

**Abbreviated Title:** *Anti- CD19 CART-cells*

**Version Date:** *September 17, 2020*

**Abbreviated Title:** Anti-CD19-CAR T cells

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

**NCI Principal Investigator:** James N Kochenderfer, M.D.  
Surgery Branch  
Center for Cancer Research,  
National Cancer Institute, National Institutes of Health  
10 Center Drive, Rm. 6B17, MSC 1203  
Telephone: 240-760-6062  
Email: [kochendj@mail.nih.gov](mailto: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

*Abbreviated Title: Anti-CD19 CAR T-cells*

*Version Date: September 17, 2020*

## **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:**

**Abbreviated Title: Anti- CD19 CART-cells****Version Date: September 17, 2020**

- 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  $\geq 1000/\mu\text{L}$ , platelet count  $\geq 45,000/\mu\text{L}$ , hemoglobin  $\geq 8\text{g/dL}$
- 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<sup>2</sup> daily for 3 days and fludarabine 30 mg/m<sup>2</sup> 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  $0.66 \times 10^6$  CAR<sup>+</sup> T cells/kg of recipient bodyweight.

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

- 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.

*Abbreviated Title: Anti-CD19 CART-cells*

*Version Date: September 17, 2020*

## **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**

PRÉCIS .....	2
TABLE OF ABBREVIATIONS .....	5
1 INTRODUCTION .....	11
1.1 Study Objectives.....	11
1.1.1 Primary Objective.....	11
1.1.2 Secondary Objectives.....	11
1.2 Background and Rationale .....	11
1.2.1 Introduction.....	11
1.2.2 B-cell malignancies: epidemiology and standard treatment.....	11
1.2.3 Allogeneic Stem Cell Transplantation for B-cell Malignancies.....	12
1.2.4 Donor Cell Infusion (DCI) .....	13
1.2.5 Mechanism of the graft-versus-malignancy (GVM) effect.....	14
1.2.6 Definition and Assessment of Graft-versus-host Disease.....	15
1.2.7 T-cell gene therapy .....	15
1.2.8 Chimeric antigen receptors preclinical background .....	16
1.2.9 CD19 .....	17
1.2.10 Clinical results with anti-CD19 CAR T cells.....	17
1.2.11 Anti-CD19 CAR development and preclinical testing .....	20
1.2.12 Rationale for immunosuppressive chemotherapy and selection of lymphocyte-depleting.....	25
1.2.13 Rationale for dose-escalation and repeated chemotherapy plus CAR T-cell treatments.....	25
1.2.14 Rationale for a fully-human CAR.....	26
1.2.15 Use of T cells from patients with malignancy versus healthy donor T cells to produce anti-CD19 CAR T cells.....	27
1.2.16 Summary of risks and potential benefits .....	28
2 ELIGIBILITY ASSESSMENT AND ENROLLMENT .....	28
2.1 Eligibility Criteria.....	28
2.1.1 Inclusion Criteria.....	29
2.1.2 Exclusion criteria:.....	31
2.2 Screening Evaluation .....	32
2.2.1 Screening activities performed after a consent for screening has been signed .....	32

2.3	Participant Registration and Status Update Procedures .....	33
2.4	Treatment Assignment Procedures: .....	33
3	STUDY IMPLEMENTATION.....	33
3.1	Study Design.....	33
3.1.1	General study plan.....	34
3.1.2	Planned repeat treatments.....	34
3.1.3	Inclusion of patients who have had allogeneic hematopoietic stem cell transplantation (alloHSCT).....	35
3.1.4	Protocol schema .....	36
3.1.5	Dose Limiting Toxicity.....	37
3.1.6	Dose Escalation.....	37
3.2	Dose Modifications/Delay.....	40
3.3	Stopping Criteria .....	41
3.4	Drug Administration.....	41
3.4.1	Leukapheresis.....	41
3.4.2	Anti-CD19-CAR-expressing T-cell preparation .....	41
3.4.3	Conditioning chemotherapy and anti-CD19 CAR T-cell administration-this can be either inpatient or outpatient.....	42
3.4.4	Potential repeat treatment.....	43
3.5	Protocol Evaluation .....	44
3.5.1	Baseline evaluations and interventions.....	44
3.5.2	Studies to be performed during the mandatory 9-day inpatient admission after cell infusion	46
3.5.3	Post-infusion outpatient evaluation.....	46
3.6	Study Calendar (Table 5).....	48
3.7	Gene-therapy-specific follow-up .....	52
3.7.1	Clinical Evaluation .....	52
3.7.2	Testing for persistence of CAR transduced cells.....	52
3.7.3	Replication competent lentivirus (RCL) testing.....	52
3.8	Criteria for Removal from Protocol Therapy and Off Study Criteria .....	53
3.8.1	Criteria for removal from protocol therapy.....	53
3.8.2	Off-study Criteria .....	53
4	CONCOMITANT MEDICATIONS/MEASURES .....	54

**Abbreviated Title: Anti- CD19 CART-cells**

**Version Date: September 17, 2020**

4.1	Routine Supportive care.....	54
4.2	Blood product support.....	55
4.3	Anti-emetics.....	55
4.4	Granulocyte colony-stimulating factor.....	55
4.5	Avoidance of corticosteroids.....	55
4.6	Guidelines for management of common acute toxicities that occur after CAR T cell infusions.....	55
5	BIOSPECIMEN COLLECTION .....	56
5.1	CORRELATIVE STUDIES FOR RESEARCH .....	56
5.1.1	Biospecimen collection before the start of the conditioning chemotherapy:.....	56
5.1.2	Biospecimen collection after CAR T-cell infusion during the required hospitalization.....	56
5.1.3	Biospecimen collection during outpatient follow-up.....	56
5.1.4	Immunological Testing.....	57
5.1.5	Additional biopsies and additional blood draws .....	58
5.1.6	Future studies.....	58
5.2	Sample Storage, Tracking and Disposition.....	58
5.2.1	Samples Sent to Figg Lab.....	59
5.2.2	Sample Storage, Tracking and Disposition.....	59
5.2.3	Sample Storage, Tracking, and Disposition for Surgery Branch.....	60
5.2.4	Protocol Completion/Sample Destruction .....	61
5.2.5	Samples for Genetic/Genomic Analysis.....	61
6	DATA COLLECTION AND EVALUATION .....	62
6.1	Data Collection.....	62
6.1.1	Adverse event recording: .....	63
6.2	Data Sharing Plans.....	63
6.2.1	Human Data Sharing Plan.....	63
6.2.2	Genomic Data Sharing Plan .....	64
6.3	Response Criteria.....	64
6.4	Toxicity Criteria.....	66
7	NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN....	66
7.1	Definitions.....	66
7.2	OHSRP Office of Compliance and Training / IRB Reporting.....	66



**Abbreviated Title: Anti- CD19 CART-cells****Version Date: September 17, 2020**

7.2.1	Expedited Reporting.....	67
7.2.2	IRB Requirements for PI Reporting at Continuing Review.....	67
7.3	NCI Clinical Director Reporting.....	67
7.4	Institutional Biosafety Committee (IBC) Reporting Criteria.....	67
7.4.1	Serious Adverse Event Reports to IBC.....	67
7.4.2	Annual Reports to IBC.....	67
7.5	NIH Required Data and Safety Monitoring Plan.....	68
7.5.1	Principal Investigator/Research Team.....	68
7.5.2	Safety Monitoring Committee (SMC).....	68
8	Sponsor Safety Reporting.....	69
8.1	Definitions.....	69
8.1.1	Adverse Event.....	69
8.1.2	Serious Adverse Event (SAE).....	69
8.1.3	Life-threatening.....	70
8.1.4	Severity.....	70
8.1.5	Relationship to Study Product.....	70
8.2	Assessment of Safety Events.....	70
8.3	Reporting of Serious Adverse Events.....	71
8.4	Reporting Pregnancy.....	71
8.4.1	Maternal exposure.....	71
8.4.2	Paternal exposure.....	71
8.5	Regulatory Reporting for Studies Conducted Under CCR-Sponsored IND.....	71
9	CLINICAL MONITORING.....	72
10	STATISTICAL CONSIDERATIONS.....	72
11	COLLABORATIVE AGREEMENTS.....	73
12	HUMAN SUBJECTS PROTECTIONS.....	73
12.1	Rationale For Subject Selection.....	73
12.2	Participation/selection rationale.....	73
12.3	Participation of Children.....	74
12.4	Participation of Subjects Unable to Give Consent.....	74
12.5	Evaluation of Benefits and Risks/Discomforts.....	75
12.5.1	Risks of exposure to Ionizing Radiation.....	75

**Abbreviated Title: Anti- CD19 CART-cells**

**Version Date: September 17, 2020**

12.5.2	Risks of Scans and Contrast .....	76
12.5.3	Risks of blood Sampling .....	76
12.5.4	Risks of Bone marrow aspiration and biopsy.....	76
12.5.5	Risks of Intravenous Catheter.....	76
12.5.6	Risks of Lumbar Puncture.....	76
12.5.7	Risks of Apheresis.....	76
12.6	Consent Process and Documentation.....	76
13	PHARMACEUTICAL INFORMATION.....	77
13.1	Lentiviral Vector Containing the anti-CD19 CAR Gene.....	77
13.2	Commercial Agents.....	77
13.2.1	Cyclophosphamide .....	77
13.2.2	FLUDARABINE.....	78
14	REFERENCES .....	79
15	APPENDICES .....	90
15.1	Appendix A: Performance Status Criteria.....	90
15.2	Appendix B: Data Collection Elements Required By Protocol.....	91
15.3	Appendix C: Guidelines for management of common toxicities that occur after CAR T-cell infusions.....	93
15.4	Appendix D: Clinical Staging and treatment of Acute GVHD.....	97
15.5	Appendix E: Infusion Instructions.....	100

## **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.<sup>1-3</sup> 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<sup>4</sup>. B cell malignancies have quite heterogeneous clinical manifestations and prognoses. Chronic lymphocytic leukemia (CLL) is a common disease that is incurable by chemotherapy<sup>5</sup>. 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<sup>6</sup>, 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<sup>6</sup>. 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<sup>7</sup>. 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<sup>8</sup>. Follicular lymphoma is a common lymphoma with an extremely variable course<sup>9</sup>. Patients with follicular lymphoma have a median survival of about 4.5 years after first relapse<sup>10,11</sup>. 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<sup>12</sup>. 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<sup>13</sup>. However, patients with DLBCL that is refractory to 1<sup>st</sup> salvage chemotherapy have response rates to 2<sup>nd</sup> salvage therapy of only 14 to 43%<sup>14-18</sup>. Patients with DLBCL refractory to 2<sup>nd</sup>-line chemotherapy have very poor prognoses with a reported median survival of only 4 months and a 1-year survival rate of only 4%<sup>18</sup>. 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%<sup>19,20</sup>. 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<sup>21-26</sup>. Khouri and coworkers reported the results of their experience treating patients with chemotherapy-sensitive follicular lymphoma with alloHSCT<sup>22</sup>. 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<sup>22</sup>. Durable remissions can also be achieved with nonmyeloablative alloHSCT in patients with advanced chronic lymphocytic leukemia (CLL)<sup>24,27</sup>, and nonmyeloablative alloHSCT is a promising treatment for relapsed mantle cell lymphoma<sup>21,28</sup>. AlloHSCT is the only curative therapy for adults with relapsed acute lymphoblastic leukemia (ALL)<sup>25</sup>.

Although alloHSCT can be an effective therapy for B-cell malignancies, many patients develop persistent or relapsed malignancy after alloHSCT<sup>24,25,28-30</sup>. Evidence for an immunologic graft-versus-malignancy effect exists for most B-cell malignancies<sup>20,21,23,30-32</sup>; 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 to DCI. 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  $1 \times 10^7$ - $5 \times 10^7$  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 after DCI 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  $1 \times 10^7$  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  $1 \times 10^8$  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  $1 \times 10^8$  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

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

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  $1 \times 10^6$  to  $1 \times 10^8$  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 histocompatibility 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<sup>56</sup>. For GVHD to occur, there must be antigenic differences between the recipient and the allograft donor<sup>56</sup>. GVHD is divided into two broad categories, acute GVHD and chronic GVHD. Characteristic features of acute GVHD include maculopapular rash, gastrointestinal disorders (nausea, diarrhea, or ileus), and cholestatic liver disease<sup>57</sup>. Chronic GVHD can manifest with a wide variety of signs and symptoms<sup>57</sup>. Formal definitions of four subsets of GVHD have been published<sup>57</sup>.

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)<sup>58,59</sup>. 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<sup>60</sup>. 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

*Abbreviated Title: Anti-CD19 CAR T-cells*

*Version Date: September 17, 2020*

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

### 1.2.8 Chimeric antigen receptors preclinical background

Preclinical experiments evaluating CAR-expressing T cells as cancer therapy were initiated in 1993.<sup>84,85</sup> These experiments led to a clinical trial of CAR-transduced T cells targeting the  $\alpha$ -folate receptor on ovarian cancer cells; no tumor regressions were observed during this clinical trial.<sup>86</sup> 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<sup>69,72-75,87-89</sup>, lentiviruses<sup>70,90-93</sup>, or transposon systems<sup>94,95</sup> 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.<sup>70,87,96</sup> 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 CD3 $\zeta$  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.<sup>78,87,91,97-99</sup>

Much of the preclinical work evaluating CARs has been performed with CARs targeting the B-cell antigen CD19.<sup>87-89,94,100-102</sup> 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.<sup>89,98</sup> These studies showed that adding CD28 moieties to CARs enhanced antigen-specific cytokine production and proliferation by anti-CD19 CAR T cells.<sup>98,103,104</sup> T cells expressing CARs with CD28 signaling moieties and CD3 $\zeta$  signaling domains were more effective than T cells expressing CARs without CD28 moieties at eradicating human leukemia cells from mice.<sup>103,104</sup> Subsequently, CARs incorporating other signaling domains from costimulatory molecules such as 4-1BB (CD137) were developed.<sup>90</sup> Anti-CD19 CARs containing the signaling domains of both 4-1BB and CD3 $\zeta$  were superior to



*Abbreviated Title: Anti-CD19 CAR T-cells*

*Version Date: September 17, 2020*

CARs containing the signaling domains of CD3 $\zeta$  without any costimulatory domains at eradicating human malignant cells from mice.<sup>91,97</sup> Similar to CD28, including 4-1BB signaling moieties in CARs led to increased CD19-specific proliferation and enhanced in vivo persistence.<sup>91</sup> 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.<sup>91,97</sup>

### 1.2.9 CD19

CD19 is commonly used as the target of CAR T cells because it is expressed on most malignant B cells <sup>105,106</sup>, but the only normal cells that express CD19 are B cells and perhaps follicular dendritic cells <sup>106,107</sup>. Importantly, CD19 is not expressed on pluripotent hematopoietic stem cells <sup>108</sup>. 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 <sup>109</sup>, yet patients that receive chemotherapy plus rituximab do not have an increased rate of common infections when compared to patients who receive chemotherapy alone <sup>110</sup>. Finally, patients can be treated with intravenous infusions of IgG if necessary to increase IgG levels<sup>111</sup>.

#### 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.<sup>69,70,72-74,112-116</sup> 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.<sup>73</sup> This clinical trial utilized a gamma retroviral vector to introduce an anti-CD19 CAR containing the signaling domains of the CD28 and CD3 $\zeta$  molecules.<sup>73</sup> 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.<sup>73</sup> 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.<sup>73</sup> The bone marrow B-cell eradication was confirmed by flow cytometry, and it persisted for over 36 weeks.<sup>73</sup> B cells were also completely absent from the blood during this time, while T cells and other blood cells recovered rapidly.<sup>73</sup> 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<sup>+</sup>, 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.<sup>72</sup>

Seven more patients were subsequently treated with the same regimen of chemotherapy, anti-CD19 CAR T cells, and high-dose IL-2.<sup>72</sup> 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.<sup>72,73</sup> In all 4 patients with B-cell depletion, the depletion lasted for over 36 weeks. The B-cell

*Abbreviated Title: Anti- CD19 CAR T-cells*

*Version Date: September 17, 2020*

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.<sup>73</sup> 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<sup>+</sup> 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.<sup>72</sup> Six of the seven evaluable patients had remissions of their malignancies. Two of the remissions were complete remissions (CRs) of CLL.<sup>72</sup> Both of these CRs were confirmed by multicolor flow cytometry of bone marrow cells.<sup>72</sup> One of these CRs lasted 24 months, and the other is ongoing at 48 months.<sup>72</sup> 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.<sup>72</sup> All of these toxicities peaked within 10 days after the cell infusion and resolved less than 3 weeks after the cell infusion.<sup>72</sup> These acute toxicities correlated with serum levels of the inflammatory cytokines tumor necrosis factor and interferon- $\gamma$ , 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.<sup>72</sup> 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<sup>117</sup>. 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

*Abbreviated Title: Anti-CD19 CAR T-cells*

*Version Date: September 17, 2020*

777 CAR<sup>+</sup> T cells/ $\mu$ L. 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  $1 \times 10^6$  anti-CD19-CAR-expressing T cells/kg bodyweight preceded by a low-dose chemotherapy regimen of 3 daily doses of cyclophosphamide  $300 \text{ mg/m}^2$  and fludarabine  $30 \text{ mg/m}^2$  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<sup>+</sup> 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<sup>92,114-116,118</sup>. Multiple groups have recently demonstrated the 70% to 90% complete remission rates when anti-CD19 CAR T cells are used to treat ALL<sup>114,116,118</sup>. 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<sup>114,116,118</sup>. 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<sup>116,118</sup>.

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 <sup>114,119,120</sup>. 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

*Abbreviated Title: Anti-CD19 CAR T-cells*

*Version Date: September 17, 2020*

(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](#).

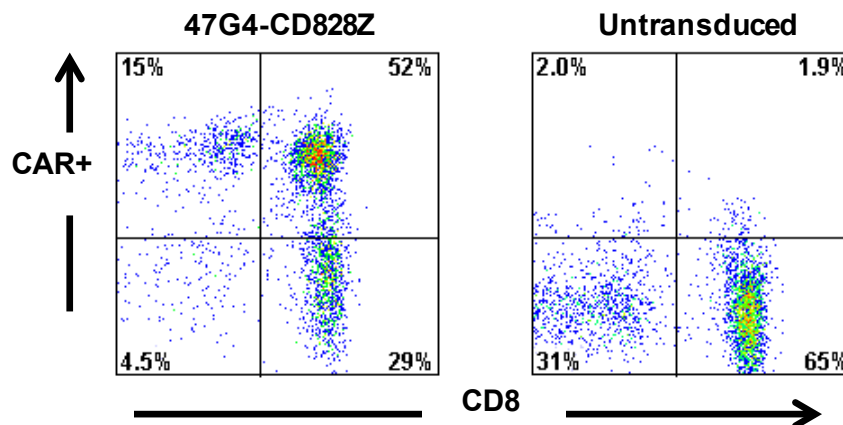
### 1.2.11 Anti-CD19 CAR development and preclinical testing

We designed a CAR that incorporated a single chain variable fragment (scFv) from the fully-human anti-CD19 antibody 47G4<sup>121</sup>. 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<sup>122</sup>. The CAR was designated LSIN-47G4-CD828Z. LSIN stands for self-inactivating lentivirus. A diagram of the CAR is shown in [Figure 1](#). After transductions, we found high levels of cell surface expression of the anti-CD19 CAR on the transduced T cells ([Figure 2](#)).

**Figure 1:** A diagram of the CAR

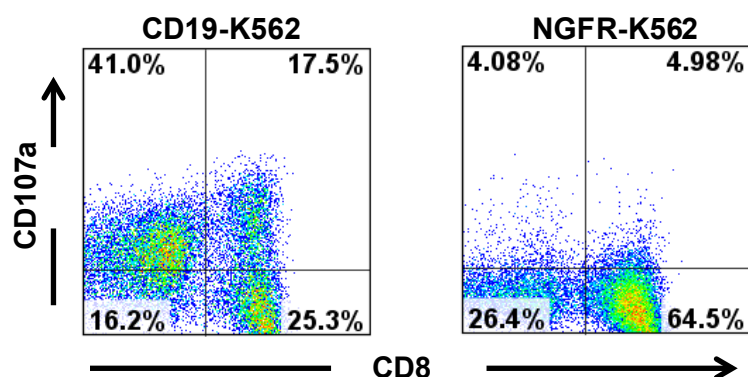


**Figure 2:** the anti-CD19 CAR on the transduced T cells



[Figure 2](#) 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<sup>+</sup> 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.

**Figure 3: Upregulation of CD107a**

**Figure 3** shows upregulation of CD107a, which indicates degranulation and correlates with cytotoxicity<sup>123</sup>, 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<sup>+</sup> lymphocytes. The T cells depicted in **Figure 3** 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 2**.

It is critical to test any new CAR for specificity. To test for specificity, we cultured CAR-expressing T cells or untransduced T cells from the same patient with target cells overnight, and then performed a standard IFN $\gamma$  (enzyme-linked immunosorbent assay) ELISA to see if T cells are activated, as indicated by IFN $\gamma$  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<sup>+</sup> target cells. We performed ELISA assays on culture supernatant from overnight co-cultures of T cells plus either CD19<sup>+</sup> or CD19-negative target cells. T cells transduced with the anti-CD19 CAR produced large amounts of IFN $\gamma$  when they were cultured overnight with the CD19-expressing cell lines but only small amounts of IFN $\gamma$  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 IFN $\gamma$  levels in picograms/mL. The CD19<sup>+</sup> target cells used in these experiments were, primary chronic lymphocytic leukemia cells (CLL), the CD19<sup>+</sup> 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

*Abbreviated Title: Anti-CD19 CART-cells*

*Version Date: September 17, 2020*

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	COL O205	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**

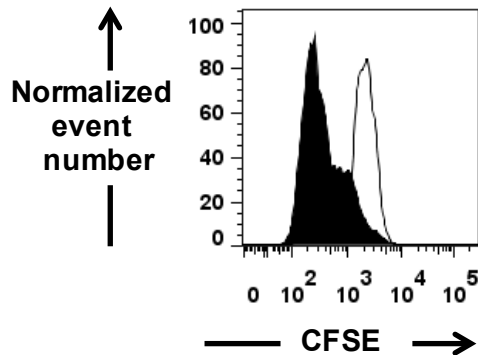
Effector T cells	NALM6	Primary cardiac microvascular endothelial cells	Primary cardiac myocytes	Primary hepatocytes	HH	CEM	T cells alone
47G4-CD828Z	13746	28	21	26	96	62	27
Untransduced	136	12	12	12	26	14	<12

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

*Abbreviated Title: Anti-CD19 CAR T-cells*  
*Version Date: September 17, 2020*

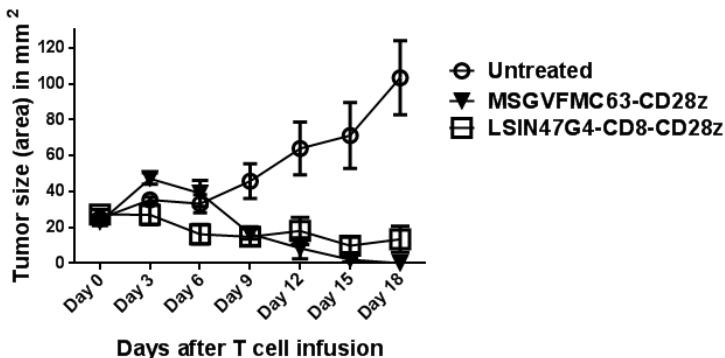
**Figure 4** shows a carboxyfluorescein diacetate, succinimidyl ester (CFSE) proliferation assay in which anti-CD19-CAR-transduced T cells were cultured for 4 days with either CD19-K562 cells or CD19-negative NGFR-K562 cells. CFSE was diluted to a greater degree, indicating more proliferation, when the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

**Figure 4:** T cells were cultured with CD19-K562 target cells



**Figure 5** 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 5**, 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.

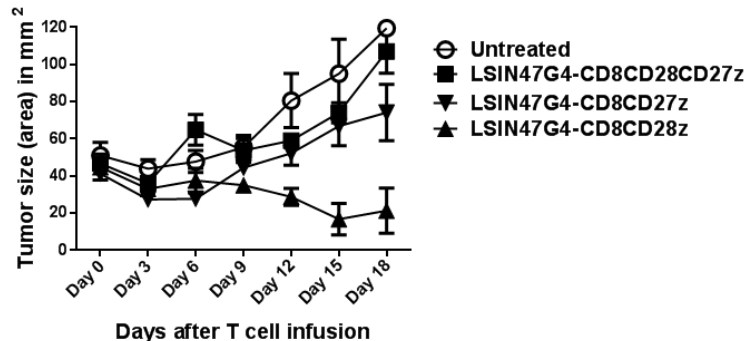
**Figure 5:** Comparison between LSIN-47G4-CD828Z and the CAR currently being used in NCI anti-CD19 CAR clinical trials



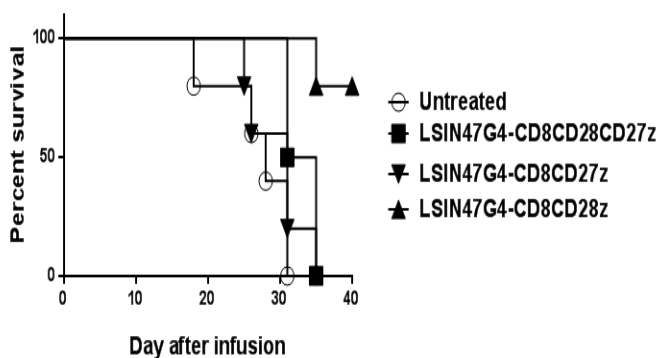
We constructed 2 CARs that had the same 47G4 scFv as LSIN-47G4-CD828Z. Each of these CARs also contained the hinge and transmembrane regions of the human CD8-alpha molecule and the cytoplasmic region of the CD3-zeta molecule. These CARs were encoded by the same lentiviral vector as LSIN-47G4-CD828Z. These CARs had different costimulatory domains than LSIN-47G4-CD828Z, either CD27 or both CD27 and CD28. We established NALM6 human

**Abbreviated Title: Anti-CD19 CAR T-cells****Version Date: September 17, 2020**

CD19<sup>+</sup> 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 (Figure 6 and Figure 7) <sup>124</sup>. 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 6:**

In the experiments depicted in (Figure 6 and Figure 7, anti-CD19-CAR T cells were infused on Day 0, and no other treatments were administered. In the experiments reported in Figure 7, 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 7**



**Abbreviated Title: Anti-CD19 CAR T-cells**

**Version Date: September 17, 2020**

### 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.<sup>1</sup> 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<sup>2</sup> of cyclophosphamide administered daily for 3 days and fludarabine 30 mg/m<sup>2</sup> 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

*Abbreviated Title: Anti-CD19 CAR T-cells*

*Version Date: September 17, 2020*

increases as the dose of T cells administered to mice increases.<sup>131-133</sup> Second, in the setting of allogeneic transplantation, relapsed malignancy is often treated with infusions of allogeneic donor lymphocytes (DCIs).<sup>134,135</sup> 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'.<sup>134,135</sup>

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 3<sup>rd</sup> 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<sup>44</sup>. 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<sup>113,136,137</sup>. 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<sup>136</sup>. 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<sup>113,137</sup>. 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

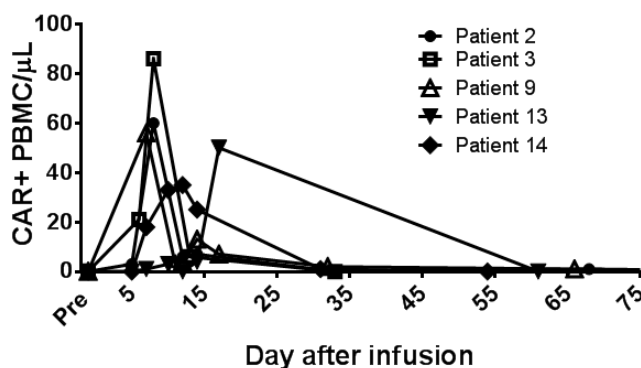
*Abbreviated Title: Anti-CD19 CAR T-cells*

*Version Date: September 17, 2020*

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 8](#))<sup>117</sup>, we hope that the fully-human anti-CD19 CAR will have longer persistence. The graphs shown in [Figure 8](#) 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 8**; absolute numbers of CAR T cells in the blood of 5 patients



#### 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)<sup>118,137</sup> and those in which the CAR T cells were from normal healthy donors (allogeneic)<sup>119,120</sup>. 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<sup>138</sup>, and might be more effective for treating malignancy<sup>139</sup>. Another potential advantage of healthy donor T cells is that they are not tolerized against antigens on the malignant cells.

*Abbreviated Title: Anti-CD19 CAR T-cells*

*Version Date: September 17, 2020*

### 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.

**Abbreviated Title: Anti- CD19 CAR T-cells****Version Date: September 17, 2020**

- 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  $1000/\text{mm}^3$  without the support of filgrastim or other growth factors.
- Platelet count greater than or equal to  $45,000/\text{mm}^3$  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.

*Abbreviated Title: Anti- CD19 CAR T-cells**Version Date: September 17, 2020*

- 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 at the NIH only are potentially eligible under these conditions:
  - At least 6 months have elapsed since the last genetically-modified T-cell therapy that the patient received and there is no evidence of replication-competent retroviruses (evidence must be provided from prior NIH gene-therapy protocol Principal Investigator) and persisting genetically-modified T cells are not detectable in the patient's blood (evidence must be provided by prior NIH gene-therapy protocol Principal Investigator).

## 2.1.2 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.

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

- Patients with current CNS involvement by malignancy (either by imaging or cerebrospinal fluid involvement or biopsy-proven).

## 2.2 SCREENING EVALUATION

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

Note: Screening evaluation testing/procedures are conducted under the separate screening protocol, 01C0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols). The following assessments must be completed within 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<sup>+</sup> 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 CO<sub>2</sub> (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid)



**Abbreviated Title: Anti- CD19 CAR T-cells****Version Date: September 17, 2020**

- CBC with differential
- PT/PTT
- Glucose-6-phosphate dehydrogenase (G6PD) deficiency screening
- Thyroid panel
- Serum cortisol
- $\beta$ -HCG pregnancy test (serum or urine) on all women of child-bearing potential

**2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES**

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

**2.4 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: $0.66 \times 10^6$ anti-CD19 CAR T cells/kg (weight based dosing) (up to a maximum dose of $18 \times 10^6$ CAR+ T cells/kg) infuse on day 0 + Cyclophosphamide: 300 mg/m <sup>2</sup> IV infusion over 30 minutes on days -5, -4 and -3 + Fludarabine: 30 mg/m <sup>2</sup> 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**

*Abbreviated Title: Anti- CD19 CAR T-cells*

*Version Date: September 17, 2020*

### 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 planned 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<sup>2</sup> daily for 3 days and fludarabine 30 mg/m<sup>2</sup> 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<sup>6</sup> 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<sup>6</sup> 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.

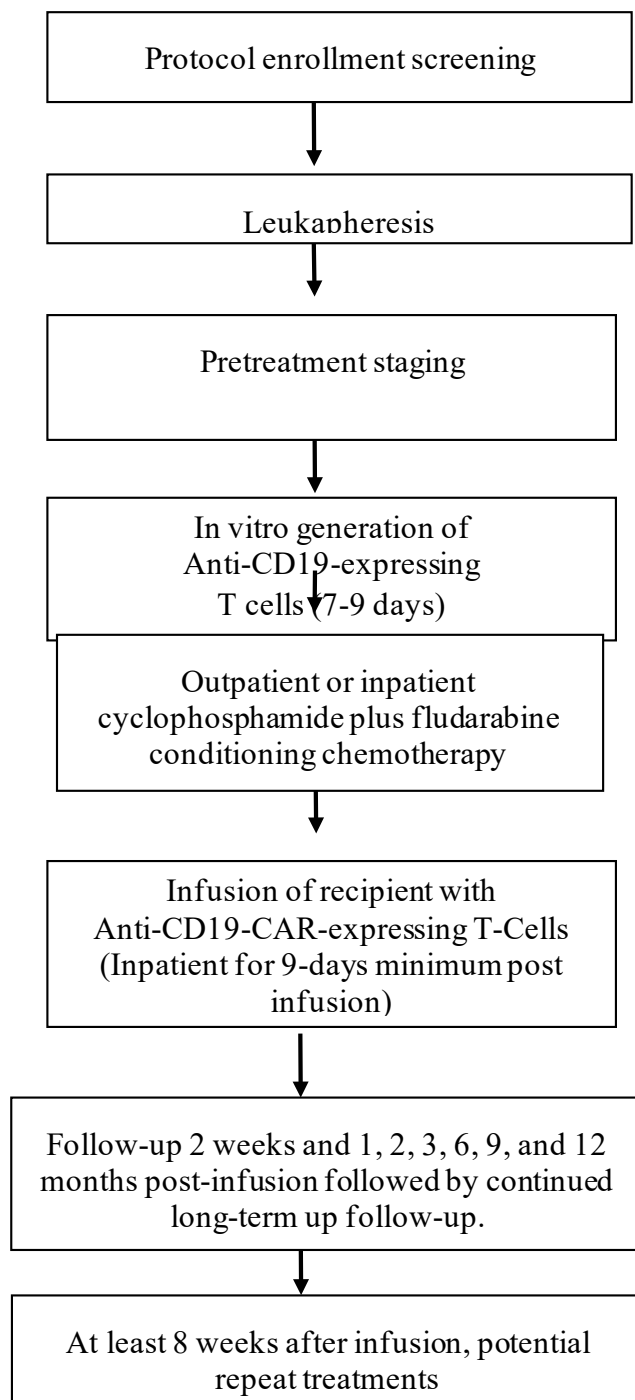
*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

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



*Abbreviated Title: Anti-CD19 CAR T-cells**Version Date: September 17, 2020*

## 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/ $\mu$ L) 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.

**Abbreviated Title: Anti- CD19 CAR T-cells****Version Date: September 17, 2020**

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**

<b>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.</b>	
<b>Dose Level</b>	<b>Dose of antri-CD19 CAR T cells</b>
Level -1	0.3x10 <sup>6</sup> CAR+ T cells per kg of recipient bodyweight
Level 1	0.66x10 <sup>6</sup> CAR+ T cells per kg of recipient bodyweight
Level 2	2.0x10 <sup>6</sup> CAR+ T cells per kg of recipient bodyweight
Level 3	6.0x10 <sup>6</sup> CAR+ T cells per kg of recipient bodyweight
Level 4	18.0x10 <sup>6</sup> 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

*Abbreviated Title: Anti- CD19 CAR T-cells*

*Version Date: September 17, 2020*

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  $18 \times 10^6$  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  $18 \times 10^6$  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**

<b>Number of Patients with DLT at a Given Dose Level</b>	<b>Escalation Decision Rule</b>
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. <ul style="list-style-type: none"> <li>• If 0 of these 3 patients experience DLT, proceed to the next dose level.</li> <li>• 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.</li> </ul>
$\leq 1$ 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



**Abbreviated Title: Anti-CD19 CAR T-cells**

**Version Date: September 17, 2020**

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

### 3.3 STOPPING CRITERIA

- If no responses of PR or CR occur after 2 patients are treated on the highest dose level for both Cohort 1 and Cohort 2, the protocol will be stopped.
- Instructions for how to proceed when toxicity occurs will be as instructed by the dose escalation section of the protocol (3.1.6).

### 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  $6-10 \times 10^9$  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 Surgery Branch Cell Production Facility SB-CPF, Bldg. 10 3W-3808. Phone: 240-858-3755 for cryopreservation at 200 to 300 million PBMC per vial. C. 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<sup>+</sup> 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  $18 \times 10^6$  CAR<sup>+</sup> 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  $18 \times 10^6$  CAR<sup>+</sup> T cells/kg). For example for a patient receiving a first dose of  $2 \times 10^6$  CAR<sup>+</sup> T cells/kg, sufficient cells need to be produced and cryopreserved for a second dose of  $6 \times 10^6$  CAR<sup>+</sup> T cells/kg and a 3<sup>rd</sup> dose of  $18 \times 10^6$  CAR<sup>+</sup> T cells/kg. Cryopreserved cells will be used for all infusions unless poor cell growth precludes obtaining enough cells rapidly enough for

**Abbreviated Title: Anti- CD19 CART-cells****Version Date: September 17, 2020**

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 Surgery Branch Cell Production Facility SB-CPF preclinical core. Each vial will contain 10-20 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<sup>+</sup> 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 2<sup>nd</sup> 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.

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

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

3.4.3.1 **Overall summary of the treatment plan**

**Table 4**

<b>Drug</b>	<b>Dose</b>	<b>Days</b>
Cyclophosphamide	300 mg/m <sup>2</sup> IV infusion over 30 minutes	Daily x 3 doses on days -5, -4, -3
Fludarabine	30 mg/m <sup>2</sup> 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,

*Abbreviated Title: Anti-CD19 CAR T-cells*

*Version Date: September 17, 2020*

-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<sup>2</sup> 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<sup>2</sup> 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<sup>2</sup> 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 15.3, 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 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.

**Abbreviated Title: Anti-CD19 CAR T-cells****Version Date: September 17, 2020**

- 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<sup>nd</sup> and prior to 3<sup>rd</sup> 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  $18 \times 10^6$  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  $18 \times 10^6$  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

**Abbreviated Title: Anti- CD19 CART-cells****Version Date: September 17, 2020**

- 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 Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755 for research purposes. 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 Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755.
- In addition to the CPT tubes, draw 16 mL of blood to obtain serum for research purposes (2 SST tubes, 8 mL per tube) within 3 days prior to the start of the chemotherapy. Send to Figg lab; For sample pick-up, page 102-11964.

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

- TBNK (T, B, and NK cell)
- Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid (to be repeated on the first day of the chemotherapy at the discretion of the PI)
- Serum quantitative immunoglobulins
- ABO typing

**Abbreviated Title: Anti-CD19 CAR T-cells****Version Date: September 17, 2020**

- 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
- $\beta$ -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<sub>2</sub> (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), 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). Send CPT tubes to the Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755. Send 1 SST tube to the Figg lab; for sample pick-up, page 102-11964.

### 3.5.3 Post-infusion outpatient evaluation

Patients will be seen at the NIH in follow-up to evaluate disease status and late problems related to CAR T-cell infusion at the following time-points: +14 (+/- 1 day), +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 (+/-

**Abbreviated Title: Anti-CD19 CAR T-cells****Version Date: September 17, 2020**

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. Send CPT tubes to the Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755. **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. Send 1 SST tube to the Figg lab; for sample pick-up, page 102-11964.

Note: **after the first year of follow-up, research blood will be reduced to 4 CPT tubes (32 mL total) during required protocol visits.** 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 Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755.
- Physical exam with vital signs and oxygen saturation
- (Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid)
- TBNK
- Blood for serum quantitative immunoglobulins
- CBC with differential. **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](#)

**Abbreviated Title: Anti-CD19 CART-cells**  
**Version Date: September 17, 2020**

### 3.6 STUDY CALENDAR (TABLE 5)

Procedures <sup>a</sup>	Screening/ Baseline	Pre-cell infusion/ Day 0	Day +7 <sup>b</sup>	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 months after day 365 up to 3 years then annually (+/- 30 days)
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#### Clinical Assessments

<i>History and PE</i>	X	X	X	X	X	X	X	X	X	X
<i>Vital signs, O2 saturation</i>	X	X	X	X	X	X	X	X	X	X
<i>Height, weight</i>	X	X								
<i>Performance Score</i>	X	X								

#### Laboratory Assessments

<i>CBC with differential</i>	X	X	X	X <sup>P</sup>	X <sup>P</sup>	X	X	X	X	X
<i>Sodium, Potassium Chloride, CO2, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Phosphorus, Alkaline Phosphatase, ALT, AST, T. Bilirubin, D. Bilirubin, LDH, Total Protein, CK, Uric Acid</i>	X	X	X	X	X	X	X	X	X	X
<i>PT/PTT</i>	X		X (also on day 4)							
<i>Thyroid Stimulating Hormone, T3</i>	X									
<i>Quantitative immunoglobulins</i>	X			X	X	X	X	X	X	X





**Abbreviated Title: Anti-CD19 CART-cells**  
**Version Date: September 17, 2020**

Procedures <sup>a</sup>	Screening/ Baseline	Pre-cell infusion/ Day 0	Day +7 <sup>b</sup>	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 months after day 365 up to 3 years then annually (+/- 30 days)
<i>Serum cortisol</i>	X									
<i>250 microgram cosyntropin test<sup>e</sup></i>	X									
<i>EKG, echocardiogram</i>	X									
<i>RCL (replication competent lentivirus)</i>	X					X	X		X	X <sup>i</sup>
<i>Central Venous catheter placement</i>		X								
<i>Donor Venous Assessment</i>	X									
<i>Bone marrow aspirate/biopsy with flow<sup>f</sup></i>	X									
<i>Research Blood</i>		X	X	X <sup>o</sup>	X	X	X	X	X	X <sup>l</sup>
<i>Research Apheresis</i>				X <sup>o</sup>						
<i>Adverse Event</i>		X	X	X	X	X	X	X	X	X
<i>Concomitant Medications</i>	X	X	X	X	X	X	X	X	X	X

<sup>a</sup> see section 2.2 and section 3.4 for details

<sup>b</sup> see section 3.4 for details of testing during hospitalization

<sup>c</sup> CT scan for lymphoma and CLL patients at the indicated time-points including within 14 days of chemo start.

<sup>d</sup> 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.

*Abbreviated Title: Anti- CD19 CART-cells*  
*Version Date: September 17, 2020*

<sup>e</sup> only if clinically indicated or if needed to document relapse suspected on CT

<sup>f</sup> 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.

<sup>g</sup> only at 2, 3, 4, 5 year post-infusion time points, see section 3.6 for details

<sup>h</sup> 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

<sup>i</sup> Annually RCL blood collection will only continue after the 1 year time-point if a previous RCL test has been positive

<sup>j</sup> Only at Baseline

<sup>k</sup> Only at Screening. Lumbar punctures may also need to be repeated after treatment if clinically indicated.

<sup>l</sup> until 5 years after infusion

<sup>m</sup> Only if positive before protocol treatment or if clinically indicated

<sup>n</sup> CT and PET will be performed only at day 30

<sup>o</sup> 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.

<sup>p</sup> 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. If all of these samples are negative for RCL, blood collection for RCL will be discontinued after the 12 month time-point.

- In case of detection of replication-competent lentivirus, the following actions will be taken:
  - a. Immediately report the finding of RCL to the FDA, the 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 removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days after 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.

*Abbreviated Title: Anti-CD19 CART-cells*

*Version Date: September 17, 2020*

- 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.

## 4 CONCOMITANT MEDICATIONS/MEASURES

### 4.1 ROUTINE SUPPORTIVE CARE

- Patients with a CD4 T-cell count less than 200/ $\mu$ L 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/ $\mu$ L are documented. If ganciclovir, valganciclovir, or foscarnet are started, valacyclovir or acyclovir will generally be discontinued.
- Patients with a CD4 T-cell count less than 200/ $\mu$ L will be maintained on valacyclovir or acyclovir. This prophylaxis will be stopped after 2 consecutive CD4 counts greater than 200/ $\mu$ L 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<sup>o</sup>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

**Abbreviated Title: Anti- CD19 CAR T-cells**

**Version Date: September 17, 2020**

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/ $\mu$ L.
- 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**

*Abbreviated Title: Anti- CD19 CAR T-cells*  
*Version Date: September 17, 2020*

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. The amount of blood that may be drawn from adult patients for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

### **5.1 CORRELATIVE STUDIES FOR RESEARCH**

#### **5.1.1 Biospecimen collection before the start of the conditioning chemotherapy:**

- One heparinized syringe containing 5 to 8 mL of bone marrow aspirate to be sent to Surgery Branch Cell Production Facility SB-CPF, Bldg. 10 3W-3808, Phone: 240-858-3755.
- 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 Surgery Branch Cell Production Facility SB-CPF, Bldg. 10 3W-3808, Phone: 240-858-3755.
- 16 mL of blood will be drawn to obtain serum for research purposes (2 SST tubes, 8 mL per tube) within 14 days prior to the start of the chemotherapy. The SST tubes will be sent to Figg lab; For sample pick-up, page 102-11964.
- 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). The 1 SST tube will be sent to the Figg lab; For sample pick-up, page 102-11964. The 6 CPT tubes sent to Surgery Branch Cell Production Facility SB-CPF, Bldg. 10 3W-3808, Phone: 240-858-3755.

#### **5.1.3 Biospecimen collection during outpatient follow-up**

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



*Abbreviated Title: Anti-CD19 CAR T-cells*

*Version Date: September 17, 2020*

- 1 SST tube (8 mL) of Research Blood will be obtained for serum collection. The SST tubes will be sent to Figg lab; For sample pick-up, page 102-11964.

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 monocytes per microliter of blood. Ex vivo immunological assays might be used to measure the antigen-specific functional activity of the CAR+ T cells and will consist of assays such as enzyme-linked immunosorbent assays (ELISAs), intracellular cytokine staining, and anti-CD107a degranulation assays. Immunological assays will be standardized by the inclusion of pre-infusion recipient PBMC and in some cases an aliquot of the engineered T cells cryopreserved at the time of infusion.
- Serum cytokine levels will also be measured by enzyme-linked immunosorbent assays (ELISAs) or similar assays.
- 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 analysis for detection of replication competent lentiviruses (RCL) by PCR **at 3 months, 6 months, and 12 months after cell administration**. Infusion cells will be tested for RCL prior to infusion by PCR targeting the VSV-G gene. Blood collection for RCL monitoring will be discontinued if all patient samples have been negative for RCL at the 12 month time-point. If any post-treatment samples are positive, further analysis of the RCL and more extensive patient follow-up will be undertaken, in consultation with the FDA. RCL PCR assays are performed by the National Gene Vector Laboratory at Indiana University. The results of these tests are maintained by the National Gene Vector Laboratory at Indiana University and by the Surgery Branch (SB) research team.
- Due to nature of these studies, it is expected that expansion of specific T-cell clones will be observed as T-cells proliferate in response to the targeted antigen. Therefore, care will be taken to track T-cell persistence, but presence of an oligoclonal T cell population does not indicate an insertional mutagenesis event. If any patient shows an increasing population of CAR gene transduced T cells at month 6 or later (by FACS staining or qPCR), the previously archived samples will be subjected to techniques that would allow

the identification of predominant clonal populations of transduced cells. Such techniques may include T cell cloning or LAM-PCR. If a predominant or monoclonal T cell clone derived from CAR gene transduced cells is identified during the follow-up, the integration site and sequence will be identified and subsequently analyzed against human genome database to determine whether the sequences are associated with any known human cancers. If a predominant integration site is observed, the T cell cloning or LAM-PCR test will be used at an interval of no more than three months after the first observation to see if the clone persists or is transient. In all instances where monoclonality is persistent and particularly in instances where there is expansion of the clone, regardless of whether or not the sequence is known to be associated with a known human cancer, the subject should be monitored closely for signs of malignancy, so that treatment, if available, may be initiated early.

#### 5.1.5 Additional biopsies and additional blood draws

Patients might be asked to undergo biopsies (up to 3) or additional blood draws for research purposes. Additional blood draws might be necessary to investigate T cell responses and serum cytokine levels in cases of clinical events such as rapid regressions of malignancy or toxicity. Biopsies including open surgical biopsies, fine needle aspirations, and core needle biopsies could be used to investigate CAR T-cell persistence or function at tumor sites. Open surgical, fine needle, or core needle biopsies might also be needed in some but not all patients to confirm continued antigen expression by tumor cells in order to meet protocol eligibility requirements for antigen expression on the tumor cells before any CAR T-cell infusion. For core needle biopsies 2 to 4 cores will be obtained. For fine needle aspirations, 1 to 3 aspirations will be performed. Standard techniques will be used for biopsies which may include CT and/or ultrasound guided biopsy. These research biopsies or blood draws are optional, and patients can participate in this trial whether or not they agree to undergo biopsies for research purposes unless the biopsies are needed to prove that the target antigen is expressed on the tumor for protocol eligibility. These biopsies will only be performed if minimal morbidity is expected based on the procedure performed and the granulocyte and platelet count. Biopsy tissue will be processed in the NIH Department of Pathology. Studies will be performed to evaluate the antigen expression by the tumor and to evaluate the presence of transduced cells. Remainder material from any clinical biopsies obtained may also be utilized for research purposes.

#### 5.1.6 Future studies

Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if 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

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

### 5.2.1 Samples Sent to Figg Lab

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

After delivery to the SB-CPF, peripheral blood mononuclear cell samples will be sent to the Head, Clinical Support Laboratory Clinical Services Program, Applied/Developmental Directorate Frederick National Laboratory for Cancer Research for processing and cryopreservation. They will be stored long-term at the NCI Frederick Repository.

### 5.2.2 Sample Storage, Tracking and Disposition

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

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

##### 5.2.2.1.1 BPC Contact Information

Please e-mail [NCIBloodcore@mail.nih.gov](mailto:NCIBloodcore@mail.nih.gov) at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact [NCIBloodcore@mail.nih.gov](mailto:NCIBloodcore@mail.nih.gov).

##### 5.2.2.1.2 Sample Data Collection

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

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

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

#### 5.2.2.1.3 Sample Storage and Destruction

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

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

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

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

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

#### 5.2.3 Sample Storage, Tracking, and Disposition for Surgery Branch

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

*Abbreviated Title: Anti- CD19 CAR T-cells*

*Version Date: September 17, 2020*

#### 5.2.4 Protocol Completion/Sample Destruction

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

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

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

#### 5.2.5 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.

##### 5.2.5.1 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.

##### 5.2.5.2 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.

##### 5.2.5.3 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 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

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

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.

## **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). 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. Document AEs from the first study intervention, Study Day 1, through 30 days after the study treatment was last administered. Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

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

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

- 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, this will be reported expeditiously per requirements in section [7.2.1](#).

#### 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 DATA SHARING PLANS

### 6.2.1 Human Data Sharing Plan

#### What data will be shared?

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

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
- Coded, linked or identified data with approved outside collaborators under appropriate agreements.

#### How and where will the data be shared?

Data will be shared through:

- An NIH-funded or approved public repository. Insert name or names: [ClinicalTrials.gov](https://clinicaltrials.gov).
- BTRIS (automatic for activities in the Clinical Center)
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

#### When will the data be shared

- Before publication.
- At the time of publication or shortly thereafter.

*Abbreviated Title: Anti-CD19 CART-cells*

*Version Date: September 17, 2020*

### 6.2.2 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 ( $\leq 1.5$  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  $\leq 1.0$  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.  $\geq 50\%$  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.



**Abbreviated Title: Anti- CD19 CART-cells****Version Date: September 17, 2020**

3. If multiple splenic and hepatic nodules are present, they must regress by  $\geq 50\%$  in the SPD. There must be a  $\geq 50\%$  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.  $\geq 50\%$  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  $\geq 1500/\text{ul}$ , platelets  $>100,000/\text{ul}$ , hemoglobin  $> 11.0$  g/dl (untransfused), and
  5. Blood B lymphocyte count  $<4,000/\text{ul}$
  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  $\geq 50\%$  decrease in peripheral B lymphocyte count from pre-treatment value, a  $\geq 50\%$  reduction in lymphadenopathy, or a  $\geq 50\%$  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  $\geq 1,500/\text{ul}$ , or a 50% improvement from pre-treatment value
    2. Platelets  $> 100,000/\text{ul}$  or 50% improvement from pre-treatment value
    3. Hemoglobin  $> 11.0 \text{ g/dl}$  (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  $\geq 50\%$  increase in the greatest diameter of any lymph node that was enlarged pre-treatment.
    2. The appearance of new palpable lymph nodes
    3. A  $\geq 50\%$  increase in the absolute number of circulating B lymphocytes (value must exceed 5,000/ul)
    4.  $\geq 50\%$  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  $> 2 \text{ gm/dl}$  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](http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm)).

## 7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

### 7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found [here](#).

### 7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING/IRB REPORTING

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

#### 7.2.1 Expedited Reporting

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

#### 7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found [here](#).

### 7.3 NCI CLINICAL DIRECTOR REPORTING

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

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

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

### 7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

#### 7.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify 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

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

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

#### 7.4.2.2 Progress Report and Data Analysis

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

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

## 7.5 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

### 7.5.1 Principal Investigator/Research Team

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

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

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

### 7.5.2 Safety Monitoring Committee (SMC)

This protocol will be periodically reviewed by an intramural Safety Monitoring Committee. Initial review will occur as soon as possible after the annual IRB continuing review date.

*Abbreviated Title: Anti-CD19 CART-cells*

*Version Date: September 17, 2020*

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 based on the risks presented in the study. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period.

The SMC review will focus on unexpected protocol-specific safety issues that are identified during the conduct of the clinical trial.

Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

## **8 SPONSOR SAFETY REPORTING**

### **8.1 DEFINITIONS**

#### **8.1.1 Adverse Event**

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

#### **8.1.2 Serious Adverse Event (SAE)**

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

- Death,
- A life-threatening adverse event (see section 8.1.3)
- Inpatient hospitalization or prolongation of existing hospitalization
  - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
  - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient convenience) is not considered a serious adverse event.
  - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

### 8.1.3 Life-threatening

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

### 8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 4.0.

### 8.1.5 Relationship to Study Product

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

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

## 8.2 ASSESSMENT OF SAFETY EVENTS

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

SAEs will be:

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

For timeframe of recording adverse events, please refer to section [6.1](#) All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor with the exception of any listed in section [8.4](#)

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

### **8.3 REPORTING OF SERIOUS ADVERSE EVENTS**

Any AE that meets a protocol-defined serious criterion or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in section **8.4**.

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

SAE reports will be submitted to the Center for Cancer Research (CCR) at:

[OSROSafety@mail.nih.gov](mailto:OSROSafety@mail.nih.gov) and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at:

<https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=157942842>

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

### **8.4 REPORTING PREGNANCY**

#### **8.4.1 Maternal exposure**

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

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

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

#### **8.4.2 Paternal exposure**

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

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

### **8.5 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND**

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

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

*Abbreviated Title: Anti-CD19 CAR T-cells*

*Version Date: September 17, 2020*

## 9 CLINICAL MONITORING

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

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

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

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

## 10 STATISTICAL CONSIDERATIONS

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.



*Abbreviated Title: Anti-CD19 CAR T-cells*

*Version Date: September 17, 2020*

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 6.2. 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 13<sup>th</sup> 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.

## **11 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.

## **12 HUMAN SUBJECTS PROTECTIONS**

### **12.1 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.

### **12.2 PARTICIPATION/SELECTION RATIONALE**

*Abbreviated Title: Anti-CD19 CAR T-cells*

*Version Date: September 17, 2020*

- 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.

### **12.3 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.

### **12.4 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 12.4), all subjects  $\geq$  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 NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects

*Abbreviated Title: Anti- CD19 CAR T-cells*

*Version Date: September 17, 2020*

who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

## **12.5 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 (see section 13 for details). First, chemotherapy that could cause cytopenias is part of the protocol. As with any chemotherapy that causes neutropenia and thrombocytopenia, this chemotherapy could cause toxicities such as infections and bleeding. The second category of toxicity is cytokine-release type toxicities such as high fevers, hypotension, and fever. A third area of toxicity is neurological toxicity such as delirium, obtundation, myoclonus, seizures, headache, and transient focal neurological toxicities including aphasia and focal paresis. Cytokine-release-type toxicities and neurological toxicities have appeared in other clinical trials of CAR T cells during the first 2 weeks after CAR T cells were infused.<sup>72,115</sup> 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 5<sup>th</sup> possible toxicity is hypogammaglobulinemia. Hypogammaglobulinemia has been a complication of many patients on clinical trials of anti-CD19 CAR-expressing T cells.<sup>72,73</sup> Hypogammaglobulinemia in these patients was routinely treated with infusions of intravenous immunoglobulins.<sup>72</sup>

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.<sup>75-77</sup>

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

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

### **12.5.1 Risks of exposure to Ionizing Radiation**

The procedures for performing the CT scans will follow clinical policies, no special procedures apply to these additional assessments for research purposes. In summary, subjects may receive additional radiation exposure from up to seven (7) CT neck +CAP, three (3) CT-Guided biopsies and seven (7) 18FDG-PET/CT scans in the first year of the study.

The total additional radiation dose for research purposes will be approximately 19.9 rem in the first year of the study. This amount is more than would be expected from everyday background radiation. Being exposed to too much radiation can cause harmful side effects such as an increase in the risk of cancer. The risk of getting cancer from the radiation exposure in this study is 2.0 out of 100 (2.0%) and of getting a fatal cancer is 1.0 out of 100 (1.0%).

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

#### 12.5.2 Risks of Scans and Contrast

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

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

#### 12.5.3 Risks of blood Sampling

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

#### 12.5.4 Risks of Bone marrow aspiration and biopsy

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

#### 12.5.5 Risks of Intravenous Catheter

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

#### 12.5.6 Risks of Lumbar Puncture

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

#### 12.5.7 Risks of Apheresis

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

### **12.6 CONSENT PROCESS AND DOCUMENTATION**

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

**Abbreviated Title: Anti-CD19 CART-cells**

**Version Date: September 17, 2020**

ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

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

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

### **13 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.

#### **13.1 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 3<sup>rd</sup> generation lentiviral vector includes the murine stem cell virus promoter, and a truncated version of the woodchuck post-transcriptional regulatory element (WPRE) designated oPRE<sup>122</sup>. 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://bmb1.od.nih.gov/sect3bsl2.htm>

#### **13.2 COMMERCIAL AGENTS**

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

##### **13.2.1 Cyclophosphamide**

###### **13.2.1.1 Source**

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

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.

#### 13.2.1.2 Administration procedures

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

### 13.2.2 FLUDARABINE

#### 13.2.2.1 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.

#### 13.2.2.2 Administration procedures

Fludarabine is administered as an IV infusion in 100 ml 0.9% sodium chloride, USP over 15 to 30 minutes. The doses will be based on body surface area (BSA).

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**Abbreviated Title: Anti- CD19 CART-cells****Version Date: September 17, 2020**

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**15 APPENDICES****15.1 APPENDIX A: PERFORMANCE STATUS CRITERIA**

<b>ECOG Performance Status Scale*</b>	
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.

**15.2 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**

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

- 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

*Abbreviated Title: Anti-CD19 CAR T-cells*

*Version Date: September 17, 2020*

### **15.3 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.<sup>72</sup>

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:

**Abbreviated Title: Anti-CD19 CAR T-cells****Version Date: September 17, 2020**

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.

*Abbreviated Title: Anti- CD19 CART-cells**Version Date: September 17, 2020*

- 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 (FIO<sub>2</sub>) 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.

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

21. If no improvement in hypotension or tachycardia occurs within 6 hours of tocilizumab infusion, consider other agents such as methylprednisolone 1 to 2 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



**15.4 APPENDIX D: CLINICAL STAGING AND TREATMENT OF ACUTE GVHD****Clinical Staging and treatment of Acute GVHD [58.59](#)**

<b>Stage</b>	<b>Skin</b>	<b>Liver</b>	<b>Gut</b>
1	Rash < 25% BSA <sup>a</sup>	Total bilirubin 2.0-2.9 mg/dl <sup>b</sup>	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

<sup>a</sup>BSA = body surface area; use “rule of nines” or burn chart to determine extent of rash.

<sup>b</sup>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.

<sup>c</sup> 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](#)**

<b>Grade<sup>e</sup></b>	<b>Skin</b>	<b>Liver</b>	<b>Gut</b>
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 <sup>d</sup>	Stage 4 <sup>e</sup>	Stage 4	Stage 4

**Abbreviated Title: Anti-CD19 CART-cells**

**Version Date: September 17, 2020**

<sup>c</sup>Criteria for grading given as minimum degree of organ involvement required to confer that grade. The highest single organ stage determines the overall grade.

<sup>d</sup>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.

<sup>e</sup>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.

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

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.

*Abbreviated Title: Anti- CD19 CART-cells*

*Version Date: September 17, 2020*

## 15.5 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.	

**Abbreviated Title: Anti- CD19 CART-cells****Version Date: September 17, 2020**

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.
<p>9. Hang a primary line of 250cc NS at a kvo rate - <b>NEW</b> bag and <b>NEW</b> tubing.</p> <p>This <b>MUST</b> be ready and infusing <b>prior</b> to the cells being delivered to the unit.</p> <p>The patient's primary IV hydration can infuse via a separate lumen while the cells are infusing, but <b>NO MEDs</b> should be administered during this time.</p> <p>Have a second bag of 250cc NS and tubing ready as an emergency line.</p>	<p>This will be the dedicated NS line for infusing the cells. Under no circumstances are any other substances to be infused into the line.</p> <p>Cell death occurs quickly – the infusion must be initiated immediately.</p> <p>Do not infuse medication during the cell infusion. If emergency meds must be administered, use the hydration or emergency NS IV line.</p> <p>This will be the emergency IV solution and can be used for medication administration.</p> <p><b>Do not use an inline filter for cells.</b></p>
<p>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.</p> <p>It is critical to be at the bedside awaiting the arrival of the cells for infusion.</p>	<p>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. <b>Cell death occurs as soon as the cells are removed from the laboratory.</b> Initiate the infusion as quickly as possible.</p>
<p>12. Prior to spiking the cell bag, two RNs will perform the identification procedure. <b>Both</b> RNs must sign the tag on the cell bag.</p>	
<p>13. Infuse the cells by infusion pump or syringe over 20-30 minutes.</p> <p>a. Piggyback the cells into the dedicated NS line; use the backflush technique to prime the line.</p> <p>b. While the cells are infusing, <b>gently</b> agitate the bag of cells <b>every few minutes</b>. When the cell bag is empty, backflush NS to rinse the bag</p>	<p>This prevents the cells from clumping in the bag.</p>

*Abbreviated Title: Anti- CD19 CART-cells**Version Date: September 17, 2020*

<p>and infuse this at the same rate as the cells; rinse bag until NS runs clear.</p> <p>c. <u>Note: in some cases cells will arrive from DTM in a syringe. In this case infuse the cells via syringe over 20-30 minutes.</u></p>	
<p>14. Measure and record VS before and after the cell infusion, q1h x 4, and then q4h after completion of the infusion.</p> <p>a. Assess and document the patient's respiratory and circulatory status post cell infusion.</p>	
<p>15. Documentation:</p> <p>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.</p> <p>b. Document the cell infusion in CRIS using the appropriate screens.</p>	