19 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[122]. The anti-CD19 CAR protein encoded by this vector contains a signal peptide from human CD8-alpha, 47G4 fully-human antibody light chain variable region (47G4 VL), linker peptide, 47G4 fully-human antibody heavy chain variable region (47G4 VH), human CD8-alpha (hinge and transmembrane), CD28 (cytoplasmic region), and the CD3-zeta (cytoplasmic region) T-cell activation domain.

The vector will be stored at –80° C in the Dept. of Transfusion Medicine, 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

11.2 CYCLOPHOSPHAMIDE

11.2.1 Source

Cyclophosphamide will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a lyophilized powder in various vial sizes.

11.2.2 Toxicities

1) Nausea and vomiting - variable; symptomatically improved with standard anti-emetics and/or benzodiazepines [e.g., lorazepam].
2) Water retention – cyclophosphamide may rarely provoke the syndrome of inappropriate antidiuretic hormone secretion and resultant hyponatremia, usually manifested 12-48 hours after IV administration.
3) Cardiomyopathy - cyclophosphamide may cause severe, sometimes lethal, hemorrhagic myocardial necrosis or congestive cardiomyopathy. Patients may present with congestive cardiomyopathy as late as 2 weeks after the last dose of cyclophosphamide. In an attempt to minimize this complication, patients with significant cardiac dysfunction are excluded from this protocol [see patient eligibility]. Congestive failure is managed according to standard medical therapeutics. Cardiac toxicity with the dose of cyclophosphamide administered on this protocol is extremely unlikely.
4) Hemorrhagic cystitis – this is a serious, potentially life-threatening complication related to injury of the bladder epithelium by cyclophosphamide metabolites.
5) Sterility
6) Less common but serious complications include pulmonary fibrosis and secondary malignancies. Less common but reversible toxicities include alopecia and skin rash.

11.2.3 Formulation and preparation

Reconstituted with sterile water for injection to yield a final concentration of 20 mg/ml as described in the package insert.

11.2.4 Stability and Storage

Vials are stored at room temperature. Following reconstitution as directed with sterile water for injection, cyclophosphamide is stable for 24 hours at room temperature or 6 days when kept at 2-8°C. Once diluted for administration, cyclophosphamide will be provided an expiration date based on the standards set by the NIH CC Pharmacy Department.

11.2.5 Administration procedures

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

11.3 **FLUDARABINE**

Source

Fludarabine monophosphate will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a white, lyophilized powder. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH. Fludarabine is stored at room temperature.

11.3.1 Toxicity

Fludarabine toxicities include myelosuppression (dose limiting toxicity), fever, nausea, vomiting, stomatitis, diarrhea, gastrointestinal bleeding, anorexia, edema, skin rashes, myalgia, headache,

agitation, hearing loss, transient episodes of somnolence and fatigue, auto-immune hemolytic anemia, auto-immune thrombocytopenia, paresthesias, peripheral neuropathy, renal, and pulmonary toxicity (interstitial pneumonitis). Severe fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status were encountered almost exclusively after very high doses of fludarabine monophosphate. Such toxicity has only rarely been demonstrated at the 25-30 mg/m2/day dosage of fludarabine. Very rarely described complications include transfusion-associated graft-versus-host disease, thrombotic thrombocytopenic purpura, and liver failure. Tumor lysis syndrome following fludarabine administration has been observed, especially in patients with advanced bulky disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed post-fludarabine, especially in heavily pre-treated individuals, and in individuals receiving fludarabine combined with other agent.

11.3.2 Formulation and preparation

FLUDARA IV should be prepared for parenteral use by aseptically adding Sterile Water for Injection, USP. When reconstituted with 2 ml of Sterile Water for Injection, each ml of the resulting solution will contain 25 mg of Fludarabine Phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7–8.5. Fludarabine will be diluted in 100 to 125ml of either 5% dextrose in water or 0.9% sodium chloride, and infused IV over 30 minutes.

11.3.3 Stability and Storage

Reconstituted FLUDARA IV should be stored in the refrigerator between 36 and 46 degrees F. Because reconstituted FLUDARA IV contains no antimicrobial preservative, care must be taken to assure the sterility of the prepared solution; for this reason, reconstituted FLUDARA IV should be used or discarded within 8 hours.

11.3.4 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).

11.3.5 Dose reduction for renal impairment

Note: in patients with a creatinine clearance calculated by the CKD-EPI equation less than 80 ml/minute/1.73 m^2 body surface area, the daily dose of fludarabine will be reduced by 20%.

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13 APPENDICES

13.1 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.

13.2 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
- CD19 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-CD19-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

1. 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
2. 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 findings, will be collected as Adverse Events and labs results.
- 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

13.3 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.[72]

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.

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. Patients at risk of syncope

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 90 mm Hg systolic blood pressure after anti-CD19 CAR T-cell infusion.
3. Heart rate greater than 100 beats per minute.

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.

11. 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 1500/microliter.
12. 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 and less than 100 mm Hg should receive a 1 L NS bolus.**
    - **Patients with a systolic blood pressure less than 85 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.

13. Patients receiving more than 1 fluid bolus for hypotension should have a stat EKG and troponin, and a cardiac echocardiogram within 24 hours of the second fluid bolus.
14. 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 4 hours.
- Oxygen requirement of more than a 4L standard nasal cannula
- Greater than grade 2 neurological toxicity

15. All patients transferred to the ICU for hypotension or tachycardia should have a stat EKG and a cardiac echocardiogram within 24 hours of the time of transfer.
16. Patients with hypotension not responding to IV fluid resuscitation should be started on norepinephrine at doses called for by standard ICU guidelines.
17. Patients should have a cardiac echocardiogram and an EKG within 12 hours of starting norepinephrine.
18. 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).
19. Anecdotal evidence suggests that the IL-6 receptor blocker tocilizumab can be an effective treatment for cytokine-release syndrome toxicities after CAR T-cell infusions. 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 4 mg/kg infused IV over 1 hour (dose should not exceed 800 mg).
    - Left ventricular ejection fraction less than 40% by echocardiogram
    - Creatinine greater than 2.5-fold higher than the most recent level prior to CAR T-cell infusion
    - Norepinephrine requirement at a dose greater than 2 μg/minute for 48 hours since the first administration of norepinephrine even if norepinephrine administration was not continuous.
    - Systolic blood pressure of 90 mm Hg cannot be maintained with norepinephrine.
    - Oxygen requirement 55% or greater fraction of inspired oxygen (FIO2) for more than 2 continuous hours.
    - Dyspnea that is severe enough to potentially require mechanical ventilation.
    - PTT or INR>2x upper limit of normal
    - Clinically-significant bleeding
    - Creatine kinase greater than 5x upper limit of normal for greater than 2 days

20. THERE IS NO EVIDENCE THAT TOCILIZUMAB HELPS NEUROLOGICAL TOXICITY, SO IT SHOULD NOT BE ADMINISTERED FOR THIS PURPOSE.

21. If no improvement in hypotension or tachycardia occurs within 6 hours of tocilizumab infusion, consider other agents such as methylprednisolone 1 to2 mg/kg every 12 hours or etanercept.
22. Avoid meperidine due to seizure risk.
23. All patients with grade 2 or greater neurological toxicities should get a neurology consult.
24. The following patients should receive dexamethasone 10 mg intravenously every 6 hours until the toxicities improve to Grade 1 or resolve or until at least 8 doses of dexamethasone have been given. Note: for seizures administer standard seizure therapies in addition to dexamethasone.
    1. Patients with Grade 3 or 4 neurological toxicities except that dexamethasone is not recommended for isolated Grade 3 headaches.
    2. Any generalized seizure

13.4 APPENDIX D

Clinical Staging and treatment of Acute GVHD [58,59]

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 [58,59]

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** Donor Cell Infusion (DCI) [57]. 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%).
   d) Progressive disease: Further progression of signs and symptoms of acute GVHD, and/or decline in performance status after the initiation of therapy.

13.5 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. 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	This prevents the cells from clumping in 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.	
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.	

Abbreviated Title: Anti-CD19 CAR PBL
CC Protocol Number: 09-C-0082 DD
IBC Number: RD-08-VII-10
OSP Number: 0809-940
NCT Number: NCT00924326
Version Date: August 23, 2018

PROTOCOL TITLE

An Assessment of the Safety and Feasibility of Administering T-Cells Expressing an Anti-CD19 Chimeric Antigen Receptor to Patients with B-Cell Lymphoma

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Investigational Agent:

Drug Name:	PG13-CD19-H3 (anti-CD19 CAR) retroviral vector-transduced autologous PBL
IND Number:	13871
Sponsor:	Center for Cancer Research
Manufacturer:	Surgery Branch Cell Production Facility

Commercial Agents: Cyclophosphamide and Fludarabine

PRÉCIS

Background:

- We have constructed a retroviral vector that encodes an anti-CD19 chimeric antigen receptor (CAR) that recognizes the CD19 antigen. This chimeric receptor also contains the signaling domains of CD28 and CD3-zeta. The retroviral vector can be used to mediate genetic transfer of this CAR to T-cells with high efficiency (> 50%) without the need to perform any selection.
- In co-cultures with CD19-expressing target cells, anti-CD19-CAR-transduced T-cells secreted significant amounts of IFN-γ and IL-2.
- We have developed a process for cryopreserving the cell product which may lead to the ability for this product to be manufactured at a central location and shipped to other institutions for treatment of a broader patient population.

Objective:

- Primary objective:
  - With the approval of Amendment S, to determine the safety and feasibility of the administration of cryopreserved anti-CD19-CAR engineered peripheral blood lymphocytes with a non-myeloablative conditioning regimen in patients with B-cell lymphomas.

Eligibility:

- Patients of 18 years of age or older must:
  - Have a CD19-expressing B-cell lymphoma
  - Be a non-responder to, or recurred after one or more standard chemotherapy-containing regimens for their malignancy
  - Currently require treatment due to progressive malignancy
  - Be deemed to be incurable by standard therapy
- Patients may not have:
  - A history of allogeneic stem cell transplantation
  - CNS disease

Design:

- PBMC obtained by leukapheresis (approximately 5.0×10^9 cells) will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin in order to stimulate T-cell proliferation.
- Transduction is initiated by exposure of approximately 1.0×10^8 to 5.0×10^8 cells to retroviral vector supernatant containing the anti-CD19 CAR.
- With the approval of Amendment S, patients will receive fludarabine and cyclophosphamide chemotherapy (NMA) for lymphodepletion, followed by cryopreserved anti-CD19-CAR-transduced T-cells.
- Patients will be followed until disease progression.
- Patients who have responded to treatment and then progress may receive one retreatment.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Prior to Amendment E

1.1.1.1 Primary Objectives

- Phases I and II:
  - Determine the safety of the administration of anti-CD19-CAR engineered peripheral blood lymphocytes and aldesleukin either with or without a non-myeloablative conditioning regimen in patients with B-cell malignancies.
- Phase II only:
  - Determine if lymphodepletion with fludarabine plus cyclophosphamide given prior to infusion of anti-CD19-CAR-transduced T-cells and aldesleukin can enhance persistence of the anti-CD19-CAR-transduced T-cells.

1.1.1.2 Secondary Objective

- Phases I and II:
  - Determine if the administration of anti-CD19-CAR engineered peripheral blood lymphocytes and aldesleukin either with or without the non-myeloablative conditioning regimen causes regression of B-cell malignancies.

1.1.2 With Approval of Amendments E and F

1.1.2.1 Primary Objective

- Determine the safety and feasibility of the administration of anti-CD19-CAR engineered peripheral blood lymphocytes and aldesleukin with a non-myeloablative conditioning regimen in patients with B-cell malignancies.

1.1.2.2 Secondary Objectives

- Determine the *in vivo* survival of the anti-CD19-CAR-transduced T-cells.
- Determine if the treatment regimen cause regression of B-cell malignancies.

1.1.3 With Approval of Amendment H (this study will no longer include aldesleukin)

1.1.3.1 Primary Objective

- Determine the safety and feasibility of the administration of anti-CD19-CAR engineered peripheral blood lymphocytes with a non-myeloablative conditioning regimen in patients with B-cell malignancies.

1.1.3.2 Secondary Objectives

- Determine the *in vivo* survival of the anti-CD19-CAR-transduced T-cells.
- Determine if the treatment regimen cause regression of B-cell malignancies.

1.1.4 With Approval of Amendment S (this study will include cryopreserved anti-CD19-CAR PBL)

1.1.4.1 Primary Objective

- Determine the safety and feasibility of the administration of cryopreserved anti-CD19-CAR engineered peripheral blood lymphocytes with a non-myeloablative conditioning regimen in patients with B-cell malignancies.

1.1.4.2 Secondary Objectives

- Determine the *in vivo* survival of the cryopreserved anti-CD19-CAR-transduced T-cells.
- Determine if the treatment regimen can cause regression of B-cell malignancies.

1.2 BACKGROUND AND RATIONALE

Adoptive transfer of tumor infiltrating lymphocytes (TIL) is an effective therapy for advanced melanoma[1]. Adoptive transfer of T-cells that were transduced with the genes of tumor-antigen-specific T-cell receptors caused regressions of melanoma in some patients[2]. Another approach to adoptive T-cell therapy is to engineer T-cells to express chimeric antigen receptors (CARs). CARs combine a single chain Fv (scFv) component that is derived from a monoclonal antibody with T-cell activation moieties[3, 4]. We have constructed and extensively tested an anti-CD19 CAR. T-cells that are transduced with gamma-retroviruses encoding the sequence of this CAR can specifically recognize and kill CD19-expressing target cells including primary chronic lymphocytic leukemia cells. These anti-CD19-CAR-transduced T-cells also produce the cytokines interferon-γ (INFγ) and IL-2 specifically in response to CD19. We propose to conduct a clinical trial in which gamma-retroviruses encoding the anti-CD19 CAR will be used to transduce T-cells from patients with advanced B-cell malignancies. Initially, the protocol will proceed in a phase I dose escalation design, with three cohorts of n=3 to determine the maximum tolerated dose. In phase I, patients will receive lymphodepleting chemotherapy consisting of cyclophosphamide and fludarabine, anti-CD19-CAR transduced T-cells, and high-dose aldesleukin. Once the MTD has been determined, the study then would proceed to the phase II portion. Patients will be randomized to two treatment arms. Patients assigned to treatment Arm 1 will receive fludarabine and cyclophosphamide chemotherapy in order to induce lymphocyte depletion prior to infusion of the anti-CD19-CAR-transduced T-cells, and high-dose aldesleukin. Patients assigned to treatment Arm 2 will receive anti-CD19-CAR transduced T-cells, and high-dose aldesleukin without chemotherapy. This trial design will allow us to rigorously assess the impact of prior lymphodepletion on the persistence of adoptively transferred T-cells in patients.

With the approval of Amendment S, the protocol will proceed in a phase I dose escalation design, with two cohorts of n=3, including a de-escalation cohort to determine the maximum tolerated dose. Patients will receive a lymphodepleting chemotherapy regimen consisting of cyclophosphamide and fludarabine followed by cryopreserved anti-CD19-CAR transduced T-cells.

With the approval of Amendment V, the protocol will proceed in a phase I dose escalation design, with one cohort of n=6 to determine the maximum tolerated dose. Patients will receive a lymphodepleting chemotherapy regimen consisting of cyclophosphamide and fludarabine followed by cryopreserved anti-CD19-CAR transduced T-cells. In this cohort, the dose of cyclophosphamide will be moderately increased to 500 mg/m^2.

Lymphodepletion has been proven in multiple murine models to dramatically increase the anti-malignancy activity of adoptively-transferred T-cells[5-7]. In addition, elimination of other host cells such as myeloid suppressor cells has also been shown to enhance anti-tumor immunity in animal models[8]. Human studies strongly suggest that chemotherapy administered before adoptive T-cell transfer can enhance the activity of adoptively-transferred T-cells[1].

1.2.1 Prior Surgery Branch Trials of Cell Transfer Therapy Using Heterogeneous TIL Plus High-Dose IL-2 Following Non-myeloablative but Lymphodepleting Chemotherapy

The NCI Surgery Branch has extensive experience at treating malignancy by adoptive transfer of autologous T-cells (**Table 1**). The best example of the ability of autologous T-cells to mediate regressions of human malignancy is the adoptive transfer of tumor infiltrating lymphocytes (TIL) for treatment of melanoma[1]. Prior to adoptive transfer of TIL, patients are lymphodepleted with the chemotherapy agents fludarabine and cyclophosphamide. Patients are lymphodepleted because in most murine models demonstrating the therapeutic effectiveness of adoptively transferred lymphocytes at mediating tumor regression, immunosuppression of the host prior to the adoptive transfer of lymphocytes was required[9]. Interestingly, a clinical trial to formally demonstrate the impact of immunosuppression on adoptively transferred T-cells in humans has not been reported. TIL were cultured from excised melanoma tumors. TIL were then expanded using the rapid expansion protocol (REP) in which TIL were cultured with OKT3, irradiated allogeneic feeder cells, and IL-2. These REPed TIL retained highly specific in vitro anti-tumor activity, often recognized several antigenic epitopes, and contained both CD8+ and CD4+ lymphocytes[1]. TIL were adoptively transferred by intravenous infusion. Patients subsequently received high-dose IL-2 and some received peptide immunization when the TIL reactivity was against known MART-1 and gp100 peptides. This regimen using REPed TIL in protocol 99-C-0158 resulted in objective cancer regressions in 51% of patients (18 of 35) with metastatic melanoma[10]. Some patients achieved a clonal repopulation of anti-tumor lymphocytes that exceeded 80% of their total circulating CD8+ lymphocytes that persisted for months after cell infusion in some cases[1]. A summary of the toxicities that occurred in the 35 patients treated with immunosuppression by fludarabine and cyclophosphamide, TIL infusion, and high-dose IL-2 is shown in **Table 2**. The combination of cyclophosphamide and fludarabine was myelosuppressive. Neutrophils nadired on day 10 after chemotherapy at 6/mm^3 and recovered to above 500/mm^3 on day 14 with support from filgrastim (G-CSF). Lymphocytes nadired at 6/mm^3 and recovered to above 500/mm^3. Platelets nadired at 5.5/mm^3 on day 8 and recovered to > 20,000/mm^3 on day 28. Patients were usually discharged between 2-3 weeks after the initiation of the chemotherapy. CD4 counts remained persistently low (below 200), which is a known side effect of immunosuppression from fludarabine.

1.2.2 Surgery Branch Trials of Cell Transfer Therapy Using Transduction of TCR Genes that Encode TCR that Recognize Tumor-Associated Antigens into Non-Reactive PBL

Transfer of antigen specific TCR genes to PBL has recently been described as a potential method of generating large numbers of reactive T-cells for anti-cancer therapy[11, 12]. Retroviral vector mediated gene transfer can be used to engineer human T-cells with high efficiency. In published work, the Surgery Branch was among the first to demonstrate that retroviral vector-mediated transfer of TCR genes could endow human PBL with anti-tumor reactivity[13]. In this study, PBL were engineered with a retroviral vector expressing a TCR gene derived from a MART-1

reactive CTL. These engineered cells reacted with MART-1 expressing cells in an HLA-A0201 restricted manner.

Based on this technology, the Surgery Branch currently has conducted five TCR gene transfer trials that have enrolled over 100 patients. In these protocols, patients with metastatic cancer who are HLA-A2 positive received a non-myeloablative but lymphodepleting preparative regimen consisting of cyclophosphamide and fludarabine, and then were treated with autologous peripheral blood lymphocytes or TIL that were genetically engineered to be reactive with melanoma tumor antigens gp100 or MART-1, or with p53. Following adoptive cell transfer, all patients received high-dose IL-2, and some patients received peptide vaccination. In four studies (04-C-0181, 04-C-0251, 07-C-0174, and 07-C-0175) patients with metastatic melanoma are being evaluated, and in one study (07-C-0003), patients with metastatic cancer whose tumors overexpress p53 are being evaluated. **Table 1** provides a summary of these studies.

To test the *in vivo* efficacy of the MART-1 TCR engineered T-cells in 04-C-0251, 17 HLA-A*0201 patients with progressive metastatic melanoma were selected for treatment[2]. All patients were refractory to prior therapy with IL-2. T-cell cultures from all 17 patients were biologically reactive, with specific secretion of interferon-γ following co-culture with MART-1 peptide pulsed T2 cells and melanoma cell lines expressing the MART-1 antigen. Gene transfer efficiencies in these lymphocytes ranged from 17% to 67% (mean value 42%). Patients received adoptive cell transfer (ACT) with MART-1 TCR transduced autologous PBL after lymphodepletion with fludarabine and cyclophosphamide. An initial cohort of three patients was treated with cells following an extended culture period of 19 days, at which point they had cell doubling times ranging from 8.7 to 11.9 days. In these patients, less than 10% of the transduced cells persisted across the time points tested during the first 30 days post-infusion and 2% or less persisted beyond 50 days. These first three patients showed no delay in the progression of disease. In an effort to administer gene-modified lymphocytes that were in their active growth phase, the culture conditions were modified to limit the *ex vivo* culture period to between 6 and 9 days after stimulation of cells with the anti-CD3 antibody OKT3. In a third cohort, larger numbers of actively dividing cells for ACT were generated by performing a rapid expansion protocol 8-9 days after the initial OKT3 stimulation. In contrast to the lack of cell persistence seen in Cohort 1, patients in cohorts 2 and 3 all exhibited persistence of the transduced cells at greater than 9% at one and four weeks post-treatment (range 9%-56%). All 13 patients examined had increased MART-1 tetramer-binding cells post-treatment and 11 of 14 had increased number of elispot positive cells[2].

Most importantly, four patients demonstrated a sustained objective regression of their metastatic melanoma assessed by standard RECIST criteria, two of the responding patients were reported in Morgan et al.[2] as patients 4 and 14 (**Figure 1**). In responding patients 4 and 14, gene marked cells in the circulation (assumed to be 1% of total body lymphocytes) expanded 1400 fold and 30 fold respectively compared to the infusion cell number. At one year post-infusion, both responding patients had sustained high levels (between 20%-70%) of circulating gene-transduced cells (**Figure 1E**). The transduced TCR included Vβ12, and these two patients displayed Vβ12 cells detectable by antibody staining between 12%-16% when followed out to >300 days post-treatment (**Figure 1F**). The responding patients 4 and 14 were also 2 of 4 patients who had greater than 1% circulating tetramer positive cells at greater than 15 days after cell infusion, and these patients demonstrated anti-TAA reactivity in *ex vivo* co-culture assays. On this clinical trial of TCR gene therapy, there were no toxicities in any patient attributed to the gene-marked cells.

Our two newest TCR studies investigate more potent TCRs which target gp100 and MART-1 and expand our studies to TCR-transduced TIL, in addition to PBL. The anti-gp100(154) TCR transduced PBL/TIL study (07-C-0174) has accrued 19 patients to date, all to the TCR transduced PBL arm. At this time, 17 patients have been evaluated for response with 3 patients experiencing PRs and 14 patients having progressive disease. Nineteen patients have been evaluated for toxicities, and most grade 3 and 4 toxicities are known toxicities of the research. Toxicities attributable to the cells include grade 1 and 2 rash, uveitis, dizziness and hearing loss. Two grade 3 toxicities (headache and vasovagal syncope) and one grade 4 event of thrombosis were unexpected events but unrelated to the research. No grade 5 events have been observed on this study. The anti-MART-1 F5 TCR transduced PBL/TIL study (07-C-0175) has accrued 22 patients to date (21 patients to the PBL arm and 1 patient to the TIL arm). At this time 20 patients have been evaluated for response with six patients experiencing a partial response. Twenty-two patients have been evaluated for toxicities, and most grade 3 and 4 toxicities are known toxicities of the research. Toxicities attributable to the cells included grade 1 and 2 rash, uveitis, dizziness, tinnitus, and hearing loss. Five grade 3 events of pain, two grade 3 neurologic toxicities (1 encephalopathy and 1 cerebrovascular ischemia) and 2 grade 4 toxicities (2 pulmonary embolisms) were unexpected events but unrelated to the research. Four grade 3 events of headache were also observed and two of these events were expected and possibly related to the research. No grade 5 events have been observed on this study.

1.2.3 Chimeric Antigen Receptors (CARs)

Targeting T-cells to tumors by transferring the genes for αβ T-cell receptors into the T-cells of cancer patients for use in adoptive transfer therapies is a promising approach to cancer immunotherapy. Another approach to adoptive T-cell therapy is to engineer T-cells to express chimeric antigen receptors (CARs). CARs are made up of a single chain variable fragment (scFv) coupled to signaling molecules that can activate the T-cells expressing the CAR[3, 4, 14]. The earliest work conducted with CARs was performed in the late 1980s[15, 16]. CARs have been designed to target many different tumor antigens and many important questions have been addressed using CARs in mouse models. Murine models have shown that syngeneic T-cells transduced with retroviruses encoding CARs protected mice from tumor challenges *in vivo*[17, 18]. Murine models indicated that including the signaling domain of CD28 in CARs enhanced tumor protection and persistence of CAR-transduced T-cells[18, 19]. Increasing the number of CAR-transduced T-cells administered to mice consistently enhanced tumor protection as well[18, 20, 21]. One murine study reported that administration of high-dose IL-2 enhanced tumor protection by CAR-transduced T-cells[21].

Our group has completed a phase I clinical trial in which ovarian carcinoma was treated with T-cells that were transduced with a CAR that was specific for the ovarian carcinoma-associated-antigen α-folate receptor (FR)[22]. This trial consisted of two cohorts. In Cohort 1, transduced T-cells that had been activated with OKT3 and transduced with an anti-FR CAR were adoptively transferred to patients. The patients then received a course of high-dose IL-2. In Cohort 2, PBMC of patients were stimulated with allogeneic PBMC and then transduced with the anti-FR CAR. The cells were adoptively transferred to the patients. Next, the patients received injections of allogeneic PBMC from the same donor used for the in vitro stimulations as a vaccine. High levels of T-cell transduction were not achieved in this study. No objective clinical responses were attained and the persistence of the transduced T-cells was generally limited to a few days[22]. Our currently proposed trial differs from this trial in many ways. First, we can achieve

much higher levels of T-cell transduction due to advances in gene transfer technology. Second, the CAR to be used in our proposed trial incorporates a CD28 signaling domain and the CD3-zeta signaling domain. In contrast the anti-FR CAR used in the earlier work did not contain a CD28 signaling domain and used an Fcγ signaling domain instead of the CD3-zeta moiety in our current receptor. The T-cell culture conditions used in the anti-FR CAR trial are significantly different than the T-cell culture conditions that we will use in our proposed trial of an anti-CD19 CAR. Finally, some of the patients in our proposed trial will be immunosuppressed prior to adoptive transfer of anti-CD19-CAR-transduced T-cells. In the earlier trial no immunosuppression was used.

Another group has reported preliminary results of a clinical trial of a CAR that is specific for carboxy-anhydrase-IX (CAIX)[23]. CAIX is expressed by renal cell carcinoma and on bile duct epithelium. The three patients treated on this trial all developed liver toxicity that was attributed to the anti-CAIX-CAR-transduced T-cells[23].

1.2.4 B-Cell Malignancies

Annually in the United States, approximately twenty-two thousand people die of B-cell malignancies[24]. B-cell malignancies have quite heterogeneous clinical manifestations and prognoses. Chronic lymphocytic leukemia (CLL) is a common disease that is incurable by chemotherapy[25]. Patients with CLL that is purine-refractory/resistant (defined as no response to a purine-based chemotherapy regimen or progression in 6 months or less after receiving such a regimen) have a median overall survival of 9-13 months[26]. Patients that have progressive disease greater than 6 months after receiving a purine-based regimen generally have a median overall survival of 3.5 years or less[27]. Another B-cell malignancy for which new therapies are urgently needed is mantle cell lymphoma. This disease is almost always incurable by chemotherapy and has an aggressive course that is characterized by short responses to chemotherapy[28]. Follicular lymphoma is a common lymphoma with an extremely variable course[29]. Patients with follicular lymphoma have a median survival of about 4.5 years after first relapse[30, 31]. Large cell lymphoma is often curable by chemotherapy, and autologous stem cell transplants are sometimes able to cure patients with relapsed large cell lymphoma[32]. However, patients with multiple relapsed large cell lymphoma have very poor prognoses and some of them are candidates for experimental therapies[33]. Allogeneic stem cell transplantation is a potentially curative treatment option for patients with advanced B-cell malignancies, but allogeneic stem cell transplantation generally has a treatment related mortality rate of 10-30%[34, 35]. Improved therapies for B-cell malignancies are clearly needed.

Our proposed trial makes use of fludarabine and cyclophosphamide to lymphodeplete the patients assigned to one of the two treatment arms. Because of the activity of fludarabine and cyclophosphamide against B-cell malignancies[26, 29, 32], we will not be able to determine whether tumor regressions that occur on this arm of the trial are due to the chemotherapy or to the adoptively transferred T-cells or a combination of both chemotherapy and T-cells. Our group has previously used IL-2 alone in the same dose and schedule proposed for this trial as a treatment for lymphoma[36]. No objective responses were observed in the eleven lymphoma patients treated with IL-2 alone[36]. Therefore, any responses that take place in the arm of our proposed trial in which patients do not receive chemotherapy can reasonably be attributed to the infused anti-CD19-CAR-transduced T-cells.

1.2.5 CD19

We have constructed a CAR that recognizes CD19. We chose to target CD19 because it is expressed on most malignant B-cells[37, 38], but the only normal cells that express CD19 are B-cells and perhaps follicular dendritic cells[38, 39]. Importantly, CD19 is not expressed on pluripotent hematopoietic stem cells[40]. While destruction of normal B-cells is a drawback to targeting CD19, several factors indicate that destruction of normal B-cells is tolerable. When patients receive the anti-CD20 monoclonal antibody rituximab, the number of normal B-cells is severely depressed for several months[41], yet patients that receive chemotherapy plus rituximab do not have an increased rate of infections when compared to patients that receive chemotherapy alone[42]. Finally, patients can be treated with intravenous infusions of IgG if necessary to increase IgG levels[43].

1.2.6 Development of an Anti-CD19 CAR for Clinical Adoptive T-Cell Transfer

The anti-CD19 CAR used in our work consists of three main components: the variable regions of the anti-CD19 monoclonal antibody FMC63[44], part of the CD28 costimulatory molecule, and the signaling domain of the CD3 zeta chain[45]. This receptor is referred to as FMC63-28. The DNA encoding this receptor was cloned into the MSGV1 retroviral vector backbone[2] to form the plasmid shown in **Figure 2A**. This plasmid is referred to as MSGV1-FMC63-28. For the remainder of this document the FMC63-28 receptor will be referred to as the anti-CD19 CAR.

We carried out extensive in vitro experiments to assess the suitability of the FMC63-28 anti-CD19 CAR for use in a clinical trial. In order to transduce T-cells with the anti-CD19 CAR, we first suspended normal peripheral blood mononuclear cells (PBMC) in IL-2-containing media and stimulated them with the anti-CD3 monoclonal antibody OKT3. Two days after the OKT3 stimulation was initiated, we spin-loaded gamma-retroviruses encoding the anti-CD19 receptor onto a retronectin-coated plate. The OKT3-activated PBMC were placed on the plate and cultured overnight. The next day, we transferred the OKT3-activated PBMC from the original virus-coated plate to a second plate that had been loaded with retroviruses encoding the anti-CD19 CAR in a manner identical to the first transduction, and the cells were cultured overnight again. Preliminary experiments using transiently produced retroviral supernatant for transductions showed that our FMC63-28 receptor functioned well in vitro; therefore, a stable producer cell clone that produces gibbon ape leukemia virus (GALV) pseudotyped retrovirus encoding the FMC63-28 anti-CD19 CAR was generated. We used supernatant from this clone for the rest of the experiments described in this document. Following transduction, the PBMC were almost 100% $CD3^+$ T-cells. Because the anti-CD19 CAR contains variable regions that were derived from a murine antibody, a goat-anti-mouse-Fab antibody can stain cells that express the anti-CD19 CAR. An example of staining of anti-CD19-transduced T-cells with the anti-Fab antibody and CD3 is shown in **Figure 2B**. In six separate transduction experiments, a mean of 57% of $CD3^+$ T-cells expressed the anti-CD19 CAR. These transduced cells produced interferon-γ (IFNγ) specifically in response to CD19-expressing target cell lines (**Figure 3**).

Next, we obtained PBMC from a patient with CLL that had previously been treated with fludarabine and rituximab. We stimulated these PBMC with OKT3 in IL-2-containing media and transduced them with retroviruses encoding the anti-CD19 CAR. We found that the T-cells from this patient proliferated well in response to OKT3 and that 54% of the $CD3^+$ cells expressed the anti-CD19 CAR. In addition, these transduced cells produced IFNγ in response to primary

allogeneic CLL cells, but not CD19-negative target cells and they killed primary allogeneic CLL cells in an in vitro cytotoxicity assay[46] (**Figure 4**).

In order to obtain the large number of T-cells necessary for clinical adoptive T-cell transfer, T-cells that have been stimulated with OKT3 and transduced are subjected to a second stimulation with OKT3 in the presence of allogeneic feeder cells in IL-2-containing media. This process is called a rapid expansion protocol (REP). The data presented in **Figure 5A** demonstrate that after being subjected to a REP, anti-CD19-CAR-transduced T-cells produce IFNγ in response to CD19-expressing target cells but not in response to CD19-negative cells. The cells can also kill primary CLL cells (**Figure 5B**). CD19-CAR-transduced T-cells produce IFNγ and IL-2 in response to target cells that were transduced with CD19, but not target cells that were transduced with the control antigen nerve growth factor receptor (NGFR) (**Figure 6**).

Like most T-cells, anti-CD19-CAR-transduced T-cells are dependent on exogenous IL-2 for survival in vitro (**Figure 7**). We have demonstrated IL-2-dependent in vitro survival of anti-CD19-CAR-transduced T-cells in many experiments. These results provide strong evidence that the transduced T-cells do not become immortalized.

1.2.7 Other Anti-CD19 CAR Clinical Trials

Other groups have constructed anti-CD19 CARs[20, 47]. Jensen and coworkers have completed a clinical trial of adoptive transfer of T-cells that were transfected with a plasmid encoding an anti-CD19 chimeric receptor[48]. The T-cells expressing the anti-CD19 receptor did not persist *in vivo* and did not cause objective remissions of lymphoma. Our proposed clinical trial differs from the completed trial of Jensen and coworkers in many ways. The most important difference is that our proposed trial will use a retroviral vector rather than plasmid transfection to transfer an anti-CD19 receptor to T-cells. Another potentially important difference is that the CAR we propose to utilize in our clinical trial contains the signaling component of CD28, but the receptor construct used by Jensen and coworkers did not include a CD28 moiety.

A second group is currently conducting a clinical trial of adoptive transfer of T-cells that have been transduced with retroviruses encoding an anti-CD19 receptor (NCI Clinical Trials PDQ, trial MSKCC trial #06-138, Principal Investigator: R. Brentjens). This trial is enrolling only CLL patients. In this trial, CAR-transduced T-cells are transferred to patients that have received either cyclophosphamide chemotherapy or no chemotherapy prior to cell transfer. Our proposed trial differs from the trial of Brentjens and coworkers in several ways. First, we plan to administer high-dose IL-2 after cell transfer. Second, we propose to randomize between administering fludarabine and cyclophosphamide chemotherapy prior to adoptive T-cell transfer or administering no chemotherapy prior to T-cell transfer. Finally, we will treat all CD19-expressing malignancies while Brentjens and coworkers are only treating CLL.

Malcolm Brenner, Helen Heslop, and coworkers are conducting a clinical trial in which anti-CD19-CAR-transduced T-cells are administered to patients with indolent B-cell malignancies (NCI Clinical Trials PDQ, trial #BCM-H-19384). In this trial, each patient is receiving a mix of T-cells in which some T-cells are transduced with a CAR that incorporates a CD28 moiety and other T-cells are transduced with a CAR lacking a CD28 moiety. The aim of the trial is to evaluate the importance of the CD28 moiety on T-cell persistence and function. Our proposed trial differs from this trial significantly. First, this trial uses no immunosuppression prior to cell infusion while our trial proposes to randomize between immunosuppression with fludarabine

plus cyclophosphamide versus no immunosuppression. Second Brenner, Heslop, and coworkers are not administering IL-2 as part of their trial.

With the approval of Amendment S, patients will receive a lymphodepleting chemotherapy regimen consisting of cyclophosphamide and fludarabine followed by cryopreserved anti-CD19-CAR transduced T-cells.

1.2.8 Rationale for Measuring Persistence of Adoptively Transferred T-Cells With or Without Lymphodepleting Chemotherapy Prior to Adoptive Transfer

We hypothesize that lymphodepletion with fludarabine and cyclophosphamide will enhance persistence of the adoptively transferred anti-CD19 T-cells. Murine studies demonstrate enhanced anti-tumor efficacy of transferred T-cells in lymphodepleted hosts compared to lymphoreplete hosts[49]. However, in previous nonrandomized Surgery Branch trials, melanoma patients were treated with tumor infiltrating lymphocytes (TIL) plus IL-2 preceded by either one dose of 25 mg/kg of cyclophosphamide or no chemotherapy[50]. There was no difference in the objective tumor response rates when patients that received chemotherapy prior to adoptive T-cell transfer were compared to patients that did not receive chemotherapy[50]. Our proposed trial will be the first randomized trial to evaluate the impact of immunosuppression on the persistence of adoptively transferred T-cells in humans. Persistence of adoptively transferred T-cells has been shown to be associated with anti-tumor efficacy[5]. An additional benefit of not treating some patients on this trial with chemotherapy is that the anti-malignancy effect of anti-CD19-CAR-transduced T-cells plus IL-2 can be evaluated without the confounding effect of chemotherapy

In the past few years a large amount of evidence has been accrued demonstrating that lymphodepletion prior to cell infusion is critical for the effectiveness of adoptive T-cell transfer[5, 49-51]. Much of this evidence has been elicited since this protocol was originally submitted. Therefore, starting with Amendment E, patients will no longer be treated with CD19-CAR engineered cells in the absence of lymphodepletion.

1.2.9 Safety Considerations

Several safety concerns regarding the infusion of large numbers of retrovirally modified tumor reactive T-cells have been addressed in our previous clinical studies. The non-myeloablative chemotherapy and the administration of high-dose IL-2 have expected toxicities discussed earlier. The immuno-myeloablative chemotherapy used in this protocol has been administered to over 100 patients and all have reconstituted their hematopoietic systems.

In other protocols we have administered over $3.0x10^{11}$ TIL with widely heterogeneous reactivity including CD4, CD8, and NK cells without difficulty. As discussed above, the expansion of tumor reactive cells is a desirable outcome following the infusion of antigen reactive T-cells. Some patients receiving gp100 or MART-1 reactive cells have developed vitiligo, uveitis, hearing loss and rash probably due to destruction of normal melanocytes though these toxicities have been manageable. In addition, two patients experienced vestibular dysfunction possibly due to the transduced cells. We do not believe the transfer of these gene modified cells has a significant risk for malignant transformation in this patient population. While the risk of insertional mutagenesis is a known possibility using retroviral vectors, this has only been observed in the setting of infants treated for XSCID using retroviral vector-mediated gene transfer into CD34+ bone marrow cells. In the case of retroviral vector-mediated gene transfer into mature T-cells, there has been no evidence of long-term toxicities associated with these

procedures since the first NCI sponsored gene transfer study in 1989. Although continued follow-up of all gene therapy patients will be required, data suggest that the introduction of retroviral vectors transduced into mature T-cells is a safe procedure. While we believe the risk of insertional mutagenesis is extremely low, the proposed protocol follows all current FDA guidelines regarding testing and follow up of patients receiving gene transduced cells.

As of April 2015, thirty-four patients have been enrolled on this study. This includes seven patients with CLL, four patients with follicular lymphoma, one patient with splenic marginal zone lymphoma, fifteen patients with diffuse large B-cell lymphoma, five patients with primary mediastinal B-cell lymphoma, one patient with non-Hodgkin lymphoma, and one with Mantle cell lymphoma. We have observed impressive clinical response rates of 70% in this study, which includes 12 patients with a partial response (PR) and 12 patients with a complete response (CR) to treatment. However, 11 patients on this study have experienced a dose limiting toxicity (DLT) resulting in the trial being placed twice on clinical hold by the FDA. Subsequently, the dose was reduced three times, IL-2 was eliminated, and the dose of cyclophosphamide was decreased by 50%. The most common toxicities observed were neurotoxicities (somnolence, aphasia, and tremors), hypotension and one incidence of renal failure. Analysis of blood samples on these patients has indicated the elevated levels of cytokines which are the likely cause of these events.

1.2.10 Cryopreservation of the Anti-CD19 CAR Transduced T-Cell Product

As noted above, we have seen substantial clinical activity in patients with B-cell lymphomas receiving these anti-CD19 gene transduced cells. The cells have most often been administered following approximately 10-12 days of growth and were given from freshly harvested cultures. This procedure involves multiple logistic problems especially if we are to develop a procedure that can be more widely disseminated. Difficulty in timing the lymphodepleting chemotherapy with completion of the culture and the need to ship cells to other institutions in subsequent protocols has led us to slightly alter the standard operating procedures for growth of cells. In the current protocol we have developed a protocol for developing cells following just six days of culture. The cells are then cryopreserved and immediately infused following thawing. We have done substantial comparability studies to show that the cells grown for six days and cryopreserved have the same phenotype and functional properties as cells that have grown for longer period of times and administered fresh. Thus, in the next cohort of patients we plan to use cells grown for six days in culture and then cryopreserved and thawed prior to administration.

In an effort to establish a more GMP-compliant cell production process, we developed a closed cell production resulting in a cryopreserved cell product at the end of 6 days. A schematic showing the process flow for the closed process is presented in **Figure 8**. Briefly, $1.0x10^9$ PBMC are stimulated in a bag with soluble OKT3 for 2 days. The stimulated PBMC (1X) are washed on a Sepax using the neat cell wash program and transferred to retronectin-coated bag containing gammaretroviral vector supernatant (1X) resulting in a final cell density of $2.0x10^8$ cells in 2X diluted vector supernatant. After an overnight incubation at 37oC, the cells are transferred to an expansion bag containing Optimizer medium and 2.5% TCSR at a density of $0.5x10^6$ cells/mL. The cells are expanded for 4 days, washed on the Sepax and then cryopreserved in bags at a concentration of $2.0x10^6$ CAR+ cells/kg in 50 mL of 50% Saline-2.5% HSA-50% Cryostor-10.

We conducted 5 engineering runs at scale using cells from lymphoma patients in parallel with our current open clinical cell production process in order to access the comparability of the closed process in terms of transduction efficiency, cell expansion and total cell number,

biological function as measured by IFNγ release following coculture with antigen-positive targets, and phenotype. The only significant difference detected between the two processes was the level of transduction. Cells transduced in the closed cell production process exhibited a significantly lower transduction efficiency as compared to the open system (**Figure 9**, 43.6 + 8.3% and 79.6 + 1.5%, n=5, respectively ($p<0.001$). We were able to achieve comparable total cell numbers (**Figure 10**, 14.5 + 4.5 x 108 and 19.6 + 13.6 x 108 total cells, respectively) as well as cell expansion (**Figure 11**, 8.2 + 2.1 and 11.4 + 5.0 fold expansion, respectively) using the 6 day cell production process. In addition, cells manufactured using the 6 day closed process are CD19-specific as evidenced by release of comparable levels of IFNγ when cocultured with CD19+ targets (Nalm6, Toledo) and not CD19- targets (K562-NGFR, CEM), (**Figure 12**). The last measure of comparability between cells generated using the 6 day closed process to those generate in our current open clinical process is based on T-cell phenotype (**Figure 13**). There was no significant difference in T-cell phenotype based on detection of CD45RA and CCR7 by FACS.

Having shown that the 6-day cell production process can yield a cell product that is comparable to our current 10-day open clinical production process, we next sought to determine what effect cryopreservation had on our T-cell product. To assess the effect of cryopreservation on the percentage of CAR+ T-cells, cell viability, expansion, function and phenotype (**Figure 17**), we conducted two additional engineering runs using cells from lymphoma patients. Where possible the 6-day cell product was evaluated pre- and post-thaw for a given parameter. In **Figure 14A**, we demonstrate that there is no significant difference in the percentage of CAR+ T-cells 3 days post-thaw suggesting that our cryopreservation protocol is not inherently detrimental to CAR expression. In addition, the CAR+ cells continued to show CD19-specific antigen recognition as measured by IFNγ release following coculture with CD19+ targets (**Figure 15A** and **Figure 15B**). The viability of the cells at thaw was 90 and 79%, respectively, for the two patients tested (**Figure 14B**). Interestingly, the viability of the cells dipped 24h post-thaw by an average of 14% (**Figure 16A**); however, the cells then quickly recovered by the next day and expanded 36- and 21-fold over a period of 7 days in the absence of an antigen-specific stimulation (**Figure 16B**). There we slight differences in T-cell phenotype following cryopreservation, but it is likely that these differences are not specific given that the FACS staining was done on different days. It is important to note that while the percentages of a particular T-cell subset might change for a given patient, there is no difference in the profile of the subsets for each patient. Thus, the cells appear comparable following cryopreservation and thaw as compared to the pre-cryopreserved cells.

As of April 1, 2014, twenty-six patients have been enrolled on this study; this includes seven patients with CLL, four patients with follicular lymphoma, one patient with splenic marginal zone lymphoma, and fourteen patients with diffuse large B-cell lymphoma. Of the twenty-six patients treated on this study, we have observed impressive clinical response rates of 77%, which include 10 patients with a partial response and 10 patients with a complete response to treatment.

The protocol was initially designed as a standard dose escalation study with the dose escalation based on our prior experience with cell therapy. Due to the dose limiting toxicities, we have modified the protocol accordingly; eliminating aldesleukin, reducing the cell dose, and decreasing the doses of both chemotherapy agents. Some of the patients treated in the first few groups had severe side effects, including difficulty breathing, inability to speak, confusion, tremors, and kidney damage.

In Cohort 1, eight patients were treated with high-dose chemotherapy (cyclophosphamide (60mg/kg x 2 days) and fludarabine (25mg/m^2 x 5 days)) followed by anti-CD19 CAR T-cells (between $4.0x10^8$ and $4.0x10^9$ cells) and high dose aldesleukin. Of these 8 patients, 7 are evaluable, one of these patients died on study due to complications related to treatment and H1N1 infection. Six of the seven evaluable patients have objective responses; 4 partial responders and 2 complete responders. Due to severe toxicities, this cohort was closed to evaluate the safety and toxicity of lower cell doses and no aldesleukin.

In Cohort 6, two patients were treated with a high-dose chemotherapy followed by anti-CD19 CAR+ cells ($0.5x10^7$ transduced cells/Kg) and no IL-2. Both patients have achieved a complete response (CR). This cohort was closed due to grade 3 neurological toxicities, so we added a dose de-escalation cohort to continue accrual.

In Cohort 8, five patients were treated with high-dose chemotherapy followed by anti-CD19 CAR+ cells ($2.5x10^6$ transduced cells/Kg) and no IL-2. One patient died due to disease and treatment (not evaluable). Three patients have achieved a complete response (CR) and one patient is a non-responder. This cohort was closed due to grade 4 neurological toxicities observed in two patients, so the dose of cyclophosphamide was reduced to 30mg/kg/day for 2 days and the dose of cells was reduced to $1.0x10^6$ transduced cells/kg.

In Cohort 9, six patients were treated with reduced cyclophosphamide dose (30mg/kg x 2 days) and fludarabine (25mg/m^2 x 5 days) followed by anti-CD19 CAR+ cells ($1.0x10^6$ transduced cells/Kg) and no IL-2. Three patients have achieved a complete response (CR) and two patients have achieved a partial response (PR). This cohort was closed due to grade 4 creatinine and neurological toxicities, therefore decreasing the total dose of cyclophosphamide by more than 50% and fludarabine by about 30%.

In Cohort 10, five patients were treated with reduced doses of cyclophosphamide (300mg/m^2 x 3 days) and fludarabine (30mg/m^2 x 3 days) followed by $1.0x10^6$ cells/kg and no aldesleukin. Four patients have confirmed partial responses (PR) and one patient is a non-responder. The majority of toxicities seen in this cohort were attributed to the chemotherapy preparative regimen and none of the toxicities attributed to the fresh cell product were dose limiting toxicities (DLTs).

With the approval of Amendment S, patients will receive Cryopreserved anti-CD19 CAR positive cells/kg followed by cyclophosphamide and fludarabine.

In Cohort 11, four patients were treated with the reduced doses of cyclophosphamide and fludarabine followed by Cryopreserved anti-CD19 CAR+ cells ($2.0x10^6$ transduced cells/kg) and no IL-2. Two patients are non-responders. Two patients have achieved partial responses.

In Cohort 12, one patient was treated with the reduced doses of cyclophosphamide and fludarabine followed by Cryopreserved anti-CD19 CAR+ cells ($6.0x10^6$ transduced cells /kg) and no IL-2. This patient is a non-responder.

In Cohort 13, one patient was treated with a moderately increased dose of cyclophosphamide and fludarabine followed by Cryopreserved anti-CD19 CAR+ cells ($2.0x10^6$ transduced cells /kg) and no IL-2. This patient has achieved a partial response (PR).

In Cohort 11 the 6-day product was cryopreserved in order to evaluate a process which could facilitate commercialization of the product by our CRADA partner. Four patients were treated in Cohort 11 and all but one patient incurred at least grade 2 toxicities. One patient was treated in

Cohort 12; this patient experienced grade 4 confusion requiring transfer to the ICU, one patient was treated in Cohort 13 and he experienced grade 3 confusion and aphasia. In a review of the data it appeared that patients who received fresh products where the cells had been in culture for 9-10 days (9-10 day product) incurred fewer toxicities and at a lesser grade than those patients who had received cryopreserved cells which had been in culture for 6 -8 days. In order to further explore the relationship between days in culture and toxicity, we are increasing the length of time in culture. In Cohort 14 the cells will be in culture for 9 days, which may be increased to 12 days depending upon the characteristics of the product. The product will continue to be cryopreserved and the dose escalation schema will continue as described in Section **3**. In addition, in order to establish a baseline which could aid in determining the cause of, or further define, these neurologic toxicities, patients enrolled in Cohort 14 may undergo lumbar puncture for CSF analysis by flow cytometry prior to treatment. Patients who have undergone LP following treatment per protocol have all shown CD19+ cells in their CSF however, it is not known to what degree these cells were present prior to treatment as the patients were all asymptomatic and had nothing remarkable seen on imaging, and thus did not meet the criteria for a lumbar puncture prior to treatment.

Note: The paragraphs highlighted in grey are no longer applicable after the approval of Amendment X.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have a CD19-expressing B-cell lymphoma. Patients with Diffuse large B-cell lymphoma, Primary Mediastinal B-cell lymphoma, and Diffuse large B-cell lymphoma transformed from follicular lymphoma must have measurable disease after at least two prior chemotherapy regimens one of which must have contained doxorubicin and rituximab.

2.1.1.2 Confirmation of diagnosis of B-cell malignancy and positivity for CD19 confirmed by the Laboratory of Pathology of the NCI. The choice of whether to use flow Cytometry or immunohistochemistry will be determined by what is the most easily available tissue sample in each patient. Immunohistochemistry will be used for lymph node biopsies, flow Cytometry will be used for peripheral blood, fine needle aspirates and bone marrow samples.

2.1.1.3 Patients must have indications for treatment for their B-cell malignancy at the time of enrollment on this trial.

2.1.1.4 Greater than or equal to 18 years of age and less than or equal to age 70.

2.1.1.5 Willing to sign a durable power of attorney.

2.1.1.6 Able to understand and sign the Informed Consent Document.

2.1.1.7 Clinical performance status of ECOG 0 or 1.

2.1.1.8 Life expectancy of greater than three months.

2.1.1.9 Patients of both genders must be willing to practice birth control from the time of enrollment on this study and for four months after treatment.

2.1.1.10 Women of child bearing potential must have a negative pregnancy test because of the potentially dangerous effects of the treatment on the fetus.

2.1.1.11 Serology

- 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 hepatitis B antigen and hepatitis C antibody unless antigen negative. If hepatitis C antibody test is positive, then patients must be tested for the presence of antigen by RT-PCR and be HCV RNA negative.

2.1.1.12 Hematology

- Absolute neutrophil count greater than or equal to 1000/mm^3 without the support of filgrastim.
- Platelet count greater than or equal to 50,000/mm^3.
- Hemoglobin greater than 8.0 g/dl.
- Lymphocyte count less than or equal to 4,000/ mm^3.

2.1.1.13 Chemistry

- Serum ALT/AST less or equal to 5 times the upper limit of normal.
- Serum creatinine less than or equal to 1.6 mg/dl.
- Total bilirubin less than or equal to 1.5 mg/dl, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dl.

2.1.1.14 More than three weeks must have elapsed since any prior systemic therapy at the time the patient receives the preparative regimen, and patients' toxicities must have recovered to a grade 1 or less (except for toxicities such as alopecia or vitiligo).

2.1.1.15 Normal cardiac ejection fraction and no evidence of pericardial effusion as determined by an echocardiogram.

2.1.2 Exclusion Criteria

2.1.2.1 Patients that require urgent therapy due to tumor mass effects such as bowel obstruction or blood vessel compression.

2.1.2.2 Patients that have active hemolytic anemia.

2.1.2.3 Patients with active brain metastases, or with a history of any CNS metastases or cerebrospinal fluid malignant cells. Note: Patients who are asymptomatic but are found to have malignant cells in the CSF on lumbar puncture prior to treatment will be considered eligible.

2.1.2.4 Women of child-bearing potential who are pregnant or breastfeeding because of the potentially dangerous effects of the treatment on the fetus or infant.

2.1.2.5 Active systemic infections, coagulation disorders or other major medical illnesses of the cardiovascular, respiratory or immune system, myocardial infarction, cardiac arrhythmias, obstructive or restrictive pulmonary disease.

2.1.2.6 Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).

2.1.2.7 Concurrent opportunistic infections (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who have decreased immune competence may be less responsive to the experimental treatment and more susceptible to its toxicities).

2.1.2.8 Concurrent systemic steroid therapy.

2.1.2.9 History of severe immediate hypersensitivity reaction to any of the agents used in this study.

2.1.2.10 History of allogeneic stem cell transplantation

2.1.2.11 Patients with cardiac atrial or cardiac ventricular lymphoma involvement.

2.2 SCREENING EVALUATION

2.2.1 Within 4 Weeks Prior to Starting the Chemotherapy Regimen

- Complete history and physical examination, including, weight and vital signs, noting in detail the exact size and location of any lesions that exist. (**Note:** patient history may be obtained within 8 weeks.)
- Chest x-ray
- EKG
- Baseline CT of the chest, abdomen and pelvis, PET scan, and brain MRI to evaluate the status of disease. Additional scans and x-rays may be performed if clinically indicated based on patients' signs and symptoms.
- HIV antibody titer and HbsAG determination, and anti HCV, (Note: may be performed within 3 months of the chemotherapy start date).
- Anti CMV antibody titer, HSV serology, and EBV panel (Note: patients who are known to be positive for any of the above do not need to be retested; may be performed within 3 months of chemotherapy start date)
- Patients with a LVEF of less than or equal to 55% will not proceed to treatment, (Note: may be performed within 8 weeks of treatment).
- CD19 staining of malignant cells by immunohistochemistry or flow cytometry (testing is permitted to be conducted at any time prior to this point).
- All patients must have a TBNK for Peripheral blood CD3 count and CD19#.
- Patients with a history of leptomeningeal disease, or signs/symptoms suggestive of leptomeningeal involvement, or with symptoms of central nervous system malignancy such as new onset severe headaches, neck stiffness, or any focal neurologic findings on physical exam will have lumbar puncture for examination of cerebral spinal fluid.

- Patients may undergo lumbar puncture (LP) for flow cytometry of the CSF in order to assess the presence of CD19 positive lymphocytes for potential correlation with neurologic toxicity. Patients who have no neurologic symptoms at the time of LP will be eligible for enrollment regardless of the results of the flow cytometry.

2.2.2 Within 14 Days Prior to Starting the Chemotherapy Regimen

- Chem 20: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid)
- Thyroid panel
- CBC with differential and platelet count
- PT/PTT
- Urinalysis and culture, if indicated

2.2.3 Within 7 Days Prior to Starting the Chemotherapy Regimen

- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential
- ECOG performance status of 0 or 1

2.3 PROTOCOL REGISTRATION

2.3.1 Prior to Registration for this Protocol

Patients will initially be registered on protocol 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols) prior to transduction of PBL cells (either fresh or cryopreserved samples), by the clinical fellow or research nurse. Once cells exceed the potency requirement and are projected to exceed the minimum number specified in the Certificate of Analysis (CoA), patients will sign the consent document for this protocol.

2.3.2 Registration Procedure

Authorized staff must register an eligible candidate with the NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the website (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to the NCI Central Registration Office at ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at the Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol. Verification of registration will be forwarded electronically via email to the research team. A recorder is available during non-working hours.

3 STUDY IMPLEMENTATION

Due to toxicities experienced during the dose escalation phase of this trial, the protocol has been amended several times to decrease the cell dose, eliminate the administration of IL-2, and administer reduced doses of cyclophosphamide and fludarabine. The description of these changes has been moved to **Appendix 2** and **Appendix 3**.

3.1 STUDY DESIGN – BEGINNING WITH AMENDMENT S

Patients with B-cell malignancies will receive a non-myeloablative conditioning regimen consisting of cyclophosphamide and fludarabine chemotherapy followed by cryopreserved anti-CD19 CAR transduced T-cells. Patients will receive one course of treatment. The start date of the course will be the start date of the chemotherapy; the end date will be the day of the first post-treatment evaluation. Patients may undergo a second treatment as described in Section **3.5**.

3.1.1 Treatment Phase: Cryopreserved PBL

Opened with Approval of Amendment S

PBMC will be obtained by leukapheresis (approximately $1.0x10^{10}$ cells). In most patients, whole PBMC will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin in order to stimulate T-cell growth. In some patients the peripheral blood might be contaminated with large numbers of CD19-expressing malignant cells. In patients with peripheral blood lymphocytes counts greater than 4000 cells per microliter, CD19-expressing cells will be depleted using the Miltenyi Biotec CliniMacs system with anti-CD19 microbeads and then CD19-depleted PBMC will be stimulated with OKT3. In all patients, 2 days after OKT3 stimulation transduction will be initiated by exposure of approximately $1.0x10^{7}$ to $5.0x10^{8}$ cells to supernatant containing the anti-CD19 CAR retroviral vector. These transduced cells will be expanded and tested for their anti-tumor activity. Successful TCR gene transfer will be determined by FACS analysis for the CAR and specific anti-tumor reactivity will be tested by cytokine release measured against CD19+ cell lines and CD19-negative cell lines. Successful CAR gene transfer for each transduced PBL population will be defined as >30% CAR positive cells and for biological activity, gamma-interferon secretion against CD19 targets must be at least 200 pg/mL. The cells administered vary depending on their growth characteristic. In prior protocols over $3.0x10^{11}$ T-cells have been safely infused to cancer patients.

Standard Operating Procedures for the cryopreservation and thawing processes will be submitted to BB-IND 13871.

With the approval of Amendment S patients will be lymphodepleted with reduced doses of fludarabine and cyclophosphamide followed by cryopreserved anti- CD19 CAR positive cells/kg (± 20%) as noted below.

With the approval of Amendment V, the dose of cyclophosphamide was increased from 300 mg/m^2/day x 3 days to 500 mg/m^2/day x 3 days. Patients will be lymphodepleted with a modest increase of cyclophosphamide and continue with the current dose of fludarabine, then followed by cryopreserved anti- CD19 CAR positive cells/kg (± 20%).

With the approval of Amendment X, the number of days in culture was increased to 9-12. Patients received the current regimen of cyclophosphamide and fludarabine followed by $2.0x10^{6}$ cryopreserved anti- CD19 CAR positive cells/kg (± 20%). With the approval of Amendment Z cells may be in culture for 6-12 days to allow for flexibility in producing a sufficient number of cells in the final product.

3.1.1.1 Dose Escalation

Note: The paragraphs highlighted in grey are no longer applicable after the approval of Amendment Z.

Beginning with Amendment X, Cohort 13 has been completed with one patient enrolled with no DLTs observed and Cohort 14 was opened.

Initially 6 patients will be enrolled in Cohort 14. If less than or equal to one DLT is seen in the first 6 patients enrolled in Cohort 14, the cohort will be expanded to a total of 12 patients to further characterize the safety of the maximum tolerated dose.

With the approval of Amendment Y, if 2 or more DLTs are encountered in Cohort 14, Cohort 11 will re-open and will be expanded to a maximum of 12 patients provided that less than 2 in 12 patients have a DLT in this cohort. If 2 or more DLTs are observed in Cohort 11, we will re-evaluate our strategy.

With the approval of Amendment Z, if ≤ 2 of 6 patients experience a DLT in Cohort 11 then the cohort will be expanded to a maximum of 18 patients provided that fewer than 4 in 18 patients have a DLT. If 4 or more DLTs are observed in 18 patients in Cohort 11, we will de-escalate to Cohort 11D. The same DLT criteria as described below will be used to define the MTD for Cohort 11D. Note: With Amendment Z, the toxicities experienced by patient #4 in Cohort 11, will no longer meet the criteria for DLT.

If 9 or greater of 18 patients in Cohort 11 experiences a PR or CR lasting more than 3 months this will be declared the MTD regimen. If fewer than 9 patients experience a PR and/or CR, then Cohort 14 will be re-opened and up to a total of 18 patients may be enrolled. The same DLT criteria as described below will be used to define the MTD.

The number of cryopreserved anti-CD19-transduced T-cells transferred for each cohort will be:

Cohort	**Treatment Regimen**		
	NMA Chemotherapy Regimen (as specified in protocol)	*Cell dose (Determined prior to cryopreservation)*	*Days in Culture*
11-Completed	Cytoxan 300mg/m^2 & Fludarabine 30mg/m^2	2.0x10^6 CAR positive cells/kg (± 20%)	6-12
11D- (De-escalation Cohort)	Cytoxan 300mg/m^2 & Fludarabine 30mg/m^2	1.0x10^6 CAR positive cells/kg (± 20%)	6-12
12-Closed	Yes	6.0x10^6 CAR positive cells/kg (± 20%)	6
13-Completed	Cytoxan 500mg/m^2 & Fludarabine 30mg/m^2	2.0x10^6 CAR positive cells/kg (± 20%)	6
14-Completed	Cytoxan 500mg/m^2 & Fludarabine 30mg/m^2	2.0x10^6 CAR positive cells/kg (± 20%)	6-12

The maximum tolerated cell dose is the highest dose at which ≤ 1 of 6 patients experienced a DLT or the highest dose level studied if DLTs are not observed at any of the dose levels.

Following administration of the cell product, neurological status will be closely monitored and urgently managed as described in Section **3.4.3**.

In addition, we will be evaluating stored serum samples in an effort to identify the particular cytokines which may be contributing to these toxicities.

3.1.1.2 Safety Assessment

Cytokine (IFN-gamma levels) and toxicity data must be analyzed prior to dose de-escalation. A one-week safety assessment period will follow regimen completion between the first patient in each cohort before a second patient will be accrued in the cohort.

3.1.2 Definition of Dose-Limiting Toxicity (DLT)

Dose-limiting toxicity is defined as follows:

- Grade 4 neutropenia lasting longer than 21 days from the day of cell transfer
- Grade 4 thrombocytopenia lasting longer than 35 days from the day of cell transfer
- All grade 3 toxicities lasting more than 3 days (including grade 3 hypotension requiring the use of pressors) and all grade 4 toxicities with the exception of:
    - Myelosuppression (includes bleeding in the setting of platelet count less than 50,000 per mcl and documented bacterial infections in the setting of neutropenia), defined as lymphopenia, decreased hemoglobin, neutropenia and thrombocytopenia" unless neutropenia and thrombocytopenia meet the DLT definition described above.
    - Expected chemotherapy toxicities as defined in Section **11**.
    - Grade 3 Fever
    - Immediate hypersensitivity reactions occurring within 2 hours of cell infusion (related to cell infusion) that are reversible to a grade 2 or less within 24 hours of cell administration with standard therapy.
    - Aphasia/dysphagia or confusion/cognitive disturbance which resolve to grade 1 or less within 2 weeks.

Note: Neurology consults and MRIs of the brain will be conducted on any subject experiencing any grade 3 or greater neurologic toxicity.

Dose Limiting Toxicities per Cohort as of 4/13/2015

Cohort	**Treatment Regimen**			
	NMA Chemotherapy Regimen	***Cell Dose***	***# of Patients per Cohort***	***# of DLTs per Cohort***
		Fresh Cells 10-25 Days in Culture		
1	Cytoxan 60mg/kg x 2 days & Fludarabine 25mg/m^2 x 5 days	$1.0x10^9$ – $1.0x10^{10}$ CAR transduced cells	8	3
6	Cytoxan 60mg/kg x 2 days & Fludarabine 25mg/m^2 x 5 days	$0.5x10^7$ CAR positive cells/kg (± 20%)	2	2
8	Cytoxan 60mg/kg x 2 days &	$2.5x10^6$ CAR positive	5	2

	Fludarabine 25mg/m^2 x 5 days	cells/kg (± 20%)		
9	Cytoxan 30mg/kg x 2 days & Fludarabine 25mg/m^2 x 5 days	1.0x10^6 CAR positive cells/kg (± 20%)	6	2
10	Cytoxan 300mg/m^2 & Fludarabine 30mg/m^2 x 3 days	1.0x10^6 CAR positive cells/kg (± 20%)	7	None
		Cryopreserved Cells 6-8 Days in Culture		
11	Cytoxan 300mg/m^2 & Fludarabine 30mg/m^2 x 3 days	2.0x10^6 CAR positive cells/kg (± 20%)	4	1
12	Cytoxan 300mg/m^2 & Fludarabine 30mg/m^2 x 3 days	6.0x10^6 CAR positive cells/kg (± 20%)	1	1
13	Cytoxan 500mg/m^2 & Fludarabine 30mg/m^2 x 3 days	2.0x10^6 CAR positive cells/kg (± 20%)	2	1
14	Cytoxan 500mg/m^2 & Fludarabine 30mg/m^2 x 3 days	2.0x10^6 CAR positive cells/kg (± 20%)	2	2

3.2 PROTOCOL STOPPING RULES

Note: The paragraphs highlighted in grey are no longer applicable after the approval of Amendment S.

The study will be halted pending discussions with the FDA and IRB if the following conditions are met:

Prior to Amendment E

- If two DLTs occur in the first cohort of the Phase I portion of this study.

With approval of Amendment E (not applicable with approval of Amendment F):

- If two DLTs occur in the first 6 patients treated on this study.
- Two or more patients develop a grade 3 or greater toxicity at any point in the study not attributable to the chemotherapy preparative regimen (or circumstances unrelated to this study).
- If 1 of the first 3 patients (OR 2 of the first 6 patients, OR 3 of the first 9 patients, OR 4 of the first 12 patients) develop DLT due to autoimmune toxicity.
- Development of EBV lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV negative subject on this or any other SB adoptive cell therapy study. Accrual of EBV negative subjects will be halted only.

With Approval of Amendments F, H, and J

As stated above, the study will be halted pending discussions with the FDA and IRB if the following conditions are met:

- If cohorts 2-7 or 9 cannot be expanded due to DLTs.

- During the expansion phase, if at any time during accrual, a cumulative total of 3 evaluable patients have experienced a DLT.
- Two or more patients develop a grade 3 or greater toxicity at any point in the study not attributable to the chemotherapy preparative (or circumstances unrelated to this study).
- If 1 of the first 3 patients (OR 2 of the first 6 patients, OR 3 of the first 9 patients, OR 4 of the first 12 patients) in cohorts 2-5 develop DLT due to autoimmune toxicity.
- Development of EBV lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV negative subject on this or any other SB adoptive cell therapy study. Accrual of EBV negative subjects will be halted only.

With Approval of Amendment X

As stated above, the study will be halted pending discussions with the FDA and IRB if the following conditions are met:

- Two or more patients develop a grade 4 or greater toxicity at any point in the study not attributable to the chemotherapy preparative (or circumstances unrelated to this study). **NOTE:** Toxicities excluded from the determination of DLTs are not included
- If 1 of the first 3 patients (OR 2 of the first 6 patients, OR 3 of the first 9 patients, OR 4 of the first 12 patients) in cohorts 11-14 develop DLT due to autoimmune toxicity.
- Development of EBV lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV negative subject on this or any other SB adoptive cell therapy study. Accrual of EBV negative subjects will be halted only.
- If one or more treatment related deaths occur due to the cell infusion, we will promptly discuss this with the IRB and FDA.

3.3 DRUG ADMINISTRATION

Note: The following dose adjustments have been made over the course of the protocol:

- With approval of Amendment H, aldesleukin was removed.
- With the approval of Amendment M, the dose of cyclophosphamide was reduced from 60 mg/kg/day to 30 mg/kg/day.
- With the approval of Amendment P, the dose of cyclophosphamide was further reduced from 30 mg/kg/day x 2 days to 300 mg/m2/day x 3 days and the dose of fludarabine was reduced from 25mg/m2/day x 5 days to 30 mg/m2 x 3 days – decreasing the total dose of cyclophosphamide by more than 50% and fludarabine by about 30%.
- With the approval of Amendment V, the dose of cyclophosphamide was increased from 300 mg/m^2/day x 3 days to 500 mg/m^2/day x 3 days.
- With the approval of Amendment Y, the dose of cyclophosphamide was reverted back to 300 mg/m2/day x 3 days.

3.3.1 Preparative Regimen with Cyclophosphamide and Fludarabine

Days -5 through -3

- 1.0L of 0.9% NaCl given at 500 mL/hour starting 2 hours prior to cyclophosphamide followed by:
- Cyclophosphamide 300mg/m^2 IV over 60 minutes followed by:
- Fludarabine 30mg/m^2 IV over 30 minutes followed by:
- An additional 1.0L 0f 0.9% NaCl given at 500 mL/hour.

Patients should be instructed to drink plenty of liquids during and for 24 hours following the chemotherapy [approximately 2 liters/24 hours]; hydration may be adjusted as clinically indicated.

3.3.2 Cell Infusion

Cells are delivered to the patient care unit by a staff member from the Tumor Immunology Cell Processing Laboratory. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), an 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 via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping. Note: with approval of amendments F-H, the dose of cells administered will be on a CAR positive cells/kg basis.

Aldesleukin will not be administered with approval of Amendment H.

Note: With the approval of Amendment S, the dose of cells administered will be cryopreserved anti-CD19 CAR positive cells/kg (±20%).

Day 0 (2-4 days after the last dose of fludarabine)

- Cells will be thawed in the TIL lab and infused intravenously (i.v.) on the Patient Care Unit over 20 to 30 minutes.

Day 1-4 (Day 0 is the day of cell infusion): Beginning with Amendment P

- 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 if the absolute neutrophil count is less than 650/microliter. Filgrastim will be discontinued as soon as the neutrophil count is 2000/microliter or higher

3.3.3 Study Calendar

Day	**-9**	**-8**	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Therapy														
Cyclophosphamide					X	X	X							
Fludarabine					X	X	X							
Cryopreserved Anti-CD19 CAR PBL[1]										X[1]				

Filgrastim[2]											X	X	X	X
Allopurinol[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
TMP/SMX[3] 160mg/800mg (example)			X	X	X	X	X	X	X	X	X	X	X	X
Fluconazole[4] (400 mg po)										X	X	X	X	X
Valacyclovir po or Acyclovir IV[5]										X	X	X	X	X

[1]Two to four days after the last dose of fludarabine

[2] 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 if the absolute neutrophil count is less than 650/microliter. Filgrastim will be discontinued as soon as the neutrophil count is 2000/microliter or higher.

[3]The TMP/SMX schedule should be adjusted to QD three times per week (Monday, Wednesday, Friday) and continue for at least six months and until CD4 > 200 X 2

[4]Continue until ANC > 1000/mm^3

[5]Continue for at least six months and until CD4 > 200 X 2.

[6]For patients at high risk of developing Tumor Lysis Syndrome only.

3.4 ON-STUDY EVALUATION

Note: Refer to Section **5** for research evaluations.

3.4.1 Prior to Starting the Preparative Regimen

- Apheresis as indicated
- Within 14 days prior to starting the preparative regimen, patients will have a complete blood count, serum chemistries performed including electrolytes, BUN, creatinine, and liver function tests. If any results are beyond the criteria established for eligibility, the patient will not proceed until the abnormalities can be resolved.

3.4.2 During the Preparative Regimen (Daily)

- Complete Blood Count
- Chem 20 equivalent: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO^2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid)
- Urinalysis

3.4.3 After Cell Infusion

- Vital signs, including neurological checks, will be monitored hourly (± 15 minutes) for four hours and then routinely (every 4-6 hours)). If the patient has been febrile (Temp 38.3 or more) at any time in the preceding 24 hours, vital signs will be monitored strictly every 4 hours.

- Neurological evaluations: If the patient experiences a grade 3 or greater neurological toxicity the following evaluations will be performed:
  - Neurology Consult - urgent
  - MRI of the Brain
  - Lumbar puncture to evaluate transduced cells in the CSF if the platelet count is greater than 50,000/mm^3. **Intrathecal dexamethasone 8mg will be administered at time of the LP, as clinically indicated.**
- Once total lymphocyte count is greater than 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly (while the patient is hospitalized). Please refer to Section **5** for additional post cell infusion evaluations.

3.4.4 During Hospitalization (Every 1-2 Days as Clinically Indicated)

- A review of systems and physical exam, including focused neurological examination
- CBC
- Chem 20 equivalent: Sodium (Na), Potassium (K), Chloride (Cl), Total CO^2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid
- Other tests will be performed as clinically indicated.

3.5 RETREATMENT

Prior to Amendment N

Patients will be evaluated 4 to 6 weeks after the initial treatment regimen (defined as the end of the last aldesleukin dose, with approval of Amendment H, defined as the end of cell infusion). If patients have a partial response to treatment and their disease subsequently progresses, they may be re-treated when progression based on the appropriate anti-malignancy response criteria (see Section **6.3**) is documented after evaluation by Principal Investigator with the same schedule that they had been given safely (grade 3 toxicity due to cell infusion which is reversible within 24 hours with supportive measures may be retreated, but patients who develop grade 4 toxicity due to cell infusion will not be retreated.) Patients must continue to meet the original eligibility criteria to be considered for retreatment. Retreatment will consist of the non-myeloablative chemotherapy regimen, cell infusion, and aldesleukin. With the approval of Amendment H, aldesleukin will not be included in the retreatment regimen. Toxicity related to cyclophosphamide or fludarabine should be stable and resolved to less than grade 1 prior to re-treatment. Re-treatment benefits and risks will be carefully explained to the patient. Patients must be reconsented prior to retreatment. Re-treatment would begin 6 to 8 weeks after the last aldesleukin dose. A maximum of one retreatment course may occur.

Following Amendment S

Patients who experience disease progression following a confirmed complete or partial response to treatment or who have residual disease at the second or subsequent assessments following the initial treatment may be re-treated. Patients will be retreated at the currently enrolling dose level, but it will not count towards establishing the MTD. Patients who develop grade 3 toxicity due to

cell infusion which is reversible within 24 hours with supportive measures may be retreated. Patients who develop grade 4 toxicity due to cell infusion will not be retreated. Patients must continue to meet the original eligibility criteria and be negative for HAMA (Biolegend human anti-mouse IgG ELISA) to be considered for retreatment. Retreatment will consist of the non-myeloablative chemotherapy regimen followed by cryopreserved cell infusion. Toxicity related to cyclophosphamide or fludarabine should be stable and resolved to less than grade 1 prior to retreatment. Retreatment benefits and risks will be carefully explained to the patient. A maximum of one retreatment course may occur.

3.6 POST-STUDY EVALUATION (FOLLOW-UP)

- All patients will return to the NIH Clinical Center for evaluation 6 weeks (± 2 weeks) following the administration of the cell product.
- Patients discharged with grade 3 or greater significant adverse events should be evaluated by referring physician within 2 weeks of discharge.

3.6.1 Time-Period of Evaluations

Patients who experience stable disease, a partial response, or a complete response or have unresolved toxicities will be evaluated as noted below:

- Week 12 (± 2 weeks)
- Every 3 months (± 1 month) x3
- Every 6 months (± 1 month) x2
- As per PI discretion for subsequent years

Note: Patients may be seen more frequently as clinically indicated.

3.6.2 Scheduled Evaluations

At each evaluation, patients will undergo:

- Physical examination
- Chem 20 equivalent: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO^2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid), Complete blood count
- Thyroid panel as clinically indicated
- TBNK
- Toxicity assessment, including a review of systems
- CT of the chest, abdomen and pelvis and PET scan. This end of course evaluation will be used to determine response of the malignancy to treatment. If clinically indicated, other scans or x-rays may be performed, e.g. brain MRI, bone scan.
- Visual symptoms will be evaluated and if changes have occurred from baseline, i.e. changes in visual acuity, an ophthalmologic consult will be performed.

- A 5-liter apheresis may be performed at the first follow up visit. If the patient is unable to undergo apheresis, approximately 96 mL of blood may be obtained. Subsequently, 60 mL of blood will be obtained at follow up visits (approximately monthly) for at least 3 months. Peripheral blood mononuclear cells will be cryopreserved so that immunologic testing may be performed.
- Detection of RCR and persistence of CAR gene transduced cells (Section **5.3**)
- Note: Long-term follow up of patients receiving gene transfer: Physical examinations will be performed and documented annually for 5 years following cell infusion to evaluate long-term safety. After 5 years, health status data will be obtained from surviving patients via telephone contact or mailed questionnaires. The long term follow up period for retroviral vectors is 15 years.
- Note: Patients who are unable or unwilling to return for follow up evaluations will be followed via phone or email contact. Patients may be asked to send laboratory, imaging and physician exam reports performed by their treating physician.

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF-STUDY CRITERIA

3.7.1 Criteria for Removal from Protocol Therapy

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

Patients will be taken off treatment (and followed until progression of disease) for the following:

- Completion of protocol therapy.
- Patient requests to be withdrawn from active therapy.
- Investigator discretion.
- Positive pregnancy test.
- Tumor lysis syndrome, defined as a 25% or greater increase in the pre-treatment values of serum phosphorous, potassium, and uric acid as well as a 25% or greater decrease in serum calcium in the setting of effective treatment of a patient with large tumor burden.

3.7.2 Off-Study Criteria

Patients will be taken off study for the following:

- Completed study follow-up period
- Participant requests to be withdrawn from study
- Progressive disease, unless the patient is eligible for a second treatment.
- Lost to follow-up
- Death

Note: Once a subject is taken off-study, no further data can be collected.

Note: Patients who are taken off-study for progressive disease or study closure may be followed on protocol 09-C-0161 (Follow-up Protocol for Subjects Previously Enrolled on NCI Surgery Branch Studies).

3.7.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office, ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 INFECTION PROPHYLAXIS

Note: Other anti-infective agents may be substituted at the discretion of the treating investigator.

4.1.1 Pneumocystis Jirovecii Pneumonia

Patients will receive the fixed combination of trimethoprim and sulfamethoxazole [SMX] as double strength (DS) tab (DS tabs = TMP 160 mg/tab, and SMX 800 mg/tab) PO daily three times a week on non-consecutive days, beginning between days -5 and -8.

Pentamidine will be substituted for TMP/SMX-DS in patients with sulfa allergies. It will be administered aerosolized at 300 mg per nebulizer within one week of chemotherapy start date and monthly thereafter.

4.1.2 Herpes Virus Prophylaxis

Patients will receive valacyclovir orally at a dose of 500 mg daily the day after chemotherapy ends, or acyclovir, 250 mg/m^2 IV every 12 hours if the patient is not able to take medication by mouth. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

Prophylaxis for pneumocystis and herpes will continue for 6 months post chemotherapy. If the CD4 count is less than 200 at 6 months post chemotherapy, prophylaxis will continue until the CD4 count is greater than 200 X 2.

4.1.3 Fungal Prophylaxis

Patients will start fluconazole 400 mg PO the day after chemotherapy concludes and continue until the absolute neutrophil count is greater than 1000/mm^3. The drug may be given IV at a dose of 400 mg in 0.9% sodium chloride USP daily in patients unable to take it orally.

4.1.4 Empiric Antibiotics

Patients will start on broad-spectrum antibiotics, either a 3rd or 4th generation cephalosporin or a quinolone for fever of 38.3^{o}C once or two temperatures of 38.0^{o}C or above at least one hour

apart, AND an ANC <500/mm^3. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications.

4.1.5 Prophylaxis and Treatment of Tumor Lysis Syndrome

Subjects deemed to be at high risk of tumor lysis syndrome should begin allopurinol at a dose of 200 mg PO every 8 hours. This should be started 2 days prior to the first dose of cyclophosphamide and continued until disease burden is reduced (e.g. peripheral blasts clear) or it is apparent that no tumor lysis has developed after 1 week of treatment.

4.2 BLOOD PRODUCT SUPPORT

Using daily CBCs as a guide, the patient will receive platelets and packed red blood cells (PRBCs) as needed. Attempts will be made to keep Hb > 8.0 gm/dL, and plts > 10,000/mm^3. All blood products with the exception of the stem cell product will be irradiated. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBCs and decrease the risk of CMV infection.

4.3 OTHER CONCOMITANT MEDICATIONS TO CONTROL SIDE EFFECTS

Concomitant medications to control side effects of therapy will be given. Meperidine (25-50 mg) will be given intravenously if severe chilling develops. Other supportive therapy will be given as required and may include acetaminophen (650 mg every 4 hours), indomethacin (50-75 mg every 6 hours) and ranitidine (150 mg every 12 hours). If patients require steroid therapy, they will be taken off treatment. Patients who require transfusions will receive irradiated blood products. Ondansetron 0.15 mg/kg/dose IV every 8 hours will be administered for nausea and vomiting. Additional antiemetics will be administered as needed for nausea and vomiting uncontrolled by ondansetron. Antibiotic coverage for central venous catheters may be provided at the discretion of the investigator.

5 BIOSPECIMEN COLLECTION

Blood and tissue are tracked at the patient level and can be linked to all protocols on which the patient has been enrolled. Samples will be used to support the specific objectives listed in the treatment protocol(s), e.g., immunologic monitoring, cytokine levels, persistence, as well as to support long term research efforts within the Surgery Branch and with collaborators as specified in protocol 03-C-0277.

5.1 RESEARCH EVALUATIONS

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.

5.1.1 Prior to Chemotherapy Administration

- 5 CPT tubes (8 mL each)
- 1 SST tube (8 mL)
- SST tube (4 mL) daily; starting day of chemotherapy

5.1.2 Prior to Cell Infusion (1-8 mL SST)

- Blood samples for analysis for detection of RCR by PCR.

- Blood samples for analysis of the cytokines IL-6, TNF-α, IFN-γ and GM-CSF.

5.1.3 Post-Cell Infusion Evaluations

Once total lymphocyte count is greater than 200/mm^3, the following samples will be drawn and sent to the TIL lab on Monday, Wednesday, and Friday x5 days, then weekly (while the patient is hospitalized):

- 5 CPT tubes (8 mL each)
- 1 SST tube (8 mL)

5.2 IMMUNOLOGICAL TESTING

- Apheresis may be performed, prior to and 4-6 weeks after the treatment. At other time points, patient peripheral blood lymphocytes (PBL) will be obtained from whole blood by purification using centrifugation on a Ficoll cushion.
- Lymphocytes will be tested directly and following in vitro culture. Direct immunological monitoring will consist of quantifying CD3+ T-cells that express the anti-CD19 CAR by FACS analysis using a goat-anti-mouse antibody as shown in **Figure 2**. *Ex vivo* immunological assays will consist of cytokine release by bulk PBL, intracellular cytokine staining as shown in **Figure 6**, and by other experimental studies such as cytolysis if sufficient cells are available. If cell numbers are limiting, preference will be given to the direct analysis of immunological activity. Immunological assays will be standardized by the inclusion of 1) pre-infusion PBMC and 2) an aliquot of the engineered PBL cryopreserved at the time of infusion. In general, differences of 2- to 3-fold in these assays are indicative of true biologic differences.

Note: The collection and analysis of research labs will be monitored by the TIL lab and not by the CCR contractor.

5.3 MONITORING GENE THERAPY TRIALS: PERSISTENCE AND RCR

- Immunological monitoring by using either anti-mouse Fab FACs staining to detect CARs on the surface of T-cells or a quantitative real-time PCR assay will be used to quantitate persistence of T-cells in the blood. Persistence will be determined at about 1 week, 4 weeks (± 2 weeks), 3 months (± 1 month), 6 months (± 1 month), and 12 months (± 1 month) after cell infusion.
- All patients will be co-enrolled on protocol 09-C-0161. Patients' blood samples will be obtained and undergo analysis for detection of RCR by PCR prior to cell infusion and RCR PCR will be performed at 3 and 6 months, and at one year post-cell administration. Blood samples will be archived annually thereafter if all previous testing has been negative with a brief clinical history. If a patient dies or develops neoplasms during this trial, efforts will be made to assay a biopsy sample for RCR. If any post-treatment samples are positive, further analysis of the RCR and more extensive patient follow-up will be undertaken, in consultation with the FDA. RCR PCR assays detect the GaLV envelop gene and are performed under contract by the Indiana University Vector Production Facility. The results of these tests are maintained by the contractor performing the RCR tests and by the Surgery Branch research team.

5.4 SAMPLE STORAGE, TRACKING, AND DISPOSITION

Blood and tissue collected during the course of this study will follow the Cell Tracking and Labeling System established by the Tumor Immunology Cell Processing Laboratory. The Cell Tracking and Labeling System is designed to unambiguously ensure that patient/data verification is consistent. The patients' cell samples (blood or tissue) are tracked by distinct identification labels that include a unique patient identifier and date of specimen collection. Cryopreserved blood and tissue samples also bear the date the sample was frozen. All cryopreserved samples are tracked for freezer location and storage criteria. All samples are stored in monitored freezers/refrigerators in 3NW Surgery Branch Laboratories at specified temperatures with alarm systems in place. Serum samples will be sent to the Clinical Pharmacology Program (CPP) for storage. Samples will be barcoded and stored on site or offsite at NCI Frederick Central Repository Services in Frederick, MD. Data is entered and stored securely in the Patient Sample Data Management System (PSDMS) utilized by the CPP, and data will be updated to the Surgery Branch central computer database weekly. All samples (blood or tissue) are entered into a central computer database with identification and storage location, and this database is backed up every night.

At the conclusion of this protocol, if additional studies are to be performed on any samples obtained during the conduct of this trial, a Request to Conduct Research for Stored Human Samples Specimens, or Data Collected in a Terminated IRB Protocol will be submitted. Otherwise, specimens will be disposed of in accordance with the environmental protection laws, regulations and guidelines of the Federal Government and the State of Maryland.

Any loss or unintentional destruction of the samples will be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

Note: Blood and tissue collected during the course of this study will be stored, tracked and disposed of as specified in protocol 03-C-0277.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) 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. Data will be entered into the NCI CCR C3D database.

All adverse events (AEs), 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 AEs until 30 days following the last dose of study therapy or until off-study, whichever comes first.

An abnormal laboratory value will be recorded in the database as an AE **only** 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.

All AEs must be recorded on the AE case report form unless otherwise noted below in Section **6.1.1**.

End of study procedures: Data will be stored according to HHS and FDA regulations, 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, the IRB will be notified.

6.1.1 Routine Adverse Event Recording

All adverse events will be recorded in the patient's medical record. Following registration through 30 days after the cell infusion adverse events will be reviewed by the research nurse and Principal Investigator and captured in the C3D database. All events occurring during the treatment phase of the study will be followed until resolution or stabilization. During the follow up period, only grade 3 and 4 and unexpected grade 2 events that are related to the treatment will be captured in C3D.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

De-identified human data generated for use in future and ongoing research will be shared through a NIH-funded or approved repository (ClinicalTrials.gov) and BTRIS. At the completion of data analysis, data will be submitted to ClinicalTrials.gov either before publication or at the time of publication or shortly thereafter. Data may also be used to support long term research efforts within the Surgery Branch and de-identified data may also be shared with collaborators as specified in protocol 03-C-0277.

6.2.2 Genomic Data Sharing Plan

All genomic analysis that requires compliance with the GDS policy is being performed on protocol 03-C-0277.

6.3 RESPONSE CRITERIA

6.3.1 Anti-Malignancy Response Criteria

6.3.1.1 Response Criteria for Lymphoma

Note: All responses must last for at least 4 weeks after treatment (Cheson et al. Revised Response Criteria for Malignant Lymphoma, Journal of Clinical Oncology 2007 [48]).

- Complete Remission (CR): CR requires all of the following:
    1. Complete disappearance of all detectable clinical evidence of disease and disease-related symptoms if present before therapy.
    2. Typically, FDG-avid lymphoma (large cell, mantle cell and follicular lymphomas are all typically FDG-avid): 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.
    3. 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.
    4. The spleen and/or liver, if considered to be enlarged before therapy on basis of physical exam or CT scan, must should be normal size on CT scan and not be palpable on physical examination and nodules thought to represent lymphoma must no longer be present.
    5. 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. The biopsy core sample must be a minimum of 20 mm in length.
- 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. Note: in patients with follicular lymphoma or

mantle-cell lymphoma, a PET scan is only indicated in patients with one or at most two residual masses that have regressed by 50% on CT scan.

- 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.

Flow cytometric, molecular or cytogenetic studies will not be used to determine response.

6.4 PERSISTENCE CRITERIA FOR ANTI-CD19 CAR TRANSDUCED T-CELLS

The absolute number of persisting anti-CD19-CAR-transduced T-cells will be calculated by multiplying the absolute peripheral blood lymphocyte (PBL) count by the percentage of PBL that express both CD3 and the anti-CD19 CAR. The percentage of PBL that express both CD3 and the anti-CD19 CAR will be determined by a flow cytometry assay that involves staining with anti-CD3 and a polyclonal goat anti-mouse antibody product that is specific for mouse IgG $F(ab')_2$ antibody fragments (Jackson ImmunoResearch).

This analysis will be performed at about 2 weeks, 4 weeks (± 2 weeks), 3 months (± 1 month), and 6 months (± 1 month) after cell infusion. The 4-week time point is the only primary analysis that will be used for the primary evaluation of the number of $CD3^+$ cells that express the anti-CD19 CAR.

6.5 TOXICITY CRITERIA

This study will utilize the CTCAE version 3.0 for toxicity and adverse event reporting. A copy of the CTCAE v3.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov).

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

7.1.2 Suspected Adverse Reaction

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

7.1.3 Unexpected Adverse Reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

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 drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- 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.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-Threatening Adverse Drug Experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

7.1.9 Non-Compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

1. Is unexpected in terms of nature, severity, or frequency in relation to
    a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
    b. the characteristics of the subject population being studied; **AND**
2. Is related or possibly related to participation in the research; **AND**
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 IRB AND CLINICAL DIRECTOR (CD) REPORTING

7.2.1 IRB and NCI CD Expedited Reporting of Unanticipated Problems, and Deaths

The Protocol PI will report on the NIH Problem Form to the IRB and the NCI Clinical Director:

- All deaths, except deaths due to progressive disease.
- All protocol deviations
- All unanticipated problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance.
3. A tabular summary of the following adverse events:
    - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
    - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;

- All Grade 5 events regardless of attribution;
- All serious events regardless of attribution.

Note: Grade 1 events are not required to be reported.

To ensure safety using this treatment, the Surgery Branch will review safety data on all protocols at the time of continuing review. Data will be presented for both the recent period and for the entire length of time the protocol has been open. The toxicity data for review will include all toxicities captured on the protocol and will be presented in individual tables as follows:

- all toxicities attributed to the cells
- all incidences of intubation including the duration of and reason for intubation

7.2.3 IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the IRB.

7.3 IND SPONSOR REPORTING CRITERIA

From the time the subject receives the investigational agent/intervention to 30 days following the last dose of study therapy, the investigator must immediately report to the sponsor, using the mandatory MedWatch Form FDA 3500A or equivalent, any serious adverse event, whether or not considered drug-related, including those listed in the protocol or Investigator's Brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. For serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention, only those events that have an attribution of at least possibly related to the agent/intervention will be reported.

- Death (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- Other serious adverse events as well as deaths due to progressive disease must be reported within one business day.

Events will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov and to the CCR PI and study coordinator.

7.3.1 Reporting Pregnancy

7.3.1.1 Maternal Exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agents (s) should be documented in box B5 of the MedWatch form "Describe Event or Problem".

Pregnancy itself is not regarded as an SAE. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)" under the Pregnancy, puerperium and perinatal conditions SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within 1 day, i.e., immediately, but no later than 24 hours of when he or she becomes aware of it.

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

7.3.1.2 Paternal Exposure

Male patients should refrain from fathering a child or donating sperm during the study and for (120 days) after the cell infusion.

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.

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 anti-CD19 CAR-transduced PBL 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 anti-CD19 CAR-transduced PBL, but are no 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 can be sent to the 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 each trial in progress or completed during the previous year. The summary is required to include the following information for each trial:

- 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 DATA AND SAFETY MONITORING PLAN

Careful evaluation to ascertain the toxicity, immunologic effects and anti-malignancy efficacy of cell infusions will be performed. Due to the nature of these studies, it is possible that expansion of specific T-cell clones will be observed as tumor reactive T-cell proliferate in response to tumor antigens. Therefore, care will be taken to track T-cell persistence both immunologically and molecularly according to plan specified in Section **6.4**.

The Principal Investigator will review all serious adverse events and will monitor the data and toxicities to identify trends monthly. The Principal Investigator will be responsible for revising the protocol as needed to maintain safety.

7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about enrollment 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. Adverse events will be reported as required above. Any safety concerns, new information that might

affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS.

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 Sponsor Monitoring Plan

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 study endpoints; adherence to the protocol, regulations, and SOPs; and human subject's 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.

7.5.3 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 STATISTICAL CONSIDERATIONS

Note: Paragraphs highlighted in grey below are no longer applicable after the approval of Amendment V.

The statistical section has been revised numerous times. Please refer to the bolded section below for the current statistical analysis for Amendment J. The primary endpoints of this trial are to determine safety and to determine whether lymphodepletion with fludarabine and cyclophosphamide enhances persistence of adoptively transferred, anti-CD19-CAR-transduced T-cells. The secondary endpoint is to determine the anti-malignancy effect of the T-cells

transduced with an anti-CD19 CAR in the patients that do not receive fludarabine and cyclophosphamide.

The initial portion of this protocol will be a phase I dose escalation design with three cohorts of a minimum of 3 patients per cohort. The number of anti-CD19-transduced T-cells transferred for each cohort will be:

Cohort 1	between	$1.0x10^9$ and $1.0x10^{10}$
Cohort 2	greater than	$1.0x10^{10}$ up to $3.0x10^{10}$
Cohort 3	greater than	$3.0x10^{10}$ up to $1.0x10^{11}$

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 assigned cohort, the patient will be enrolled in the appropriate cohort for the number of cells infused. Should a single patient experience a dose limiting toxicity at a particular dose level, three more patients would be treated at that dose to confirm that no greater than 1/6 patients have a DLT prior to proceeding to the next higher level. If a level with 2 or more DLTs in 3-6 patients has been identified, three 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 prior to starting the phase II portion of the trial.

In phase II of the trial patients will be randomized to two treatment arms. Patients assigned to one treatment arm will receive fludarabine and cyclophosphamide lymphodepleting chemotherapy prior to receiving the engineered PBL cells while patients assigned to the other treatment arm will not receive chemotherapy. One to four days after completion of chemotherapy, anti-CD19 CAR-transduced T-cells will be infused. After cell infusion, all patients will receive IV aldesleukin therapy (720,000 IU/kg every 8 hours for a maximum of 15 doses). The randomization performed during phase II of this trial will be stratified so that each arm will receive an equal number of patients with a total CD3 count greater than or equal to 500 cells/microliter, and each arm will receive an equal number of patients with a CD3 count less than 500 cells/microliter. Randomization will also be stratified so that an equal number of patients with circulating malignant cells (leukemia) will be entered on each arm of the phase II part of the trial. For this trial, a patient with circulating malignant cells (leukemia) will be defined as a patient with a pre-treatment peripheral blood lymphocyte count of greater than 4000 lymphocytes per microliter.

The degree of persistence of anti-CD19-CAR-transduced T-cells will be evaluated by a quantitative measure in all patients, and will be compared directly between the two arms in order to determine if the use of fludarabine and cyclophosphamide lymphodepleting chemotherapy prior to receiving the engineered PBL cells will enhance persistence. Persistence will be determined as described in Section **5.3**. The 4-week time point will be used for the primary evaluation of percentage of $CD3^+$ cells that express the anti-CD19 CAR. With 17 patients in each of the two randomized arms, there will be 80% power to identify a difference in the mean persistence parameter between the two arms which will be equal to one standard deviation of the values within each arm (effect size 1.0), using a 0.05 two-tailed two-sample t-test. In a previous trial of TCR gene transfer, in patients that received fludarabine and cyclophosphamide for lymphodepletion, 9-56% of transduced cells persisted at 1 to 4 weeks post-infusion. We anticipate that the persistence of cells will be decreased without the lymphodepleting

chemotherapy. In practice, if the persistence measure is not normally distributed in both arms ($p<0.05$ by a Shapiro-Wilks test in either arm) then a Wilcoxon rank sum test will be used.

Anti-malignancy effects will be measured by clinical response as noted in Section **6.3**, and will be reported using 95% confidence intervals, separately for CLL and for lymphoma. As there are no requirements for a particular number of each type of disease, the results will be interpreted cautiously in the context of a pilot study which may be used to guide determination of parameters for study in future protocols if warranted.

In order to complete enrollment to this protocol, up to 18 patients may be needed for the phase I portion of the trial, and an additional 34 for the phase II portion. Thus, at an accrual rate of 2 patients per month, up to 2.5 years may be required to enroll up to 52 patients onto this trial.

With Amendment E (closed with approval of Amendment F), the trial is being refocused to evaluate the safety and toxicity of the regimen studied at dose level 1, between $1.0x10^9$ and $1.0x10^{10}$ cells. This has been selected as the dose to use in all patients on this study as a result of the experience reported to date with this regimen. Under this Amendment, the study will initially enroll up to 6 evaluable patients who will receive this level of cells (4 patients have already been treated), and if no more than 1 of the 6 has a DLT, then accrual will continue up to a total of 18 evaluable patients, subject to not exceeding 3 patients in total with a DLT. If at any time during accrual, a cumulative total of 3 evaluable patients have experienced a DLT, then no further patients will be enrolled. The upper one sided 90% confidence interval bound on 3/18 is 0.334, which is marginally tolerable, while the upper one sided 90% confidence interval bound on 4/18 is 0.396. At earlier accrual points the upper 90% confidence interval bounds are higher: 3/15 has a bound of 0.393 and 4/15 has an upper 90% confidence interval bound of 0.464. Thus, 3/18 with a DLT is consistent with approximately 1/3 or fewer patients having a DLT, while having 3 patients with DLT occur at an earlier point, or having >3 patients with DLT is consistent with >1/3 of patients having a DLT. These latter two conditions would be considered consistent with excessive toxicity.

With approval of Amendment F, the trial is being refocused to evaluate the safety and toxicity of the regimen studied at lower escalating dose levels since 3 DLTs were observed at dose level 1 (between $1.0x10^9$ and $1.0x10^{10}$ cells). For Cohort 2, 3 patients will be treated with NMA, $0.5x10^7$ CAR positive cells/kg (± 20%) and high dose aldesleukin (720,000 IU/kg). This has been selected as the starting dose due to PK modeling of interferon gamma levels and toxicity data from the first 8 patients treated on this study. If one DLT is observed in a patient treated in Cohort 2, after analysis of the IFN-gamma cytokine data, 3 additional patients will be treated at this dose level ($0.5x10^7$ CAR positive cells/kg (± 20%) after receiving NMA followed by a reduced dose of aldesleukin (72,000 IU/kg every 8 hours for a maximum of 15 doses) (Cohort 3). This 10-fold reduction of aldesleukin was previously investigated in a study of patients with renal cancer. The incidence of grade 3 and 4 aldesleukin related toxicities was less in patients treated with 72,000 IU/kg every 8 hours than those treated with the higher dose of 720,000 IU/kg aldesleukin, every 8 hours (Yang, J.C., et al., J Clin Oncol. 2003, 21(16):3127-32).

If no DLTs are observed in the 3 patients in Cohort 2, after analysis of the IFN-gamma cytokine data, 3 additional patients will be treated with NMA, $1.0x10^7$ CAR positive cells/kg (± 20%) high dose aldesleukin (720,000 IU/kg) (Cohort 4). If one DLT is observed in a patient treated in Cohort 4, 3 additional patients will be treated at this dose level ($1.0x10^7$ CAR positive cells/kg (±

20%) after receiving NMA followed by a reduced dose of aldesleukin (72,000 IU/kg every 8 hours for a maximum of 15 doses) (Cohort 5).

Cohort	Treatment Regimen		
	NMA Chemotherapy Regimen (as specified in protocol)	*Cell Dose*	*IL-2 Dose*
1 (Closed with Amendment F)	Yes	between $1.0x10^9$ and $1.0x10^{10}$ cells	High dose (720,000 IU/kg)
2	Yes	$0.5x10^7$ CAR positive cells/kg (± 20%)	High dose (720,000 IU/kg)
3[1]	Yes	$0.5x10^7$ CAR positive cells/kg (± 20%)	Low dose (72,000 IU/kg)
4	Yes	$1.0x10^7$ CAR positive cells/kg (± 20%)	High dose (720,000 IU/kg)
5[2]	Yes	$1.0x10^7$ CAR positive cells/kg (± 20%)	Low dose (72,000 IU/kg)

[1] Cohort 3 will be skipped if there are no DLTs in Cohort 2.

[2] Cohort 5 will be skipped if there are no DLTs in Cohort 4.

The highest dose cohort evaluated with no DLTs in 3 patients (either 3, 4 or 5) will be expanded to a total of 18 evaluable patients, subject to not exceeding 3 patients in total with a DLT. If at any time during accrual, a cumulative total of 3 evaluable patients have experienced a DLT, then no further patients will be enrolled. The upper one sided 90% confidence interval bound on 3/18 is 0.334, which is marginally tolerable, while the upper one sided 90% confidence interval bound on 4/18 is 0.396. At earlier accrual points the upper 90% confidence interval bounds are higher: 3/15 has a bound of 0.393 and 4/15 has an upper 90% confidence interval bound of 0.464. Thus, 3/18 with a DLT is consistent with approximately 1/3 or fewer patients having a DLT, while having 3 patients with DLT occur at an earlier point, or having >3 patients with DLT is consistent with >1/3 of patients having a DLT. These latter two conditions would be considered consistent with excessive toxicity.

Accrual will be halted to the study if none of the cohorts can be expanded due to DLTs.

At the conclusion of the study, toxicities will be tabulated according to worst grade per patient of each type of toxicity, and the fraction experiencing a DLT will be reported, along with appropriate confidence intervals.

In order to complete enrollment to this protocol, up to 35 patients may be needed in total (including the 8 patients already accrued to this study, the 9 patients for the dose escalation under Amendment F, the 15 additional patients included in the expanded cohort described above, and 3 additional patients in case patients need to be replaced for any reason. Thus, at an accrual rate of 6-8 patients per year, up to 4 years may be required to enroll up to 35 patients onto this trial.

Following approval of Amendment H, only two dose cohorts will be open for accrual: cohorts 6 and 7. For Cohort 6, 3 patients will be treated with NMA, and $0.5x10^7$ CAR positive cells/kg (± 20%). If one DLT is observed in a patient treated in Cohort 6, after analysis of the IFN-gamma cytokine data, accrual will be halted pending discussions with the FDA and IRB. If no DLTs are observed in the 3 patients in Cohort 6, after analysis of the IFN-gamma cytokine data, 3 additional patients will be treated with NMA, and $1.0x10^7$ CAR positive cells/kg (± 20%) in Cohort 7. If one DLT is observed in a patient treated in Cohort 7, 3 additional patients will be treated in Cohort 6. If 1 of 6 patients in Cohort 6 experiences a DLT, after analysis of the IFN-gamma cytokine data, accrual will be halted pending discussions with the FDA and IRB.

The highest dose cohort evaluated with no DLTs in 3 or more patients will be expanded to a total of 18 evaluable patients, subject to not exceeding 3 patients in total with a DLT.

Accrual will be halted to the study if neither cohorts 6 nor 7 can be expanded

due to DLTs.

In November 2011, the first patient in Cohort 6 experienced a DLT of grade 3 confusion (possible aphasia), and grade 3 neuropathy (facial droop) possibly related to the cell therapy. The serum IFN-gamma levels were low in this patient (range 9-44 pg/mL) following treatment with cells. These events were discussed with the FDA, and the FDA indicated that we can treat an additional patient in Cohort 6 (with approval of Amendment I). If this additional patient does not experience at DLT, Cohort 6 will be expanded to a total of 6 patients. If any additional patients treated in Cohort 6 experiences a DLT, after analysis of the IFN-gamma cytokine data, accrual will be halted pending discussions with the FDA and IRB. If no additional DLTs are observed in the 6 patients in Cohort 6, after analysis of the IFN-gamma cytokine data, 3 patients will be treated with NMA, and $1.0x10^7$ CAR positive cells/kg (± 20%) in Cohort 7. If one DLT is observed in a patient treated in Cohort 7, 3 additional patients will be accrued to Cohort 7. If 2 DLTs occur in 6 or less patients in Cohort 7, no additional patients will be accrued at this dose level. The highest dose cohort evaluated with no DLTs in 3 or more patients, or 1 DLT in 6 patients will be expanded to a total of 18 evaluable patients, subject to not exceeding 3 patients in total with a DLT.

In February 2012, the second patient in Cohort 6 experienced DLTs. The SAEs consisted of grade 3 hypotension and grade 3 confusion (possibly aphasia) related to the cell therapy. These toxicities have resolved and the patient has been discharged. After discussions with the FDA, cohorts 6 and 7 were closed and two new dose de-escalation cohorts were opened with approval of Amendment J as follows:

Cohort	Treatment Regimen		
	NMA Chemotherapy Regimen (as specified in protocol)	*Cell Dose*	*IL-2 Dose*
8 (De-escalation Dose 1)	Yes	$2.5x10^6$ CAR positive cells/kg (± 20%)	None
9 (De-escalation Dose 2)	Yes	$1.0x10^6$ CAR positive cells/kg (± 20%)	None

Cohort 8 will initially accrue 3 patients. If no DLTs are observed in these 3 patients, three additional patients will be accrued. If DLTs are observed in ≤ 1 patient out of 6 patients treated,

this cohort will be expanded as described below. If a DLT is observed in one of the first 3 patients, after analysis of the cytokine data, 3 additional patients can be accrued to this cohort. If more than 1 out of 6 patients in this cohort experience a DLT, this cohort will be closed, and patients will be accrued to Cohort 9. Cohort 9 will proceed in a similar fashion as Cohort 8. If more than 1 out of 6 patients in this cohort experience a DLT, this cohort will be closed. Subsequent dose reductions will be discussed with the FDA prior to initiation.

The highest dose cohort evaluated with DLTs in ≤ 1 patient in 6 patients treated will be expanded to a total of 18 evaluable patients, subject to not exceeding 3 patients in total with a DLT. If at any time during accrual, a cumulative total of 3 evaluable patients have experienced a DLT, then no further patients will be enrolled. The upper one sided 90% confidence interval bound on 3/18 is 0.334, which is marginally tolerable, while the upper one sided 90% confidence interval bound on 4/18 is 0.396. At earlier accrual points the upper 90% confidence interval bounds are higher: 3/15 has a bound of 0.393 and 4/15 has an upper 90% confidence interval bound of 0.464. Thus, 3/18 with a DLT is consistent with approximately 1/3 or fewer patients having a DLT, while having 3 patients with DLT occur at an earlier point, or having >3 patients with DLT is consistent with >1/3 of patients having a DLT. These latter two conditions would be considered consistent with excessive toxicity.

After discussions with the FDA, Cohort 8 was closed with Amendment M and patients will be lymphodepleted with fludarabine and a reduced dose of cyclophosphamide – 30mg/kg/day for 2 days and will receive $1.0x10^6$ CAR positive cells/kg (± 20%) in Cohort 9.

Cohort 9 will initially accrue 3 patients. If no DLTs are observed in these 3 patients, three additional patients will be accrued. If DLTs are observed in ≤ 1 patient out of 6 patients treated, this cohort will be expanded as described below. If a DLT is observed in one of the first 3 patients, after analysis of the cytokine data, 3 additional patients can be accrued to this cohort. If more than 1 out of 6 patients in this cohort experience a DLT, this cohort will be closed. Subsequent dose reductions will be discussed with the FDA prior to initiation.

If ≤ 1 patient in 6 patients treated develops a DLT then the cohort will be expanded to a total of 18 evaluable patients, subject to not exceeding 3 patients in total with a DLT.

Starting with Amendment S

The primary endpoints of this trial are to determine the safety and feasibility of the administration of cryopreserved anti-CD19-CAR engineered peripheral blood lymphocytes following a non-myeloablative conditioning regimen in patients with B-cell malignancies. The secondary endpoints are to determine the *in vivo* survival of the cryopreserved anti-CD19-CAR-transduced T-cells and determine if the treatment regimen cause regression of B-cell malignancies.

Starting with Amendment S, Cohort 10 was closed and only two dose cohorts will be open, including a de-escalation cohort: cohorts 11, 11D, and 12.

Initially 3 patients will be enrolled in Cohort 11. If 1 patient experiences a DLT the cohort will be expanded to 6 patients, if no additional DLTs are seen, patients will enroll in Cohort 12. If less than or equal to one DLT is seen in the first 6 patients enrolled in Cohort 12, the cohort will be expanded to a total of 12 patients as long as less than 1/3 of patients have a DLT in this cohort in order to further characterize the safety of the maximum tolerated dose.

If 2 or more DLTs are encountered in Cohort 12, Cohort 11 will be expanded to a maximum of 12 patients provided that less than 1/3 of patients have a DLT in this cohort.

If more than one in six patients experiences a DLT in Cohort 11, patients will be enrolled in Cohort 11D. If less than or equal to one DLT is seen in the first 6 patients enrolled in Cohort 11D, the cohort will be expanded to a total of 12 patients as long as less than 1/3 of patients have a DLT in this cohort in order to further characterize the safety of the maximum tolerated dose.

Cohort	**Treatment Regimen**		
	NMA Chemotherapy Regimen (as specified in protocol)	*Cell Dose: Determined Prior to Cryopreservation*	*IL-2 Dose*
11	Yes	$2.0x10^6$ CAR positive cells/kg (± 20%)	None
11D (De-escalation Cohort)	Yes	$1.0x10^6$ CAR positive cells/kg (± 20%)	None
12	Yes	$6.0x10^6$ CAR positive cells/kg (± 20%)	None

With the approval of Amendment X, Cohort 13 was completed and Cohort 14 has been opened.

With the approval of Amendment Y, the dose de-escalation for Cohort 14 was corrected to state that Cohort 11 would be the de-escalation cohort. With the approval of Amendment Z, the Dose Escalation and DLT sections have been revised.

The primary endpoints of this trial are to determine the safety and feasibility of the administration of cryopreserved anti-CD19-CAR engineered peripheral blood lymphocytes following a non-myeloablative conditioning regimen in patients with B-cell malignancies. The secondary endpoints are to determine the *in vivo* survival of the cryopreserved anti-CD19-CAR-transduced T-cells and determine if the treatment regimen cause regression of B-cell malignancies.

Refer to Section **3.1.1** for additional details.

The maximum tolerated cell dose is the highest dose at which < =1 of 6 patients experienced a DLT or the highest dose level studied if DLTs are not observed at any of the dose levels.

At the conclusion of the study, toxicities will be tabulated according to worst grade per patient of each type of toxicity, and the fraction experiencing a DLT will be reported, along with appropriate confidence intervals.

In order to complete enrollment to this protocol, up to 72 patients may be needed. This includes 37 patients already accrued to this study, up to 14 additional patients for Cohort 11 (four patients are already enrolled in this cohort), and either up to 18 patients for Cohort 11D, or, an additional 16 patients for Cohort 14, and 3 additional patients in the event patients need to be replaced for any reason. Thus, at an accrual rate of 6-12 patients per year, up to 3 additional years may be required to enroll up to 72 patients onto this trial.

9 COLLABORATIVE AGREEMENTS

We have established a Cooperative Research and Development Agreement (CRADAs #02716 and #03168) with Kite Pharma, Inc., and will be sharing data with them.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

The patients to be entered in this protocol have B-cell malignancies which are refractory to standard therapy, and 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 drug metabolism or 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.

10.2 PARTICIPATION OF CHILDREN

The use of the non-myeloablative regimen in this protocol is a major procedure which entails serious discomforts and hazards for the patient, such that fatal complications are possible. It is therefore only appropriate to carry out this experimental procedure in the context of life threatening metastatic cancer. 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. Should results of this study indicate efficacy in treating B-cell malignancies, which is not responsive to other standard forms of therapy, future research can be conducted in the pediatric population to evaluate potential benefit in that patient population.

10.3 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 **10.5**), all 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. 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 MAS Policy 87-4 and OHSRP 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.

10.4 Evaluation of Benefits and Risks/Discomforts

The experimental treatment has a chance to provide clinical benefit though this is unknown. The risks in this treatment are detailed in Section **1.2.9**. The goal of this study is to improve upon the number of patients who may benefit from adoptive cell therapy by using patients' own transduced T-cells without the need to identify anti-tumor T-cells uniquely from each patient as was required in several prior protocols. The success of this effort cannot be predicted at this time. Because all patients in this protocol have B-cell malignancies and limited life expectancies the potential benefit is thought to outweigh the potential risks.

10.5 Risk/Benefit Analysis

Because all patients in this protocol have B-cell malignancies and limited life expectancies, the potential benefit is thought to outweigh the potential risks. The risk/benefit analysis for adults with the capacity to consent, as well as for adults who may become unable to provide consent, is greater than minimal risk with the prospect of direct benefit.

10.6 Consent Process and Documentation

Patients initially sign a consent document when they agree to have PBMC obtained for study and growth on protocol 03-C-0277. If the lymphocytes can be generated for infusion and the patient meets the thorough screening for eligibility, the patient, with family members or friends at the request of the patient, will be presented with a detailed description of the protocol treatment. The specific requirements, objectives, and potential advantages and disadvantages will be presented. The informed consent document is given to the patient, who is requested to review it and to ask questions prior to agreeing to participate in the treatment portion of this protocol. The patient is reassured that participation on trial is entirely voluntary and that he/she can withdraw or decide against treatment at any time without adverse consequences. The Principal Investigator, associate investigator, or clinical fellow is responsible for obtaining written consent from the patient.

10.6.1 Telephone Consent

The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

11 PHARMACEUTICAL INFORMATION

11.1 Investigational Regimen

11.1.1 Anti-CD19 CAR transduced PBL

The procedure for expanding the human PBL and the Certificate of Analysis are similar to those approved by the Food and Drug Administration, and used at the NCI in ongoing protocols

evaluating cell therapy in the Surgery Branch (most recently 07-C-0174, 07-C-0175, 08-C-0121, 08-C-0155, and 08-C-0162), and is included in **Appendix 1** and in the IND submission for these cells. The PBL will be transduced with retroviral supernatant containing the anti-CD19 CAR.

11.1.1.1 Retroviral Vector Containing the anti-CD19 CAR Gene

The retroviral vector supernatant (PG13-CD19-H3) encoding a chimeric antigen receptor (CAR) directed against the B-cell antigen, CD19, was prepared and preserved following cGMP conditions in the Surgery Branch Vector Production Facility (SBVPF). The retroviral vector utilizes the MSGV1 retroviral vector backbone and consists of 7026 bps including the 5' LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor sites, FMC63-based (anti-CD19 FMC63-28) CAR protein containing a signal peptide (human GM-CSF receptor), FMC63 light chain variable region (FMC63 VL), linker peptide, FMC63 heavy chain variable region (FMC63 VH), CD28 (hinge, transmembrane and cytoplasmic region), and TCR zeta (cytoplasmic region), followed by the murine stem cell virus 3'LTR. The physical titer will be determined by RNA dot blot according to sponsor certificate.

The supernate will be stored at SBPVF upon the completion of production at –80° C or shipped on dry ice and stored at Cryonix, Rockville, MD. Both storage facilities are equipped with around-the-clock temperature monitoring. Upon request, supernatant will be delivered on dry ice to be used in *in vitro* transductions of PBL. There will be no re-use of the same unit of supernate for different patients. Retroviral titer has been shown to be stable after immediate thawing and immediate administration (coating the tissue culture wells previously coated with Retronectin). 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.

11.1.1.2 OKT3

OKT3 will be obtained by the Surgery Branch Laboratory from commercial sources.

Formulation: Muromonab-CD3 (Ortho), NSC #618843, is provided as a sterile, clear, colorless solution at a concentration of 1 mg/mL in 5 mL ampoules. The solution may contain a few fine, translucent protein particles. The antibody is dissolved in a buffered solution at pH of 6.5 to 7.5. The solution contains 2.25 mg of monobasic sodium phosphate, 9 mg of dibasic sodium phosphate, 43 mg of sodium chloride and 1 mg of polysorbate 80 per 5 mL of water for injection.

Storage/Stability: Ampules should be stored in a refrigerator at 2-8° C. Solution should not be frozen or shaken. Each ampule bears an expiration date.

11.1.2 Fludarabine

(Please refer to package insert for complete product information)

Description: Fludarabine phosphate is a synthetic purine nucleoside that differs from physiologic nucleosides in that the sugar moiety is arabinose instead of ribose or deoxyribose. Fludarabine is a purine antagonist antimetabolite.

How Supplied: It will be purchased by the NIH Clinical Pharmacy Department from commercial sources. Fludarabine is supplied in a 50 mg vial as a fludarabine phosphate powder in the form of a white, lyophilized solid cake.

Stability: Following reconstitution with 2 mL of sterile water for injection to a concentration of 25 mg/mL, the solution has a pH of 7.7. The fludarabine powder is stable for at least 18 months at 2-8°C; when reconstituted, fludarabine is stable for at least 16 days at room temperature. Because no preservative is present, reconstituted fludarabine will typically be administered within 8 hours. Specialized references should be consulted for specific compatibility information. Fludarabine is dephosphorylated in serum, transported intracellularly and converted to the nucleotide fludarabine triphosphate; this 2-fluoro-ara-ATP molecule is thought to be required for the drug's cytotoxic effects. Fludarabine inhibits DNA polymerase, ribnucleotide reductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.

Storage: Intact vials should be stored refrigerated (2-8°C).

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). If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Appendix 4**.

Toxicities: At doses of 25 mg/m2/day for 5 days, the primary side effect is myelosuppression; however, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Serious opportunistic infections have occurred in CLL patients treated with fludarabine. Hemolytic anemia has been reported after one or more courses of fludarabine with or without a prior history of a positive Coomb's test; fatal hemolytic anemia has been reported. In addition, bone marrow fibrosis has been observed after fludarabine therapy. Other common adverse effects include malaise, fever, chills, fatigue, anorexia, nausea and vomiting, and weakness. Irreversible and potentially fatal central nervous system toxicity in the form of progressive encephalopathy, blindness, and coma is only rarely observed at the currently administered doses of fludarabine. More common neurologic side effects at the current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesia, visual or hearing disturbances, and sleep disorders. Adverse respiratory effects of fludarabine include cough, dyspnea, allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of CLL. Treatment on previous adoptive cell therapy protocols in the Surgery Branch have caused persistently low (below 200) CD4 counts, and one patient developed polyneuropathy manifested by vision blindness, and motor and sensory defects.

11.1.3 Cyclophosphamide

(Refer to FDA-approved package insert for complete product information)

Description: Cyclophosphamide is a nitrogen mustard-derivative alkylating agent. Following conversion to active metabolites in the liver, cyclophosphamide functions as an alkyating agent; the drug also possesses potent immunosuppressive activity. The serum half-life after IV administration ranges from 3-12 hours; the drug and/or its metabolites can be detected in the serum for up to 72 hours after administration.

How Supplied: Cyclophosphamide will be obtained from commercially available sources by the Clinical Center Pharmacy Department.

Stability: Following reconstitution as directed with sterile water for injection, cyclophosphamide is stable for 24 hours at room temperature or 6 days when kept at 2-8°C.

Administration: It will be diluted in 250 mL D5W and infused over one hour. The dose will be based on the patient's body weight. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Appendix 4**.

Toxicities: Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea and vomiting, rash and alopecia occur, especially after high-dose cyclophosphamide; diarrhea, hemorrhagic colitis, infertility, and mucosal and oral ulceration have been reported. Sterile hemorrhagic cystitis occurs in about 20% of patients; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Although the incidence of hemorrhagic cystitis associated with cyclophosphamide appears to be lower than that associated with ifosfamide, mesna (sodium 2-mercaptoethanesulfonate) has been used prophylactically as a uroprotective agent in patients receiving cyclophosphamide. Prophylactic mesna is not effective in preventing hemorrhagic cystitis in all patients. Patients who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in patients with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, patients should be watched closely for cyclophosphamide toxicity (due to allopurinol induction of hepatic microsomal enzymes). At high doses, cyclophosphamide can result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain without edema occurs. At high doses, cyclophosphamide can result in cardiotoxicity. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from a syndrome of acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis have occurred following IV administration. Mesna (sodium 2-mercaptoethanesulphonate; given by IV injection) is a synthetic sulfhydryl compound that can chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

11.2 SUPPORT MEDICATIONS

11.2.1 Mesna (Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex, NSC-113891)

(Please refer to the FDA-approved package insert for complete product information)

Description: Mesna will be obtained commercially by the Clinical Center Pharmacy Department and is supplied as a 100 mg/mL solution.

Storage: Intact ampoules are stored at room temperature.

Stability: Diluted solutions (1-20 mg/mL) are physically and chemically stable for at least 24 hours under refrigeration. Mesna is chemically stable at room temperature for 48-72 hours in D5W, 48-72 hour in D5W/0.45% NaCl, or 24 hours in 0.9% NaCl.

Administration: Dilute to concentrations less than or equal to 20 mg mesna/mL fluid in D5W or 0.9% NaCl and to be administered intravenously as a continuous infusion. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Appendix 4**.

Toxicities: Nausea, vomiting, and diarrhea.

11.2.2 Filgrastim (Granulocyte Colony-Stimulating Factor, G-CSF, Filgrastim, Neupogen)

Filgrastim will be obtained commercially by the Clinical Center Pharmacy Department and is supplied in 300 ug/mL and 480 ug/1.6 mL vials. G-CSF should be refrigerated and not allowed to freeze. The product bears the expiration date. The product should not be shaken. It is generally stable for at least 10 months when refrigerated. The appropriate dose is drawn up into a syringe. G-CSF will be given as a daily subcutaneous injection. The side effects of G-CSF are skin rash, myalgia and bone pain, an increase of preexisting inflammatory conditions, enlarged spleen with occasional associated low platelet counts, alopecia (with prolonged use) elevated blood chemistry levels.

11.2.3 Trimethoprim and Sulfamethoxazole Double Strength (TMP / SMX DS)

TMP/SMX DS will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of PCP pneumonia. The oral dose is 1 tablet PO daily three times a week (MUST be on non-consecutive days) beginning on day -7 and continuing for at least 6 months and until the CD4 count is greater than 200 on 2 consecutive lab studies. Like other sulfa drugs, TMP/SMX DS can cause allergies, fever, photosensitivity, nausea, and vomiting. Allergies typically develop as a widespread itchy red rash with fever eight to fourteen days after beginning the standard dose. Neutropenia, a reduction in the number of neutrophils, can also occur.

11.2.3.1 Aerosolized Pentamidine in Place of TMP/SMX DS

Patients with sulfa allergies will receive aerosolized Pentamidine 300 mg per nebulizer with one week prior to admission and continued monthly until the CD4 count is above 200 on two consecutive follow up lab studies and for at least 6 months post chemotherapy. Pentamidine Isethionate will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of PCP infections. It is supplied in 300 mg vials of lyophilized powder and will be administered via nebulizer. Toxicities reported with the use of Pentamidine include metallic taste, coughing, bronchospasm in heavy smokers and asthmatics; increased incidence of spontaneous pneumothorax in patients with previous PCP infection or pneumatoceles, or hypoglycemia.

11.2.4 Herpes Virus Prophylaxis

11.2.4.1 Valacyclovir (Valtrex)

Valacyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used orally to prevent the occurrence of herpes virus infections in patients with positive HSV serology. It is supplied in 500 mg tablets. Valcyclovir will be started the day after the last dose of fludarabine at a dose of 500 mg orally daily if the patient is able to tolerate oral intake. See package insert for dosing adjustments in patients with renal impairment. Common side effects include headache, upset stomach, nausea, vomiting, diarrhea or constipation. Rare serious side effects include hemolytic uremic syndrome and thrombotic thrombocytopenic purpura.

11.2.4.2 Acyclovir

Acyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of herpes virus infections in patients who cannot take oral medications. It is supplied as powder for injection in 500 mg/vials. Reconstitute

in 10 mL of sterile water for injection to a concentration of 50 mg/mL. Reconstituted solutions should be used within 12 hours. IV solutions should be diluted to a concentration of 7mg/mL or less and infused over 1 hour to avoid renal damage. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Stomach upset, headache or nausea, rash or hives; peripheral edema; pain, elevated liver function tests; and leukopenia, diarrhea, lymphadenopathy, myalgias, visual abnormalities and elevated creatinine have been reported. Hair loss from prolonged use has been reported. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

11.2.5 Fluconazole

Fluconazole will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prophylax against fungal infections. It is available in 200 mg tablets. It can cause headache, nausea, vomiting, diarrhea or abdominal pain, and liver damage which may be irreversible. It can cause rashes and itching, which in rare cases has caused Stevens Johnson Syndrome. It has several significant drug interactions. The package insert should be consulted prior to prescribing. For IV administration in patients who cannot tolerate the oral preparation, Fluconazole comes in 2 mg/mL solution for injection, and prepared according to Clinical Center Pharmacy standard procedures. It should be administered at a maximum IV rate of 200 mg/hr.

11.2.6 Ondansetron Hydrochloride

Ondansetron hydrochloride will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to control nausea and vomiting during the chemotherapy preparative regimen. It can cause headache, dizziness, myalgias, drowsiness, malaise, and weakness. Less common side effects include chest pain, hypotension, pruritis, constipation and urinary retention. Consult the package insert for specific dosing instructions.

11.2.7 Allopurinol

Allopurinol will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used as prophylaxis or treatment of patients with or at high risk for Tumor Lysis Syndrome. Dosage is approximately 100 mg/m^2/dose po TID (maximum dose 200 mg TID). The most common side effects include hypersensitivity, rash, nausea, vomiting, renal insufficiency, and hepatic dysfunction. Allopurinol should be stopped immediately if rash develops. Consult the package insert for a complete list of all side effects.

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13 TABLES, FIGURES, AND APPENDICES

13.1 TABLE 1: SURGERY BRANCH CELL THERAPY STUDIES

Data as of 09/30/2008

Study #/ Disease	Cellular Product Administered	Arms (n)	Chemotherapy, Cytokines, and Immunizations	# of Cells	Response	Reference
98-C-0095 in patients with metastatic melanoma	Cloned Peripheral Blood Lymphocytes (PBL)/ Tumor infiltrating lymphocytes (TIL)	1. Cloned PBL/TIL intravenously (12)	None	1.5 to $34.8x10^9$ cells	NR[d]	Dudley, ME, *et al.* 2001
		2. IV Cloned PBL/TIL intravenously (6[a])	SQ IL-2 (125,000 IU/kg/d X 12 d)			
		3. IV Cloned PBL/TIL intravenously (6[b])	HD IL-2 (720,000 IU/kg 3X/d to tolerance (max 12)			
		4. IV Cloned PBL/TIL (3[c])	Gp100:209-217(210M)			
99-C-0158 in patients with metastatic melanoma	Tumor infiltrating lymphocytes (TIL)	1. *In vitro* expanded cloned T-cells intravenously (3)	30 mg/kg cyclophosphamide X2 days, 25 mg/kg fludarabine X5 days	$0.9x10^9$ to $24.2x10^9$ cells (avg. $10.4x10^9$)	NR	Dudley, ME, *et al.* 2002
		2. *In vitro* expanded cloned T-cells intravenously (3)	60 mg/kg cyclophosphamide X2 days, 25 mg/kg fludarabine X5 days		NR	
		3. *In vitro* expanded cloned T-cells intravenously (3)	60 mg/kg cyclophosphamide X2 days, 25 mg/kg fludarabine X5 days plus IV IL-2 (72,000 IU/kg 3X/day X5 days)	$0.9x10^9$ to $24.2x10^9$ cells (avg. $10.4x10^9$)	NR	
		4. *In vitro* expanded cloned T-cells intravenously (6)	[60 mg/kg cyclophosphamide X2 days, 25 mg/kg fludarabine X5 days][g] plus IV IL-2 (720,000 IU/kg 3X/day to tolerance (max 12)		NR	
		5. Autologous tumor reactive REP'd[e] bulk TIL cells intravenously (35)	Chemotherapy[g] plus IV HD IL-2 [(720,000 IU/kg 3X/day to tolerance (max 15)][h] with or without immunization with gp100:209-217(210M) or MART-1:26-35(27L) in Montanide ISA-51™ QD X5, then Qwk X3.	1.1 to $16.0x10^{10}$ cells (avg. $6.3x10^{10}$)	18/35 (51%)[f] 3 CRs 15 PRs	Dudley, ME, *et al.* 2003, Dudley, ME, *et al.* 2005
		6. Autologous tumor reactive REP'd[e] bulk TIL cells intravenously (6)	Chemotherapy[g] plus Low Dose IL-2[k] with or without immunization with gp100:209-217(210M) peptide or MART-1:26-	$8.3x10^9$ to $52.9x10^9$ cells (avg. $33.7x10^9$)	3PR 2NR 1 TE[j]	

			35(27L) peptide in Montanide ISA-51™ QD X5, then Qwk X3.			
04-C-0181 in patients with metastatic melanoma	Gp100 TCR engineered T-cells (PBL or TIL)	1. Escalating doses of anti-gp100 TCR transduced PBL (3)	Chemotherapy[g] plus IV HD IL-2[h] with 1mg gp100:209-217(210M) in Montanide ISA-51™ QD X5, then Qwk X3	Up to $30x10^9$	NR	NA
		2. Anti-gp100 TCR CD8+ enriched PBL (8)	Chemotherapy[g] plus IV HD IL-2[h] with $6X10^9$ pfu rFgp100P209 IV; rFgp100P209 IV and IL-2 repeated 28 days later.	Up to $30x10^9$	NR	
		3. Escalating doses of anti-gp100 TCR transduced TIL (3)	Chemotherapy[g] plus IV HD IL-2[h] with $6X10^9$ pfu rFgp100P209 IV; rFgp100P209 IV and IL-2 repeated 28 days later.	Up to $30x10^9$	1 PR[i]	
04-C-0251 in patients with metastatic melanoma	MART-1 TCR engineered T-cells (PBL or TIL)	1. Escalating doses of anti-MART-1 TCR transduced PBL (18)	Chemotherapy[g] plus IV HD IL-2[h] with MART-1:26-35(27L) in Montanide ISA-51™ QD X5, then Qwk X3.	Up to $30x10^9$	2PRs[i]	Morgan, *et al.* Science, 2006 Oct 6;314(5796): 126-9
		2. Escalating doses of anti-MART-1 CD8+ TCR transduced PBL (6)	Chemotherapy[g] plus IV HD IL-2[h] with MART-1:27-35 in Montanide ISA-51™ QD X5, then Qwk X3	Up to $30x10^9$	1 PR[i]	
		3. Escalating doses of anti-MART-1 CD8+ TCR transduced PBL (8)	Chemotherapy[g] plus IV HD IL-2[h] with MART-1:26-35(27L) in Montanide ISA-51™ QD X5, then Qwk X3	Up to $30x10^9$	1 PR[i]	
		4. Escalating doses of anti-MART-1 TCR transduced TIL (3)	Chemotherapy[g] plus IV HD IL-2[h] with MART-1:26-35(27L) in Montanide ISA-51™ QD X5, then Qwk X3	Up to $30x10^9$	NR	
		5. Escalating doses of anti-MART-1 TCR transduced PBL plus 1200 TBI (4)	Chemotherapy[g] and 1200 TBI plus IV HD IL-2[h] with MART-1:26-35(27L) in Montanide ISA-51™ QD X5, then Qwk X3	Up to $30x10^9$	NR	
07-C-0003 in melanoma/ renal cell or other histologies	Anti-p53 TCR engineered T-cells (PBL)	1. Melanoma or renal cell cancer: Up to 50 X 10^9 cells (PBL) (2)	Chemotherapy[g] plus IV HD IL-2[h]	Up to $50x10^9$	NR	NA
		2. Other histologies: Up to 50 X 10^9 cells (PBL) (10)	Chemotherapy[g] plus IV HD IL-2[h]	Up to $50x10^9$	1 PR[i]	

07-C-0174 in patients with metastatic melanoma	Anti-gp100(154) TCR engineered T-cells (PBL)	Anti-gp100(154) TCR transduced PBL at a dose ranging from 0.5 X 10^9 cells up to 300 x10^9 (19)	Chemotherapy[g] plus IV HD IL-2[h]	1.7x10^9 to 110.0x10^9 cells (avg. 31.5x10^9)	3 PR 14 NR 2 TE[j]	NA
07-C-0175 in patients with metastatic melanoma	Anti-MART-1 F5 TCR engineered T-cells (PBL or TIL)	Anti-MART-1 F5 TCR transduced PBL at a dose ranging from 0.5 X 10^9 cells up to 300 x10^9 cells (21)	Chemotherapy[g] plus IV HD IL-2[h]	1.45x10^9 to 110.0x10^9 cells (avg. 34.2x10^9)	6 PR 12 NR 3 TE[j]	NA
		Anti-MART-1 F5 TCR transduced TIL at a dose ranging from 0.5 X 10^9 cells up to 300 x10^9 cells	Chemotherapy[g] plus IV HD IL-2[h]	84x10^9 cells	1 TE	NA
07-C-0176 in patients with metastatic melanoma	Young TIL	Young TIL at a dose ranging from 1.0 X 10^9 cells up to 300 x10^9 cells (26)	Chemotherapy[g] plus IV HD IL-2[h]	11.1x10^9 to 124x10^9 cells (avg. 55.6x10^9)	3 PR 19 NR 2 TE[j] 2 NT[j]	NA

[a] Previously treated with cells alone

[b] Five previously treated with cells alone, one new patient for first cell treatment

[c] All three patients had previously been treated with cell alone and cells with IL-2

[d] NR: No Response

[e] Rapid Expansion Protocol

[f] Responses defined: CR (complete response) is disappearance of all clinical evidence of disease; PR (partial response) defined as > 50% reduction in the sum of the products of the perpendicular diameters for at least one month and no increase in any lesion and no new lesions.

[g] Chemotherapy at maximum dose: 60 mg/kg cyclophosphamide X2 days, 25 mg/kg fludarabine X5 days

[h] High dose (HD) IL-2: 720,000 IU/kg 3X/day to tolerance (max 15)

[i] Responses defined according to RECIST criteria

[j] TE: Too early to evaluate, NT: not treated

[k] Low dose IL-2: 250,000 IU/kg subcutaneously daily for 5 days. After a two day rest, IL-2 will be administered at a dose of 125,000 IU/kg subcutaneously daily for 5 days for the next five weeks (2 days rest per week).

13.2 TABLE 2: TIME IN HOSPITAL AND NON-HEMATOLOGICAL GRADE 3 AND 4 TOXICITIES RELATED TO LYMPHODEPLETING CHEMOTHERAPY AND CELL TRANSFER

Attribute measured	Duration, Number or Type	Number of Patients (%)
Days in Hospital[1]	6-10	6 (17%)
	11-15	18 (51%)
	16-20	4 (11%)
	21-25	7 (20%)
pRBC Transfusions	0	2 (6%)
	1-5	18 (51%)
	6-10	13 (37%)
	11-15	2(6%)
Platelet Transfusions	0	6 (17%)
	1-5	21 (60%)
	6-10	5 (14%)
	11-15	2 (6%)
	16-20	1 (3%)
Autoimmunity	Uveitis	5 (14%)
	Vitiligo	13 (37%)
Opportunistic Infections	Herpes zoster	3 (9%)
	Pneumocystis pneumonia	2 (6%)
	EBV-B cell lymphoma	1 (3%)
	RSV pneumonia	1 (3%)
Other	Febrile neutropenia	13 (37%)
	Intubated for dyspnea	3 (9%)
	Cortical blindness	1 (3%)

[1]Measured from the day of cell administration to discharge

13.3 FIGURE 1: CANCER REGRESSION IN TWO PATIENTS

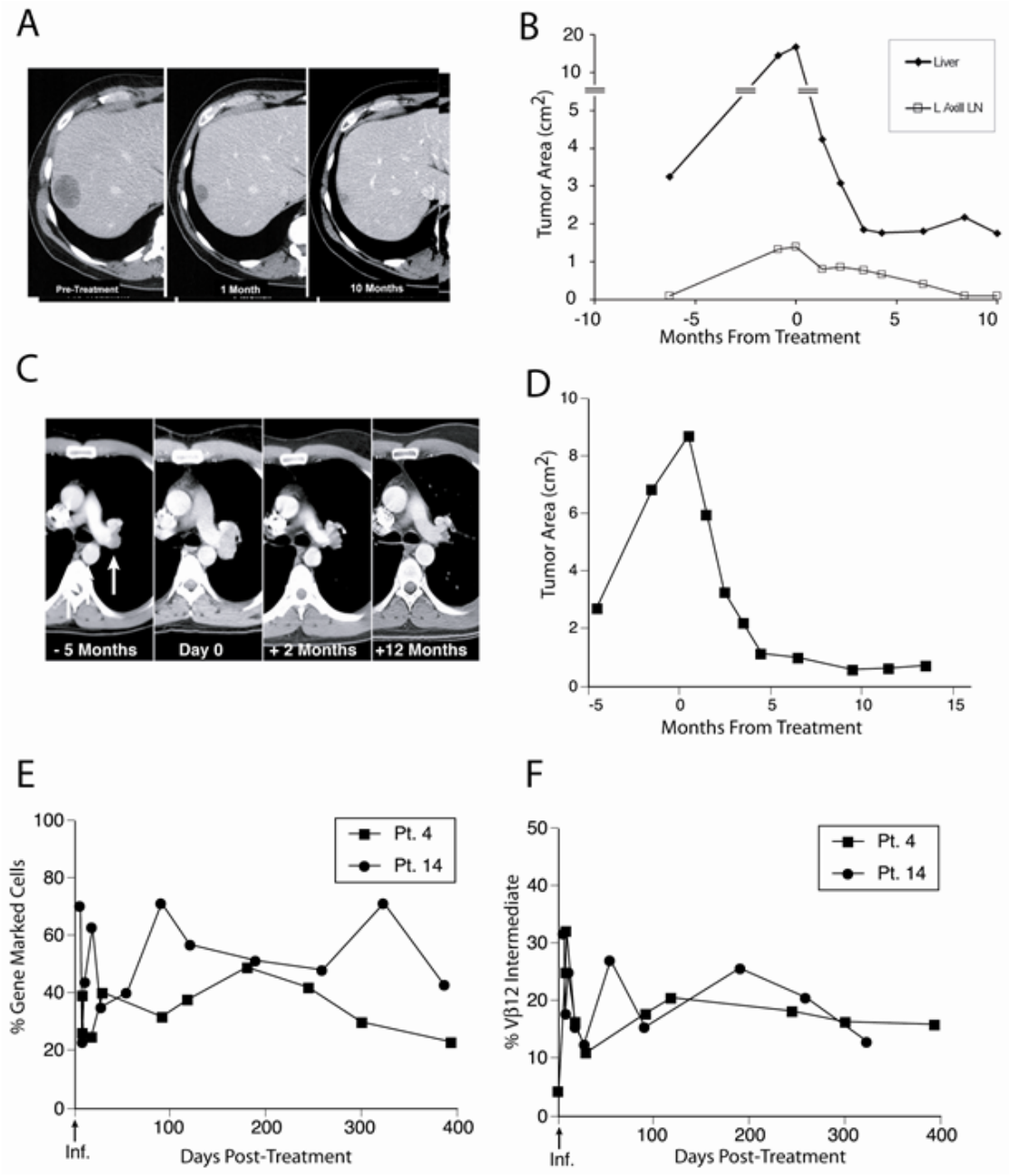

Figure 1. Cancer regression in two patients. **A.** CT images of patient 4 liver metastasis; pre-treatment, one month and 10 months post-treatment with TCR engineered T-cells. **B.** Size of liver and axillary tumors and tempo of regression of tumor sites in patient 4 (treatment time = day 0). **C.** CT images of patient 14 hilar lymph node metastasis; pre-treatment, beginning of treatment (Day 0), and two months and 12 months post-treatment. **D.** Size of tumor and tempo of regression in patient 14. **E.** Quantitation of gene marked cells in patients 4 and 14 PBMC was determined by real-time quantitative PCR. Day of infusion (Inf.) indicated by arrow. **F.** The percentage of CD8+/Vβ12 cells in the intermediate gate in the circulation of patients 4 and 14.

13.4 FIGURE 1: NORMAL PBMC CAN BE TRANSDUCED WITH AN ANTI-CD19 CAR

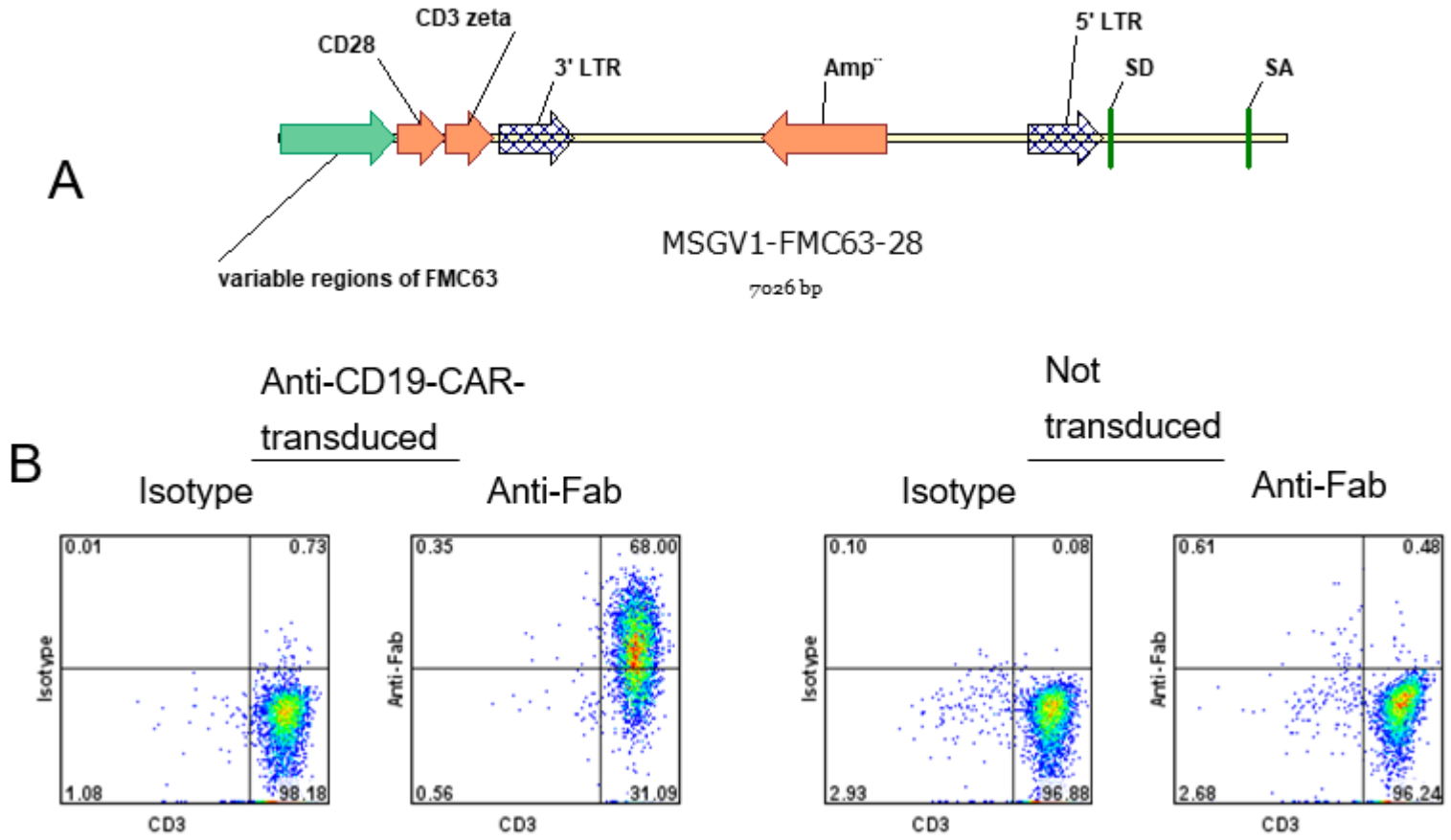

Figure 2. Normal PBMC can be transduced with an anti-CD19 CAR. A. Linearized plasmid map of the MSGV1-FMC63-28 gamma-retroviral vector that encodes the FMC63-28 anti-CD19 chimeric antigen receptor. **B.** Expression of the anti-CD19 chimeric receptor can be detected on the surface of transduced T-cells by staining with a goat-anti-mouse Fab antibody. Plots are gated on live lymphocytes.

13.5 FIGURE 2: PBMC TRANSDUCED WITH ANTI-CD19 CAR RECOGNIZE CD19-EXPRESSING TARGET CELLS

	CD19+ targets			CD19 negative targets					
Effector cells	SupB15	NALM6	Toledo	624	A549	TC71	CEM	K562	Effectors alone
Anti-CD19 CAR	**14800**	**15150**	**15150**	38	19	63	8	24	4
SP6 Control CAR	23	35	36	83	124	46	22	184	14
Donor 1 Nontransduced	13	54	39	7	5	7	16	8	3

Figure 3. PBMC that were transduced with an anti-CD19 CAR specifically recognize CD19-expressing target cells. 100,000 effector cells were cultured overnight with 100,000 target cells, and an interferon-γ ELISA was performed. All values are pg/mL of interferon-γ (mean of duplicate wells). Effector cells were either anti-CD19-CAR-transduced T-cells, T-cells transduced with the control CAR SP6, or non-transduced T-cells from the same patient stimulated with OKT3 in the same manner. SupB15 and NALM6 are CD19-expressing leukemia cell lines, Toledo is a CD19-expressing large cell lymphoma cell line. 624, A549, TC71, CEM and K562 are immortalized CD19 negative cell lines. The control CAR SP6 is specific for the chemical 2,4,6-TNP.

13.6 FIGURE 3: T-CELLS FROM A CLL PATIENT POST FLUDARABINE/RITUXIMAB THERAPY

A

Effector cells	CD19+ targets		CD19 negative targets			Effectors alone
	CLL1	CLL2	Allogeneic T cell	CEM	K562	
anti-CD19 CAR-transduced	**72,000**	**47,000**	1945	32	490	24
Nontransduced	132	345	305	36	52	18

B

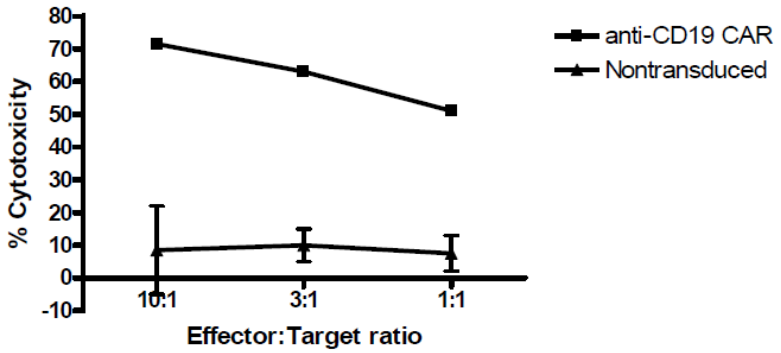

Figure 4. T-cells from a CLL patient post fludarabine/rituximab therapy can be transduced with an anti-CD19 CAR and then produce interferon-γ in response to a primary allogeneic CLL cells and kill primary allogeneic CLL cells. A. 100,000 effector cells were cultured overnight with 100,000 target cells, and an interferon-γ ELISA was performed. All values are pg/mL of interferon-γ (mean of duplicate wells). Effector cells were either anti-CD19-CAR-transduced T-cells from a CLL patient or non-transduced T-cells from the same patient stimulated with OKT3 in the same manner. CLL1 and CLL2 are primary CLL cells from two different patients. Allogeneic T-cell targets were 7 days post OKT3 stimulation. CEM and K562 are immortalized CD19- negative leukemia cell lines. **B.** The same effector cell cultures were tested for their ability to kill allogeneic primary CLL cells. Effector cells transduced with the anti-CD19 CAR killed the CLL cells, while non-transduced T-cells from the same donor that were cultured identically did not kill CLL cells.

13.7 FIGURE 4: ANTI-CD19-TRANSDUCED T-CELLS MAINTAIN FUNCTION FOLLOWING RAPID EXPANSION

A

	CD19+ targets			CD19 negative targets					
Effector cells	bv173	SupB15	CLL3	MDA231	624	TC71	A549	CEM	Effectors alone
Anti-CD19 CAR	**16070**	**7758**	**2321**	343	108	65	37	20	20
SP6 Control CAR	78	16	27	3044	342	60	198	15	10

B

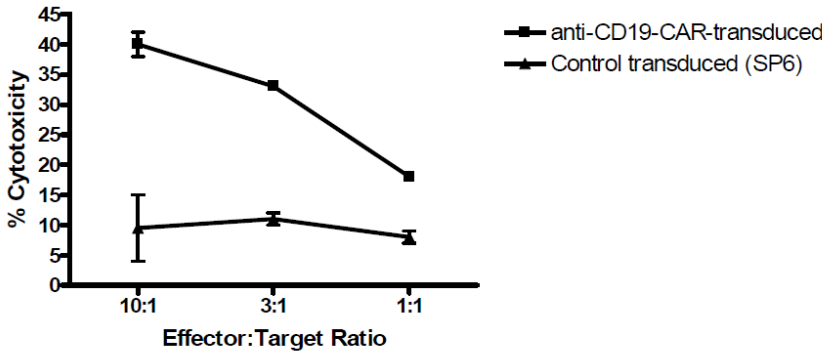

Figure 5. Following rapid expansion to a number of cells sufficient for clinical adoptive T-cell transfer, anti-CD19-transduced T-cells maintain function. A. 100,000 effector cells that were 9 days post REP initiation were cultured overnight with 100,000 target cells, and an interferon-γ ELISA was performed. All values are pg/mL of interferon-γ (mean of duplicate wells). Effector cells were either anti-CD19-CAR-transduced T-cells or T-cells transduced with the control CAR SP6 from the same patient stimulated with OKT3 in the same manner. SupB15 and bv173 are CD19-expressing leukemia cell lines, CLL3 are primary CLL cells. MDA231, A549, TC71, CEM and K562 are immortalized CD19 negative cell lines. The control CAR SP6 is specific for the chemical 2,4,6-TNP. **B** Twenty-one days after the initiation of rapid expansion, anti-CD19 CAR transduced T-cells can kill allogeneic primary CLL cells while T-cells transduced with the control CAR SP6 cannot.

13.8 FIGURE 5: T-CELLS PRODUCE IFN-GAMMA AND IL-2 IN RESPONSE TO CD-19-EXPRESSING TARGET CELLS FOLLOWING RAPID EXPANSION

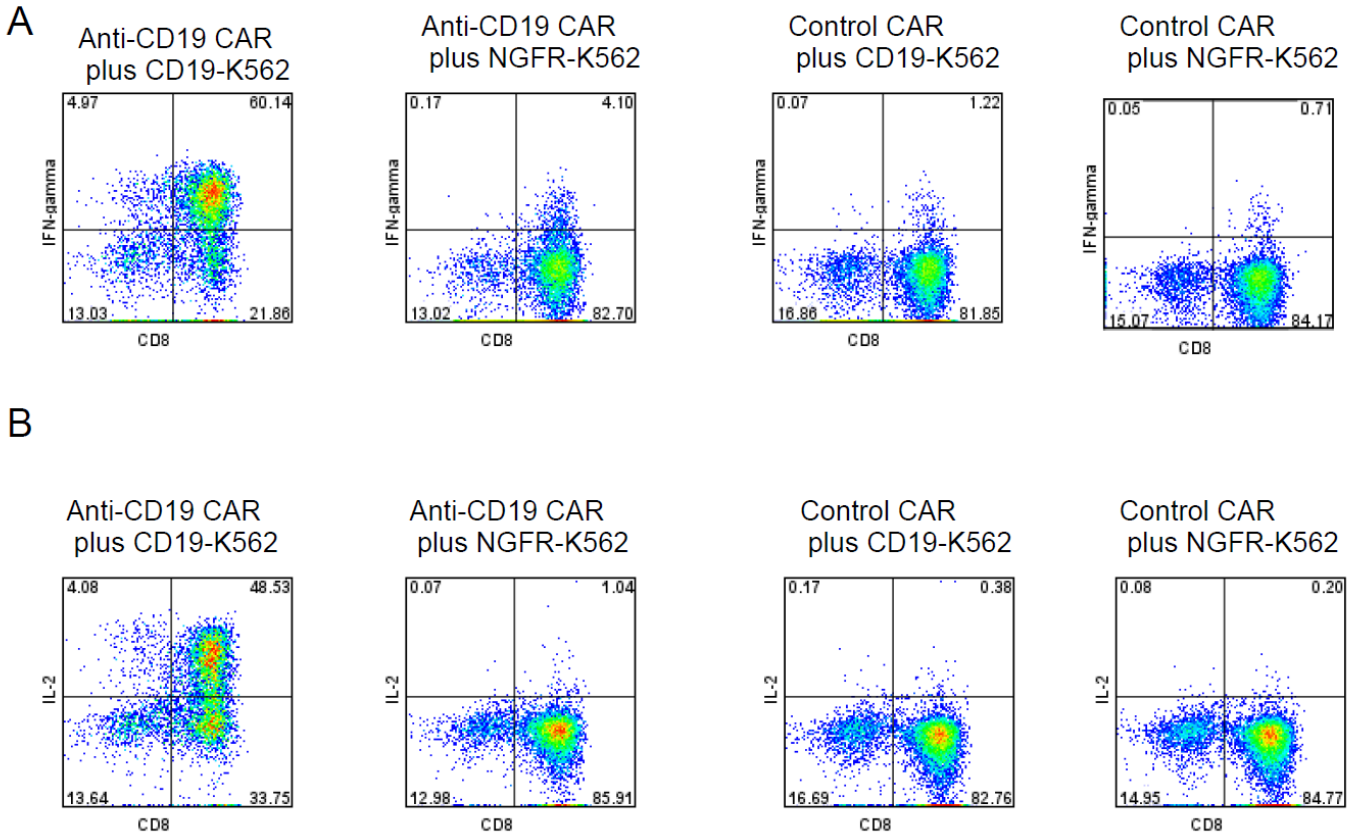

Figure 6. Fourteen days after initiation of rapid expansion a large percentage of T-cells produce IFNγ (Figure 6A) and IL-2 (Figure 6B) specifically in response to CD19-expressing target cells. Anti-CD19-CAR-transduced or control-transduced T-cells were cultured with K562 cell transduced with either full-length CD19 or nerve growth factor receptor (NGFR) for 5 hours and then intracellular cytokine staining was performed. Control-transduced T-cells were transduced with the SP6 CAR. The SP6 CAR is described in the legend for Figure 3. The K562 target cells were identical except that CD19-K562 cells were transduced with a retrovirus encoding full-length CD19 and NGFR-K562 cells were transduced with retroviruses encoding NGFR.

13.9 Figure 6: Anti-CD19-CAR-Transduced T-Cells Dependent Upon Exogenous IL-2 for In Vitro Survival

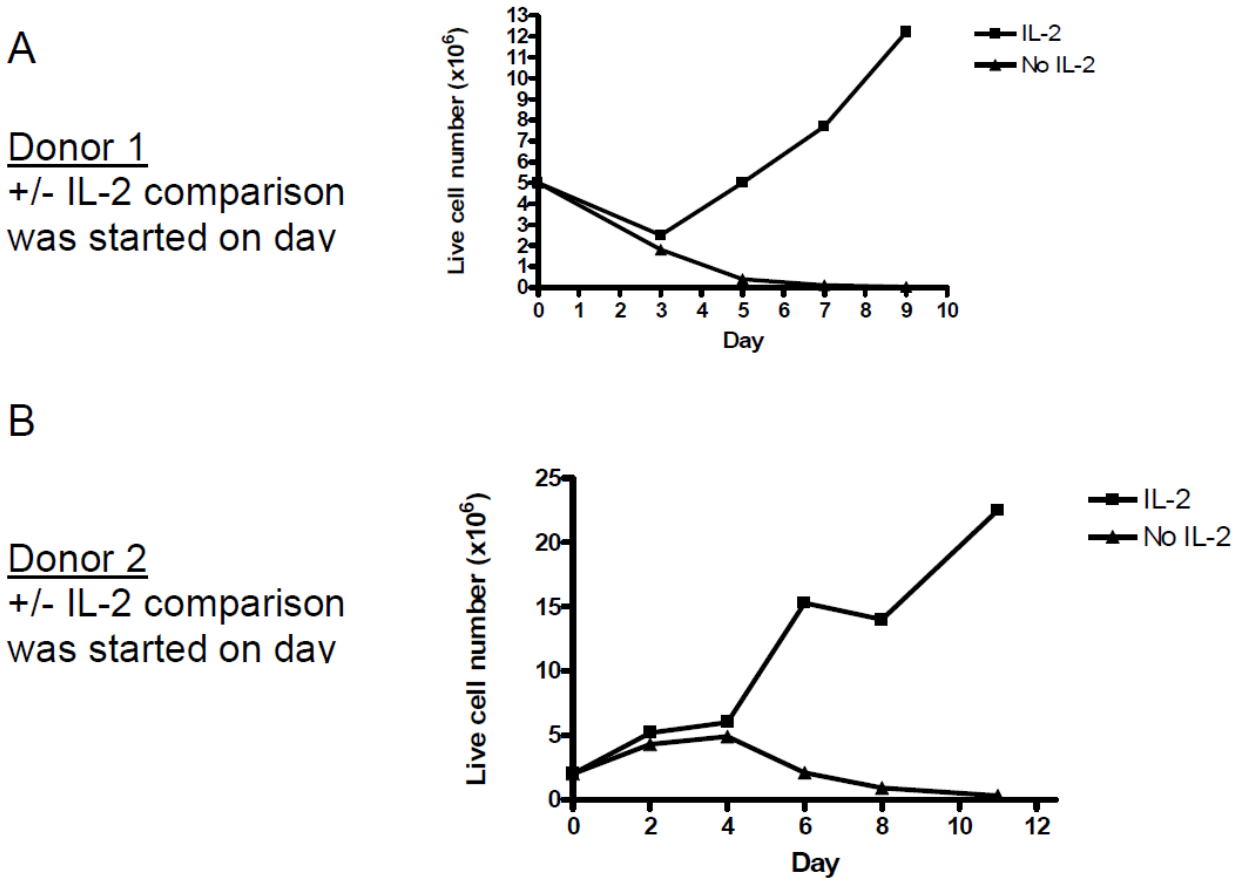

Figure 7. Anti-CD19-CAR-transduced T-cells are dependent upon exogenous IL-2 for in vitro survival. At the indicated times after initiation of a REP equal numbers of T-cells were either cultured with or without IL-2 and the number of live cells was determined by trypan blue staining on the indicated days.

13.10 FIGURE 7: OVERVIEW OF THE 6-DAY CRYOPRESERVED CELL PRODUCTION PROCESS

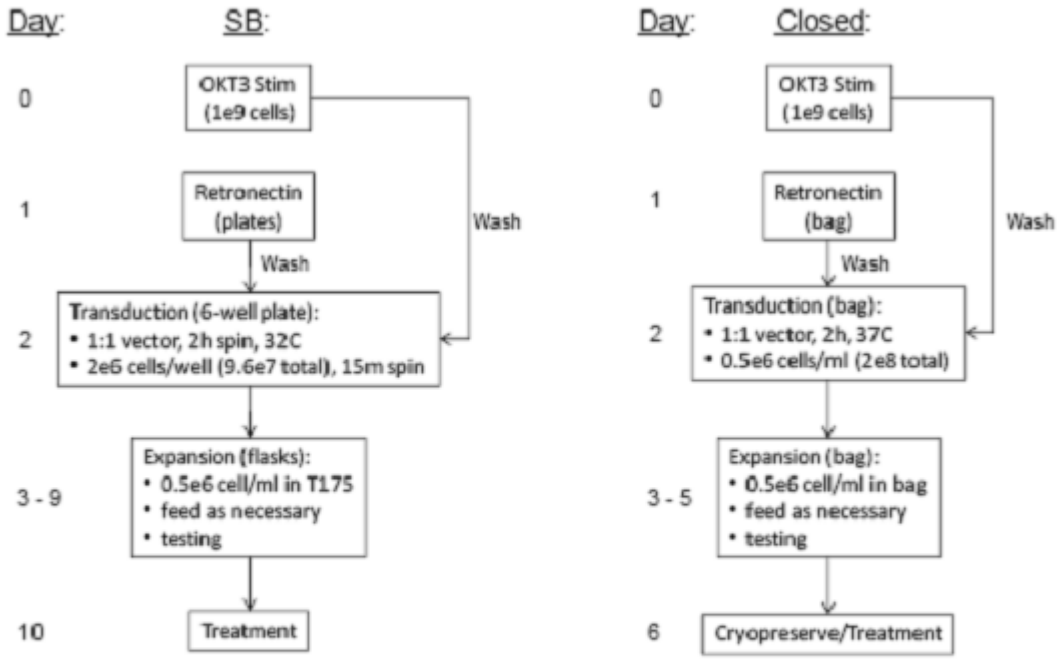

Figure 8. Overview of the 6 day cryopreserved cell production process. The schematic shows a comparison between the current 10 day Surgery Branch (SB) open cell production process to our new 6 day closed cell production process with cryopreservation.

13.11 FIGURE 8: COMPARISON OF TRANSDUCTION EFFICIENCY OF PBMC IN CLOSED VS OPEN SYSTEM

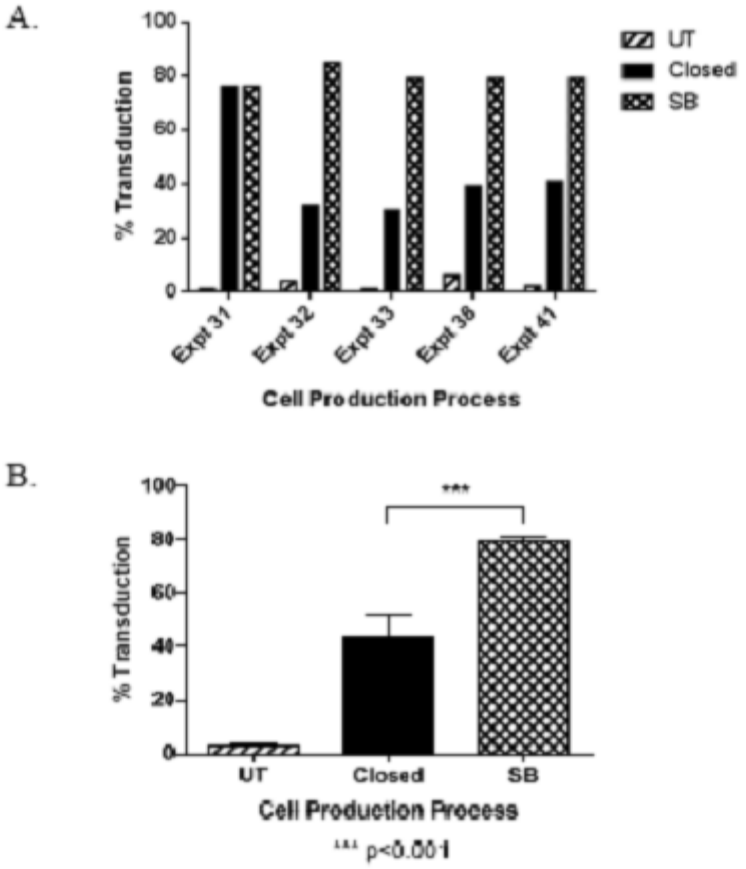

Figure 9. Comparison of transduction efficiency of PBMC in a closed versus open system. The transduction of PBMC with a Gammaretroviral vector encoding an anti-CD19 CAR in a closed bag system (black bars) was compared to The current Surgery Branch (SB) open (stippled bars) plate transduction platform. In both cases, $1x10^6$ PBMC were stimulated with soluble OKT3 for 2 days, followed transduction of PBMC at a density of $0.5x10^6$ cells/ml with a 1:1 diluted vector supernatant. A) In four of five experiments, transduction of PBMC in bags was significantly lower when compared to our current clinical plate transduction process (range 30 – 76%). B) Summary data for all five engineering runs at scale. Transduction in bags was significantly lower as compared to plate transductions, $43.6 \pm 8.3\%$ and $79.6 \pm 1.5\%$, n=5, respectively ($p<0.001$).

13.12 FIGURE 9: TOTAL CELL NUMBER OF PBMC FOLLOWING TRANSDUCTION IN CLOSED VS OPEN SYSTEM

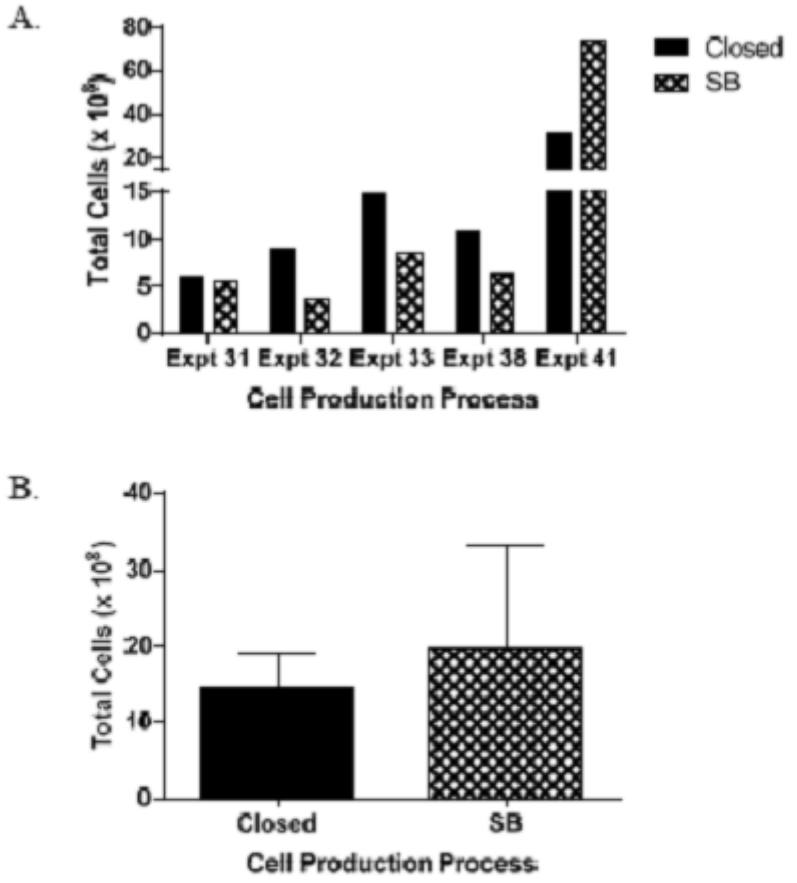

Figure 10. Total cell number of PBMC following transduction in a closed versus open system. Following PBMC transduction, the cells were washed and resuspended in cell expansion medium. For the closed process, cells were transferred into bags via sterile connection and the SB cells were expanded in upright T175 flasks. Total cell counts we calculated over 6 days for both the closed (black bars) and open (SB, stippled bars) process. A) After 6 days, total cell counts from five experiments ranged from 6.2 – 32.0 x 10^8 total cells and the open process range was between 3.7 – 74.0 x 10^8 total cells. B) There was no significant difference in total cell number when cells were expanded in the closed or open process (14.5 $\pm$ 4.5 x 10^8 and 19.6 $\pm$ 13.6 x 10^8 total cells, respectively).

13.13 FIGURE 10: CELL EXPANSION OF PBMC FOLLOWING TRANSDUCTION IN CLOSED VS OPEN SYSTEM

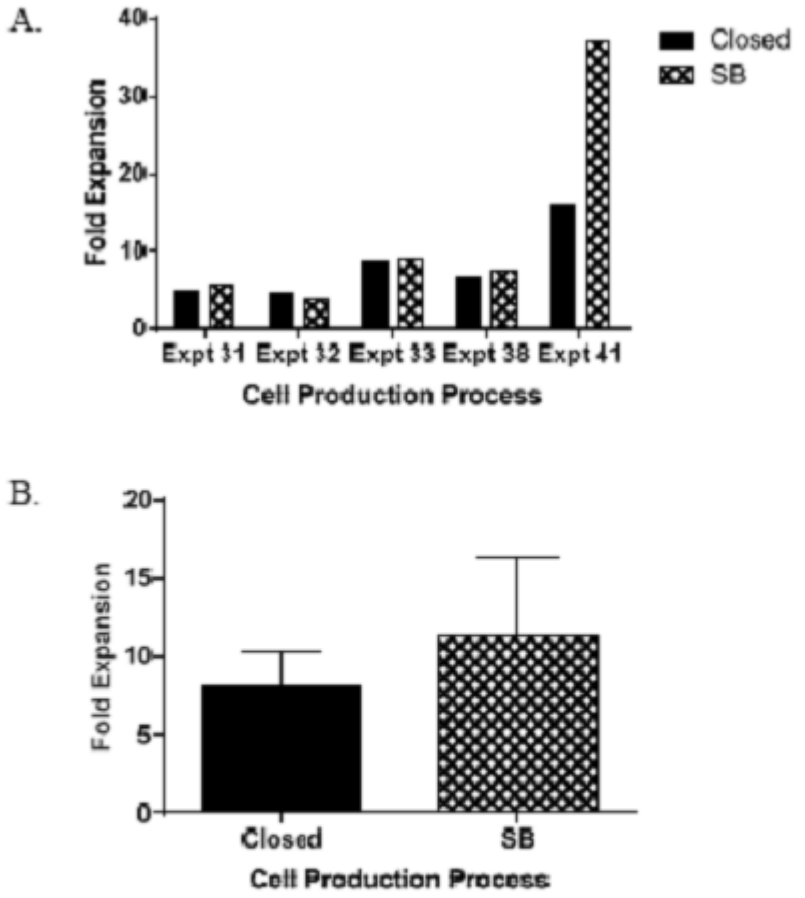

Figure 11. Cell expansion of PBMC following transduction in a closed versus open system. Following PBMC transduction, the cells were washed and resuspended in cell expansion medium. For the closed process, cells were transferred into bags via sterile connection and the SB cells were expanded in upright T175 flasks. Fold cell expansion was calculated over 6 days for both the closed (black bars) and open (SB, stippled bars) process. A) After 6 days, cells expanded the closed system in five experiments ranged from 4.5 – 16 fold as compared to 3.9 – 37 fold in the SB open process. B) There was no significant difference in total cell expansion in the closed or open process (8.2 $\pm$ 2.1 and 11.4 $\pm$ 5.0 fold expansion, respectively).

13.14 FIGURE 11: COMPARISON OF IFN-GAMMA RELEASE OF CELLS TRANSDUCED AND EXPANDED IN CLOSED VS OPEN SYSTEM

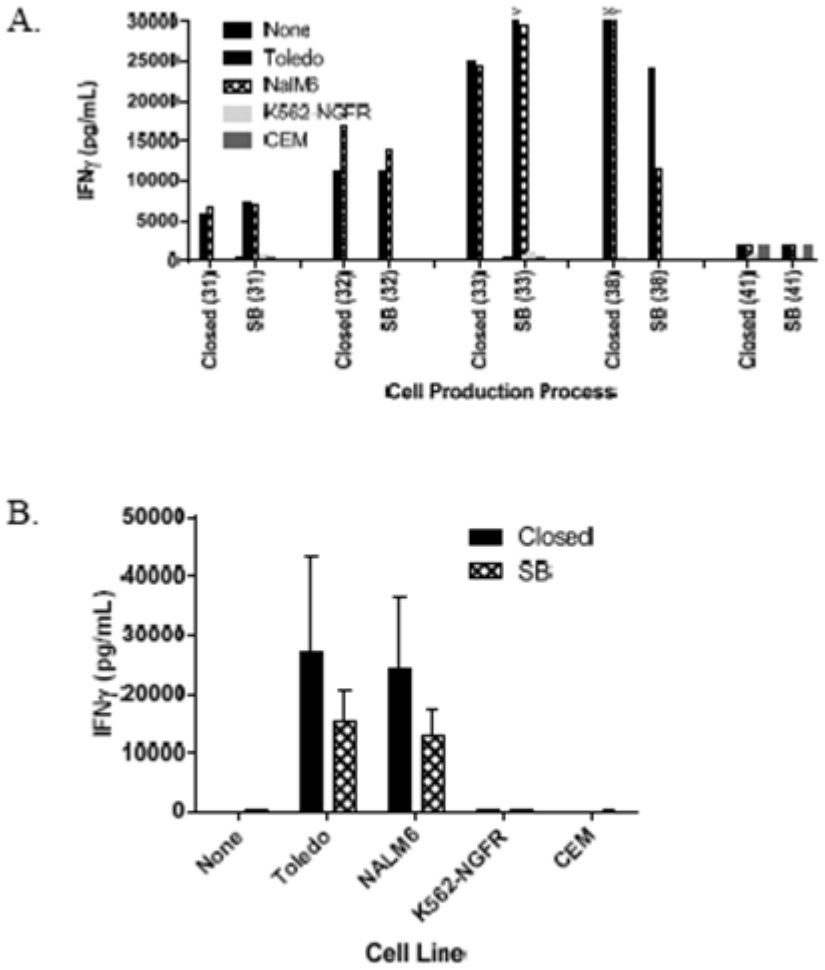

Figure 12. Comparison of IFNγ release of cells transduced and expanded in a closed versus open system. Following PBMC transduction, the cells were washed and resuspended in cell expansion medium grown for 6 days in bags (closed) or in upright T175 flasks (SB, open). IFNγ release was measured by ELISA following coculture with either CD19+ (Toledo, Nalm6) or CD19- (K562-NGFR, CEM) targets. A) IFNγ release from 5 independent experiments showing no difference in IFNγ within any single experiment. B) There was no significant difference in IFNγ release when cells were grown for 6 days in the closed or open for any of the cell lines tested.

Figure 12: Comparison of T-Cell Phenotype for PBMC Transduced and Expanded in Closed vs Open System

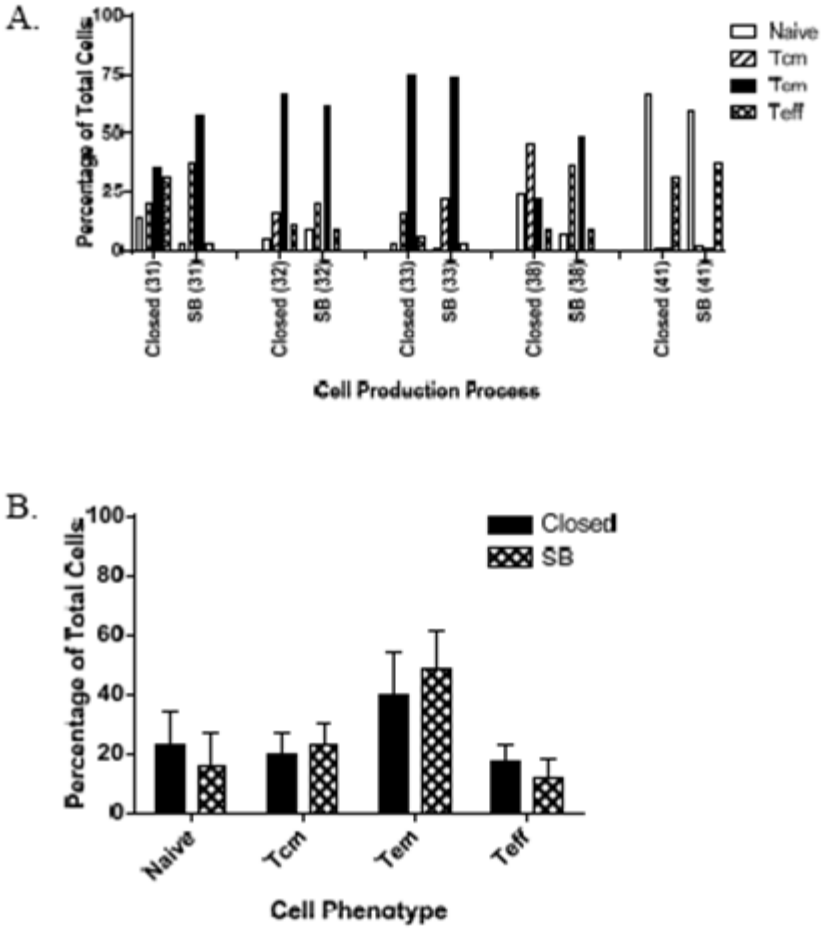

Figure 13. Comparison of T cell phenotype for PBMC transduced and expanded in a closed versus open system. Following PBMC transduction, the cells were washed and resuspended in cell expansion medium grown for 6 days in bags or in upright T175 flasks. T cell phenotype was measured by FACS following staining with anti-CD45RA and anti-CCR7 antibodies. A) T cell phenotype from 5 independent experiments was measured and showed little to no difference in phenotype within any single experiment. B) There was no significant difference in the percentage of each T cell subset measured when cells were grown for 6 days in the closed (black bars) or open (stippled bars).

13.15 FIGURE 13: COMPARISON OF PERCENTAGE AND VIABILITY OF CAR+ T-CELLS MANUFACTURED IN 6-DAY CLOSED PROCESS

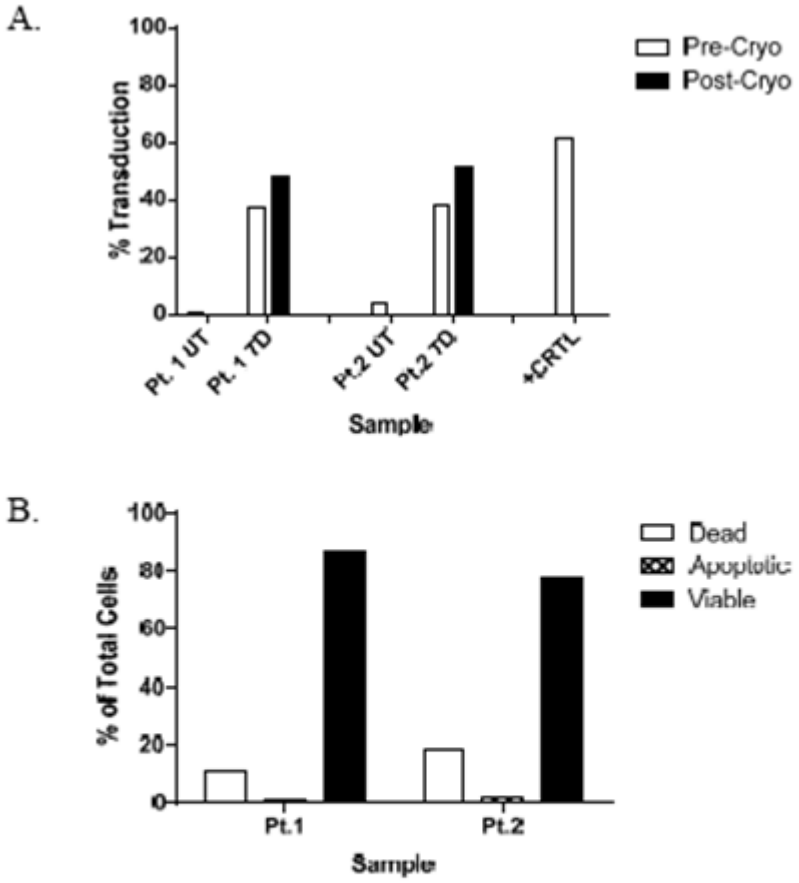

Figure 14. Comparison of the percentage and viability of CAR+ T cells manufactured in a 6 day closed process. PBMC were stimulated, transduced and expanded in bags for a total of 6 days and then cryopreserved in cryobags at a density of 8.0×10^6 cells/ml in 50ml saline-2.5% HSA-5% DMSO. A) The percentage of CD19 CAR+ T cells was measured by FACS on day 6 prior to cryopreservation and then again on day 3 post-thaw for two patients. There was no significant difference in the level of CAR+ T cells after cryopreservation (patient 1, 37.6% pre and 48.3% post; patient 2, 38.5% pre and 51.0% post). B) The viability of the cryopreserved cell product was assessed by annexin/7AAD staining. The viability of the cells at the time of thaw for patient 1 and 2 was 87% and 78%, respectively.

13.16 FIGURE 14: COMPARISON OF IFN-GAMMA RELEASE OF CAR+ T-CELLS MANUFACTURED IN 6-DAY CLOSED PROCESS AFTER CRYOPRESERVATION

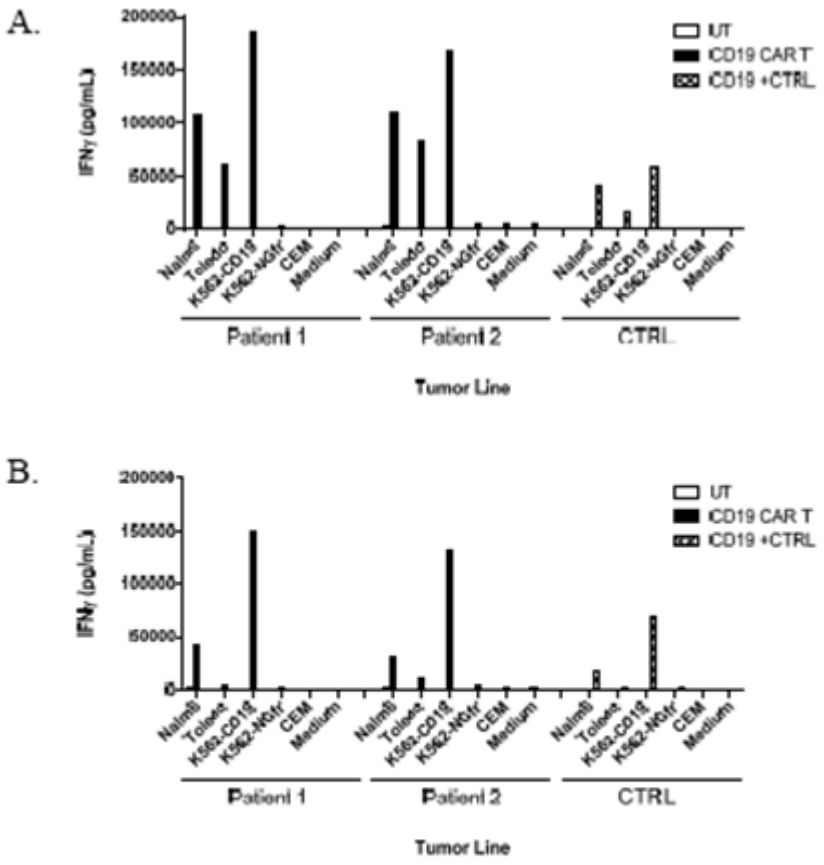

Figure 15. Comparison of IFNγ release of CAR+ T cells manufacrured in a 6 day closed process after cryopreservation. PBMC were stimulated, transduced and expanded in bags for a total of 6 days and cryopreserved. After thaw (day 0), the cells were expanded in Optimizer plus 2.5% TCSR and 50 IU/ml IL-2 for 3 days. A) On day 6, prior to cryopreservation, CD19 CAR+ T cells demonstrated specific IFNγ release to CD19+ tumor targets (Nalm6, Toldedo, K562-CD19) but not to CD19- targets (K562-NGFR, CEM). B) On day 3 post-thaw, T cells were assessed for IFNγ release against CD19+ and CD19- targets. Both patients showed high levels of specific IFNγ production suggesting the cells are still CD19 CAR+ and able to recognize antigen-specific target cells.

13.17 FIGURE 15: COMPARISON OF VIABILITY AND FOLD EXPANSION OF CAR+ T-CELLS MANUFACTURED IN 6-DAY CLOSED PROCESS AFTER CRYOPRESERVATION

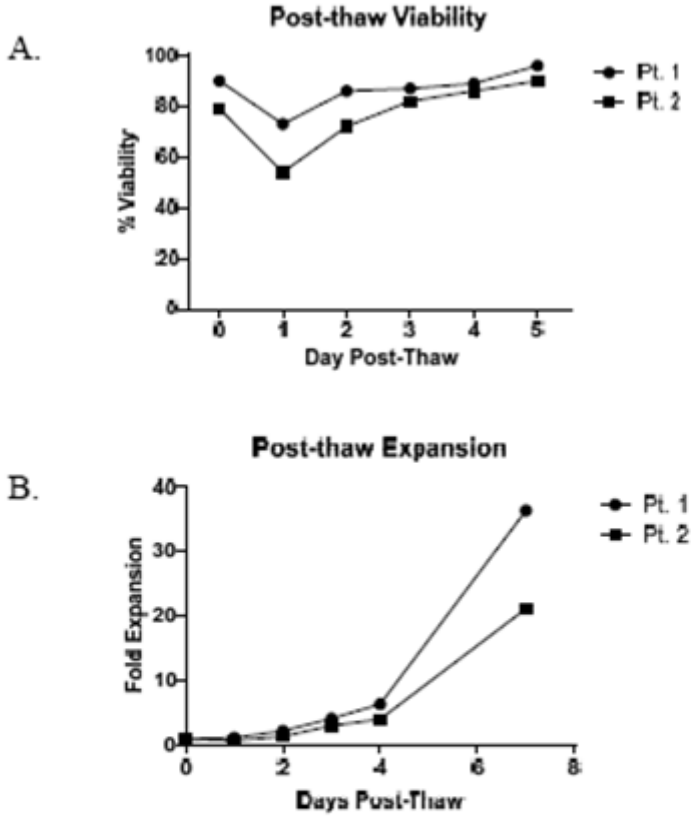

Figure 16. Comparison of the viability and fold expansion of CAR+ T cells manufactured in a 6 day closed process after cryopreservation. PBMC were stimulated, transduced and expanded in bags for a total of 6 days and cryopreserved. After thaw (day 0), the cells were washed and resuspended in Optimizer plus 2.5% TCSR and 50 IU/ml IL-2. The cells were monitored for daily for viability as well as there ability to expand post-thaw. A) Cell viability at the time of thaw was 90.0% (patient 1) and 79.9% (patient 2). A slight decrease in viability (13-15%) was observed 24h post-thaw for both patients; however cell viability recovered at 48h and continued to increase at least 90% viability over a 5 day period. B) Cell expansion post-thaw was measured. The cells expanded 36- and 21-fold, respectively, over a 7 day period.

13.18 FIGURE 16: PHENOTYPE OF CD19 CAR+ T-CELLS MANUFACTURED IN 6-DAY CLOSED PROCESS BEFORE AND AFTER CRYOPRESERVATION

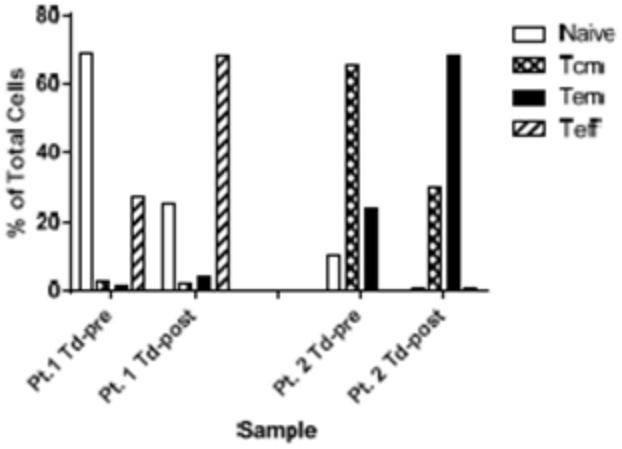

Figure 17. Phenotype of CD19 CAR+ T cells manufactured in a 6 day closed process before and after cryopreservation. PBMC were stimulated, transduced and expanded in bags for a total of 6 days and cryopreserved. After thaw (day 0), the cells were expanded in Optimizer plus 2.5% TCSR and 50 IU/ml IL-2 for 3 days. T cell phenotype was measured by FACS following staining with anti-CD45RA and anti-CCR7 antibodies. While there are slight changes in the percentages of the cell subsets represent pre- and post-cryopreservation for both patients, the overall population subsets are still represented.

13.19 APPENDIX 1: CERTIFICATE OF ANALYSIS

Anti-CD19 CAR-Transduced PBL (09-C-0082)

Date of preparation of final product:

Patient:

Tests performed on final product: Number of days in culture:

Test	*Method*	*Limits*	*Result*	*Initials/ Date*
Cell viability[1]	trypan blue exclusion	>70%		
CAR positive cells/patient weight (kg)	Calculated Value	Between $0.2x10^7$ and $1.0x10^7$ CAR positive cells/kg		
Tumor reactivity[2]	γ-IFN release vs. CD19-expressing target cell lines	>200 pg/mL		
Microbiological studies	gram stain[1]	no micro-organisms seen		
	aerobic culture[3]	no growth		
	fungal culture[3]	no growth		
	anaerobic culture[3]	no growth		
	mycoplasma test[4]	negative		
Endotoxin	limulus assay[1]	<5 E.U./kg		
RCR	S+L- Assay[3] RCR-PCR[5]	negative		

[1] Performed on sample of the final product immediately prior to cryopreservation. Results are available at the time of infusion. Total viable cell number is dependent on CAR+ cells patient's weight.

[2] Performed 2-10 post transduction. Results are available at the time of infusion.

[3] Sample collected from the final product prior to cryopreservation. Results will not be available before cells are infused into the patient.

[4] Performed 2-10 days prior to infusion. Results are available at the time of infusion.

[5] A sample will be saved from the final product prior to cryopreservation. If the culture period after retroviral transduction is less than 96 hours the sample will be archived. If the culture period exceeds 96 hours after retroviral transduction, the assay will be performed and results will be available at the time of cell infusion.

Prepared by: _____ Date: _____

QC sign-off: _____ Date: _____

Qualified laboratory or Clinical Supervisor

13.20 APPENDIX 2: DESCRIPTION OF DOSE DE-ESCALATIONS (COHORTS 1-7)

This trial will consist of two phases. In phase I, a dose escalation of the number of anti-CD19-CAR-transduced T-cells will be carried out. All patients in this phase of the trial will be lymphodepleted with fludarabine and cyclophosphamide, and all patients will receive aldesleukin (720,000 IU/kg every 8 hours for a maximum of 15 doses). Patients with platelet counts between 75,000 and 99,999 cells/mm^3 will receive half the cyclophosphamide dose that patients with platelet counts of 100,000/mm^3 or greater will receive. After a maximum tolerated cell dose is determined in phase I of the trial, phase II of the trial will begin.

Starting with approval of Amendment E (to be discontinued with approval of Amendment F), this trial is being refocused to evaluate the safety and feasibility of administering between $1.0x10^9$ and $1.0x10^{10}$ anti CD19-CAR transduced cells and aldesleukin (720,000 IU/kg every 8 hours for a maximum of 15 doses) following a preparative conditioning regimen of fludarabine and cyclophosphamide. We have accrued 4 patients at this dose level with one patient experiencing a DLT. If we observe no more DLTs in the next 2 patients treated for a total of 1/6 patients treated, accrual will continue up to 18 patients. If at any time during accrual a cumulative total of 3 evaluable patients have experienced a DLT, then no further patients will be enrolled. If DLTs are encountered in up to 3/18 patients, the trial will be considered safe and feasible. A one-week safety assessment period will follow regimen completion (defined as the last dose of aldesleukin) between each patient in the first 6 patients and a two-week safety assessment period will follow between the 6^{th} and 7^{th} patient before expansion of the trial to 18 patients.

With approval of Amendment F (to be discontinued with the approval of Amendment H), the trial is being refocused to evaluate the safety and toxicity of the regimen studied at lower escalating dose levels since 3 DLTs were observed at dose level 1 (between $1.0x10^9$ and $1.0x10^{10}$ cells). The dose of cells administered will be on a CAR positive cells/kg basis. If a DLT is observed at the revised dose levels, the dose of aldesleukin may be reduced to 72,000 IU/kg every 8 hours for a maximum of 15 doses.

Phase I – Dose Escalation (not applicable after approval of Amendment E)

The initial portion of this protocol will be a phase I dose escalation design with three cohorts of a minimum of 3 patients per cohort. The number of anti-CD19-transduced T-cells transferred for each cohort will be:

Cohort 1	between	$1.0x10^9$ and $1.0x10^{10}$
Cohort 2	greater than	$1.0x10^{10}$ up to $3.0x10^{10}$
Cohort 3	greater than	$3.0x10^{10}$ up to $1.0x10^{11}$

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 assigned cohort, the patient will be enrolled in the appropriate cohort for the number of cells infused. If a DLT occurs in an additional patient entered at a lower dose due to cell growth limitations, accrual will continue at this level as described in the dose-escalation scheme below. Accrual will be halted at the higher level until the cohort at the lower level is complete as described below.

Should a single patient experience a dose limiting toxicity at a particular dose level, three more patients would be treated at that dose to confirm that no greater than 1/6 patients have a DLT prior to proceeding to the next higher level. If a level with 2 or more DLTs in 3-6 patients has been identified, three 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 prior to starting the phase II portion of the trial. After the maximum tolerated dose is determined, the phase II portion of the trial will begin.

A one-week safety assessment period will follow regimen completion (defined as the last dose of aldesleukin) between each patient in a cohort except for the last patient in each cohort for which a two-week safety assessment period will follow regimen completion (defined as the last dose of aldesleukin) before a patient will be accrued to the next cohort.

Upon completion of the Phase I portion of the study, the adverse events observed in this cohort will be reported to and evaluated by the IRB before proceeding to the Phase II portion.

Phase II – Randomization to Chemotherapy Versus No Chemotherapy (not applicable after approval of Amendment E)

In phase II of the trial, patients will be randomized to two treatment arms. Patients assigned treatment arm 1 will receive fludarabine and cyclophosphamide lymphodepleting chemotherapy prior to receiving the engineered PBL cells while patients assigned to treatment arm 2 will not receive chemotherapy. Randomization will also be stratified so that an equal number of patients with circulating malignant cells (leukemia) will be entered on each arm of the phase II part of the trial.

One to four days after completion of chemotherapy, anti-CD19 CAR-transduced T-cells will be infused. After cell infusion, IV aldesleukin therapy will be initiated (720,000 IU/kg every 8 hours for a maximum of 15 doses). The randomization performed during phase II of this trial will be stratified so that each arm will receive an equal number of patients with a total peripheral blood CD3 count greater than or equal to 500 cells/microliter, and each arm will receive an equal number of patients with a CD3 count less than 500 cells/microliter. In addition, the randomization will be stratified so that each arm will receive an equal number of patients with peripheral blood involvement with malignant cells. Patients with peripheral blood involvement with malignant cells are defined as patients with pre-treatment peripheral blood lymphocyte counts greater than 4000 lymphocytes per microliters.

During both phases multiple immunologic assays will be carried out on peripheral blood T-cells to determine persistence of anti-CD19-CAR-transduced T-cells as well as their function. During phase II an assessment of the anti-malignancy effect of anti-CD19-CAR-transduced T-cells will be made. All patients will be followed for anti-malignancy response, survival and toxicity.

Dose Escalation and Cohort Expansion (with approval of Amendment F, not applicable after approval of Amendment H)

With approval of Amendment F, the trial is being refocused to evaluate the safety and toxicity of the regimen studied at lower escalating dose levels since 3 DLTs were observed at dose level 1 (between $1.0x10^9$ and $1.0x10^{10}$ cells). For cohort 2, 3 patients will be treated with NMA, $0.5x10^7$ CAR positive cells/kg (± 20%), and high dose aldesleukin (720,000 IU/kg). This starting dose of cells has been selected due to PK modeling of interferon gamma levels and toxicity data from the first 8 patients treated on this study. If one DLT is observed in a patient treated in Cohort 2, after

analysis of the IFN-gamma cytokine data, 3 additional patients will be treated at this dose level ($0.5x10^7$ CAR positive cells/kg (± 20%) after receiving NMA followed by a reduced dose of aldesleukin (72,000 IU/kg every 8 hours for a maximum of 15 doses) (Cohort 3). This 10-fold reduction of aldesleukin was previously investigated in a study of patients with renal cancer. The incidence of grade 3 and 4 aldesleukin related toxicities was less in patients treated with 72,000 IU/kg every 8 hours than those treated with the higher dose of 720,000 IU/kg aldesleukin, every 8 hours (Yang, J.C., et al., J Clin Oncol. 2003, 21(16):3127-32).

If no DLTs are observed in the 3 patients in Cohort 2, after analysis of the IFN-gamma cytokine data, 3 additional patients will be treated with NMA, $1.0x10^7$ CAR positive cells/kg (± 20%) and high dose aldesleukin (Cohort 4). If one DLT is observed in a patient treated in Cohort 4, 3 additional patients will be treated at this dose level ($1.0x10^7$ CAR positive cells/kg (± 20%) after receiving NMA followed by a reduced dose of aldesleukin (72,000 IU/kg every 8 hours for a maximum of 15 doses) (Cohort 5).

Cohort	**Treatment Regimen**		
	NMA Chemotherapy Regimen (as specified in protocol)	*Cell Dose*	*IL-2 Dose*
1 (Closed with Amendment F)	Yes	between $1.0x10^9$ and $1.0x10^{10}$ cells	High dose (720,000 IU/kg)
2	Yes	$0.5x10^7$ CAR positive cells/kg (± 20%)	High dose (720,000 IU/kg)
3[1]	Yes	$0.5x10^7$ CAR positive cells/kg (± 20%)	Low dose (72,000 IU/kg)
4	Yes	$1.0x10^7$ CAR positive cells/kg (± 20%)	High dose (720,000 IU/kg)
5[2]	Yes	$1.0x10^7$ CAR positive cells/kg (± 20%)	Low dose (72,000 IU/kg)

[1] Cohort 3 will be skipped if there are no DLTs in Cohort 2.

[2] Cohort 5 will be skipped if there are no DLTs in Cohort 4.

The highest dose cohort evaluated with no DLTs in 3 patients (either 3, 4 or 5) will be expanded to a total of 18 evaluable patients, subject to not exceeding 3 patients in total with a DLT.

Safety Assessment

Cytokine (IFN-gamma levels) and toxicity data must be analyzed prior to dose escalation. A one-week safety assessment period will follow regimen completion (defined as the last dose of aldesleukin) between each patient in a cohort except for the last patient in each cohort for which a two-week safety assessment period will follow regimen completion (defined as the last dose of aldesleukin) before a patient will be accrued to the next cohort. A two-week safety assessment period will follow between the 3th and 4th patient before expansion of the trial to 18 patients.

Dose Escalation and Cohort Expansion (with approval of Amendment H, closed with Amendment J)

With Amendment H, cohorts 2-5 will be closed and two new cohorts will be opened at the same cell dose level as cohorts 2 and 4 but without aldesleukin. These cohorts will be designated cohort 6 and 7. For Cohort 6, 3 patients will be treated with NMA, and $0.5x10^7$ CAR positive cells/kg (± 20%). If one DLT is observed in a patient treated in Cohort 6, after analysis of the IFN-gamma cytokine data, accrual will be halted pending discussions with the FDA and IRB.

In November 2011, the first patient in Cohort 6 experienced a DLT of grade 3 confusion (possible aphasia), and grade 3 neuropathy (facial droop) possibly related to the cell therapy. The serum IFN-gamma levels were low in this patient (range 9-44 pg/mL) following treatment with cells. These events were discussed with the FDA, and the FDA indicated that we can treat an additional patient in Cohort 6 (with approval of Amendment I). If this additional patient does not experience at DLT, Cohort 6 will be expanded to a total of 6 patients. If any additional patients treated in Cohort 6 experiences a DLT, after analysis of the IFN-gamma cytokine data, accrual will be halted pending discussions with the FDA and IRB.

If no additional DLTs are observed in the 6 patients in Cohort 6, after analysis of the IFN-gamma cytokine data, 3 patients will be treated with NMA, and $1.0x10^7$ CAR positive cells/kg (± 20%) in Cohort 7. If one DLT is observed in a patient treated in Cohort 7, 3 additional patients will be accrued to Cohort 7. If 2 DLTs occur in 6 or less patients in Cohort 7, no additional patients will be accrued at this dose level.

Cohort	**Treatment Regimen**		
	NMA Chemotherapy Regimen (as specified in protocol)	*Cell Dose*	*IL-2 Dose*
1 (Closed with Amendment F)	Yes	between $1.0x10^9$ and $1.0x10^{10}$ cells	High dose (720,000 IU/kg)
2 (Closed with Amendment H)	Yes	$0.5x10^7$ CAR positive cells/kg (± 20%)	High dose (720,000 IU/kg)
3 (Closed with Amendment H)	Yes	$0.5x10^7$ CAR positive cells/kg (± 20%)	Low dose (72,000 IU/kg)
4 (Closed with Amendment H)	Yes	$1.0x10^7$ CAR positive cells/kg (± 20%)	High dose (720,000 IU/kg)
5 (Closed with Amendment H)	Yes	$1.0x10^7$ CAR positive cells/kg (± 20%)	Low dose (72,000 IU/kg)
6 (Closed with Amendment J)	Yes	$0.5x10^7$ CAR positive cells/kg (± 20%)	None
7 (Closed with Amendment J)	Yes	$1.0x10^7$ CAR positive cells/kg (± 20%)	None

The highest dose cohort evaluated with no DLTs in 3 or more patients, or 1 DLT in 6 patients will be expanded to a total of 18 evaluable patients, subject to not exceeding 3 patients in total with a DLT.

Safety Assessment

Cytokine (IFN-gamma levels) and toxicity data must be analyzed prior to dose escalation. A one-week safety assessment period will follow regimen completion between each patient in a cohort except for the last patient in each cohort for which a two-week safety assessment period will follow regimen completion before a patient will be accrued to the next cohort. A two-week safety assessment period will follow the last patient treated in the dose escalation cohort before expansion of the trial to 18 patients.

Dose De-escalation and Cohort Expansion

In February 2012, the second patient in Cohort 6 experienced DLTs. The SAEs consisted of grade 3 hypotension and grade 3 confusion (possibly aphasia) related to the cell therapy. These toxicities have resolved and the patient has been discharged. After discussions with the FDA, cohort 6 and 7 were closed and two new dose de-escalation cohorts were opened as follows:

13.21 APPENDIX 3: DESCRIPTION OF DOSE ESCALATION FOR FRESH ANTI-CD19 TIL (COHORTS 8-10)

PBMC will be obtained by leukapheresis (approximately $1.0x10^{10}$ cells). In most patients, whole PBMC will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin in order to stimulate T-cell growth. In some patients the peripheral blood might be contaminated with large numbers of CD19-expressing malignant cells. In patients with peripheral blood lymphocytes counts greater than 4000 cells per microliter, CD19-expressing cells will be depleted using the Miltenyi Biotec CliniMacs system with anti-CD19 microbeads and then CD19-depleted PBMC will be stimulated with OKT3. In all patients, 2 days after OKT3 stimulation transduction will be initiated by exposure of approximately $1.0x10^{7}$ to $5.0x10^{8}$ cells to supernatant containing the anti-CD19 CAR retroviral vector. These transduced cells will be expanded and tested for their anti-tumor activity. Successful TCR gene transfer will be determined by FACS analysis for the CAR and specific anti-tumor reactivity will be tested by cytokine release measured against CD19+ cell lines and CD19-negative cell lines. Successful CAR gene transfer for each transduced PBL population will be defined as >30% CAR positive cells and for biological activity, gamma-interferon secretion against CD19 targets must be at least 200 pg/mL. The cells administered vary depending on their growth characteristic. In prior protocols over $3.0x10^{11}$ T-cells have been safely infused to cancer patients.

Due to toxicities experienced during the dose escalation phase of this trial, the protocol has been amended several times to decrease the dose and eliminate the administration of IL-2. The description of these changes has been moved to **Appendix 2**.

Beginning with Amendment M patients will be lymphodepleted with fludarabine and a reduced dose of cyclophosphamide – 30mg/kg/day for 2 days and will receive $1.0x10^{6}$ CAR positive cells/kg (± 20%) as noted below.

Cohort	**Treatment Regimen**		
	NMA Chemotherapy Regimen (as specified in protocol)	*Cell Dose*	*IL-2 Dose*
8 (De-escalation Dose 1)	Yes	$2.5x10^{6}$ CAR positive cells/kg (± 20%)	None
9 (De-escalation Dose 2)	Yes	$1.0x10^{6}$ CAR positive cells/kg (± 20%)	None
10 (De-escalation Dose 2)	Yes – dose of both agents reduced	$1.0x10^{6}$ CAR positive cells/kg (± 20%)	None

Cohort 8 will initially accrue 3 patients. If no DLTs are observed in these 3 patients, three additional patients will be accrued. If DLTs are observed in ≤ 1 patient out of 6 patients treated, this cohort will be expanded as described below. If a DLT is observed in one of the first 3 patients, after analysis of the cytokine data, 3 additional patients can be accrued to this cohort. If more than 1 out of 6 patients in this cohort experience a DLT, this cohort will be closed, and patients will be accrued to cohort 9. Cohort 8 was closed with Amendment M.

Cohort 9 will initially accrue 3 patients. If no DLTs are observed in these 3 patients, three additional patients will be accrued. If DLTs are observed in ≤ 1 patient out of 6 patients treated,

this cohort will be expanded as described below. If a DLT is observed in one of the first 3 patients, after analysis of the cytokine data, 3 additional patients can be accrued to this cohort. If more than 1 out of 6 patients in this cohort experience a DLT, this cohort will be closed. Subsequent dose reductions will be discussed with the FDA prior to initiation. If ≤ 1 patient in 6 patients treated develops a DLT then the 12 remaining patients will be enrolled in cohort 10 for a total of 18 evaluable patients at a dose level of $1.0x10^6$ CAR positive cells/kg (± 20%), subject to not exceeding 3 patients in total with a DLT.

Following administration of the cell product, neurological status will be closely monitored and urgently managed as described in Section **3.4.3**.

In addition, we will be evaluating stored serum samples in an effort to identify the particular cytokines which may be contributing to these toxicities.

Safety Assessment

Cytokine (IFN-gamma levels) and toxicity data must be analyzed prior to dose de-escalation. A one-week safety assessment period will follow regimen completion between each patient in a cohort except for the last patient in each cohort for which a two-week safety assessment period will follow regimen completion before a patient will be accrued to the next cohort. Beginning with Amendment R, the one week safety assessment period between patients will no longer be required during the expansion phase of the protocol for the remaining patients to be enrolled in Cohort 10.

The study will be halted pending discussions with the FDA and IRB if the following conditions are met:

Prior to Amendment E

- If two DLTs occur in the first cohort of the Phase I portion of this study.
- Two or more patients develop a grade 3 or greater toxicity at any point in the study not attributable to the chemotherapy preparative regimen (or circumstances unrelated to this study).
- If 1 of the first 3 patients (OR 2 of the first 6 patients, OR 3 of the first 9 patients, OR 4 of the first 12 patients) develop DLT due to autoimmune toxicity.
- Development of EBV lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV negative subject on this or any other SB adoptive cell therapy study. Accrual of EBV negative subjects will be halted only.

With Approval of Amendment E (not applicable with approval of Amendment F)

- If two DLTs occur in the first 6 patients treated on this study.
- Two or more patients develop a grade 3 or greater toxicity at any point in the study not attributable to the chemotherapy preparative regimen (or circumstances unrelated to this study).
- If 1 of the first 3 patients (OR 2 of the first 6 patients, OR 3 of the first 9 patients, OR 4 of the first 12 patients) develop DLT due to autoimmune toxicity.

- Development of EBV lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV negative subject on this or any other SB adoptive cell therapy study. Accrual of EBV negative subjects will be halted only.

With Approval of Amendments F, H, and J

As stated above, the study will be halted pending discussions with the FDA and IRB if the following conditions are met:

- If cohorts 2-7 or 9 cannot be expanded due to DLTs.
- During the expansion phase, if at any time during accrual, a cumulative total of 3 evaluable patients have experienced a DLT.
- Two or more patients develop a grade 3 or greater toxicity at any point in the study not attributable to the chemotherapy preparative (or circumstances unrelated to this study).
- If 1 of the first 3 patients (OR 2 of the first 6 patients, OR 3 of the first 9 patients, OR 4 of the first 12 patients) in cohorts 2-5 develop DLT due to autoimmune toxicity.
- Development of EBV lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV negative subject on this or any other SB adoptive cell therapy study. Accrual of EBV negative subjects will be halted only.

13.22 APPENDIX 4: MODIFICATION OF DOSE CALCULATIONS* IN PATIENTS WHOSE BMI IS GREATER THAN 35

Beginning with Amendment M, actual body weight will be used for dose calculations of all treatment agents.

1. BMI Determination:

   BMI = weight (kg) / [height (m)]2

2. Calculation of ideal body weight:

   Male = 50 kg + 2.3 (number of inches over 60 inches)

   Example: Ideal body weight of 5'10" male

   50 + 2.3 (10) = 73 kg

   Female = 45.5 kg + 2.3 (number of inches over 60 inches)

   Example: Ideal body weight of 5'3" female

   45.5 + 2.3 (3) = 57 kg

3. Calculation of "practical weight":

   Calculate the average of the actual and the ideal body weights. This is the practical weight to be used in calculating the doses of chemotherapy and associated agents designated in the protocol.

*Practical weight will NOT be used in the calculation of dose for aldesleukin.