**Abbreviated Title:** Anti-CD19 and anti-CD20 CAR T cells

**CC Protocol #:** *20-C-0008A*

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**Title:** T Cells Expressing Fully-human Anti-CD19 and Anti-CD20 Chimeric Antigen Receptors for Treating B-cell malignancies and Hodgkin Lymphoma

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**Investigational Agents:**

| | |
|---|---|
| Drug Name: | Anti-CD19 and anti-CD20 CAR T cells |
| IND Number: | 19203 |
| Sponsor: | Center for Cancer Research |
| Manufacturer: | NIH DTM |

**Commercial Agents:** Cyclophosphamide, Fludarabine, OKT3

## PRÉCIS

**Background:**

- Improved treatments for a variety of treatment-resistant malignancies including B-cell lymphomas, and chronic lymphocytic leukemia (CLL) and Hodgkin lymphoma 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 patients with leukemia or lymphoma. These results have established anti-CD19 CAR T cells as an important therapy for relapsed lymphoma, but only about 40% of patients receiving anti-CD19 CAR T cells have durable complete remissions.
- Most B-cell malignancies express CD19 and CD20, but expression of CD19 and CD20 can be lost or diminished.
- The malignant cells of Hodgkin lymphoma, Hodgkin Reed-Sternberg cells, originate from B cells, which is the rationale for treating Hodgkin lymphoma with T cells targeting CD19 and CD20.
- CD19 and CD20 are not expressed by normal cells except for B cells and some plasma cells.
- We have constructed a novel gene therapy construct that encodes a fully-human anti-CD19 CAR with a CD28 domain and a fully-human anti-CD20 CAR with a 4-1BB domain.
- T cells expressing this CAR construct, called Hu1928-Hu20BB, can specifically recognize CD19 and CD20-expressing target cells in vitro and eradicate CD19 or CD20-expressing tumors in mice.
- One CAR expressed in this CAR construct, Hu19-CD828Z has been tested in humans before. The other CAR in the total construct, Hu20-CD8BBZ, 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 and anti-CD20 CAR construct to patients with advanced B-cell malignancies and Hodgkin lymphoma.

**Exploratory**

- Evaluate serum cytokine levels and associations with anti-malignancy efficacy and toxicity
- Evaluate clinical predictors of anti-lymphoma responses and toxicity.
- Evaluate phenotype of infused CAR T cells and CAR T cells from the blood of patients.

**Eligibility:**

- Patients must have any B-cell lymphoma, or CLL/SLL, Gray-zone lymphoma, nodular lymphocyte-predominance Hodgkin lymphoma, or classical Hodgkin lymphoma with any CD19 or CD20 expression on Reed-Sternberg cells. Lower grade lymphomas transformed to DLBCL are potentially eligible as is primary mediastinal B-cell lymphoma and all other subtypes of DLBCL. Burkitt and mantle cell lymphoma are potentially eligible.
- 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 1.5 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 hepatitis B or hepatitis C.
- Absolute neutrophil count≥1000/μL, platelet count≥50,000/μL, hemoglobin≥8g/dL
- Serum ALT and AST less or equal to 3 times the upper limit of the institutional normal unless liver involvement by malignancy is demonstrated.
- At least 14 days must elapse between the time of any prior systemic treatment (including corticosteroids) and protocol-required leukapheresis or CAR T-cell infusion. Thirty days must elapse from therapy with antibodies targeting CD19 or CD20 and CAR T-cell infusion.
- The patient's malignancy will need to be assessed for CD19 and C20 expression by flow cytometry or immunohistochemistry performed at the NIH. If unstained, paraffin-embedded bone marrow or lymphoma tissue sections are available from prior biopsies, these can be used to determine CD19 and CD20 expression by immunohistochemistry; otherwise, patients will need to come to the NIH for a biopsy to determine CD19 and CD20 expression. The sample for CD19 and CD20 expression must come from a biopsy obtained after any CD19 or CD20-targeted therapies such as monoclonal antibodies if such antibodies or CAR T-cell therapies have been received by the patient.
- For classical Hodgkin lymphoma only, a biopsy from any time from any institution that shows any CD19 or CD20 expression on Reed-Sternberg cells is adequate for eligibility. CD19 or CD20 expression on the Reed-Sternberg cells that is weak or only present on some Reed-Sternberg cells by immunohistochemistry is compatible with protocol eligibility.
- For all lymphoma types except for classical Hodgkin lymphoma, either CD19 or CD20 expression must be "uniform". "Uniform" CD19 or CD20 expression is defined as no

obvious lymphoma population lacking antigen expression can be present. Antigen expression can be assessed by either immunohistochemistry or flow cytometry.

**Design:**

- This is a phase I dose-escalation trial
- Patients will undergo leukapheresis
- T-cells obtained by leukapheresis will be genetically modified to express the Hu1928-Hu20BB CAR construct.
- Patients will receive a lymphocyte-depleting chemotherapy conditioning regimen with the intent of enhancing the activity of the infused CAR-expressing T cells.
- The chemotherapy conditioning regimen is cyclophosphamide 500 mg/$m^2$ daily for 3 days and fludarabine 30 mg/$m^2$ daily for 3 days. Fludarabine will be given on the same days as the cyclophosphamide.
- Two days after the chemotherapy ends, patients will receive an infusion of anti-CAR-expressing T cells.
- The initial dose level of this dose-escalation trial will be $0.66x10^6$ $CAR^+$ T cells/kg of recipient bodyweight.
- The cell dose administered will be escalated until a maximum tolerated dose is determined.
- Following the T-cell infusion, there is a mandatory 14-day inpatient hospitalization to monitor for toxicity.
- Outpatient follow-up is planned for 2 weeks,16 days (neurologic checks), 21 days (neurologic checks) and 1, 2, 3, 4, 6, 9, and 12 months after the CAR T-cell infusion; less frequent follow-up is required more than 1 year after infusion. Long-term gene-therapy follow-up for a total of 15 years after infusion is required.

## TABLE OF ABBREVIATIONS

| | |
|---|---|
| Allo-HSCT | 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 |
| CR | Complete remission |
| CRO | Central Registration Office |
| CRP | C-reactive protein |
| 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 |
| 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 |
| RCR | Replication competent retroviruses |
| 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 |

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## STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

# 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 and anti-CD20 CAR construct to patients with advanced B-cell malignancies and Hodgkin lymphoma.

### 1.1.2 Secondary Objective(s)

- Evaluate the in vivo persistence and peak blood levels of CAR T cells.
- Assess for evidence of anti-malignancy activity by T cells expressing both an anti-CD19 CAR and an anti-CD20 CAR
- Assess the relationship of CD19 and CD20 expression on treatment outcomes.

### 1.1.3 Exploratory Objective(s)

- Evaluate serum cytokine levels and associations with anti-malignancy efficacy and toxicity
- Evaluate clinical predictors of anti-lymphoma responses and toxicity.
- Evaluate phenotype of infused CAR T cells and CAR T cells from the blood of patients.

## 1.2 BACKGROUND AND RATIONALE

### 1.2.1 Introduction

We have developed anti-CD19 CAR T-cell therapies for B-cell malignancies, but we have found that some cases of B-cell lymphoma are CD19-negative before CAR T-cell treatment, and other cases lose CD19 expression after CAR T-cell treatment. To address the issue of CD19-negative B-cell lymphomas, we have designed a CAR construct that leads to simultaneous expression of 2 CARs on the same T cell, one CAR targeting CD19 and the other targeting CD20. Both CD19 and CD20 are B-cell antigens with very restricted tissue expression patterns. The anti-CD19 CAR expressed by this construct is Hu19-CD828Z that has been previously tested as a single CAR in a phase I clinical trial. In this trial, 55% of patients obtained complete remissions, and toxicity was manageable and limited to expected CAR-related toxicities. Four of 20 patients on this trial of Hu19-CD828Z lost CD19 expression, and this loss of CD19 expression is a major factor prompting us to develop CAR T-cell strategies targeting 2 antigens simultaneously. CD20 is a B-cell antigen that is expressed at high levels on most B-cell lymphomas, especially the most common B-cell lymphoma, diffuse large B-cell lymphoma. CD20 is the target of the commonly-used monoclonal antibody rituximab. Consistent with the limited expression pattern of CD20, Rituximab is remarkably free of toxicities. We chose CD20 as the second target, along with CD19, for the CAR construct to be tested in this clinical protocol because CD20 has high expression on many lymphomas and an expression pattern that is apparently limited to B cells. We designed an anti-CD20 CAR called Hu20-CD8BBZ. This CAR has a fully-human anti-CD20 single-chain variable fragment (scFv) and also includes a 4-1BB costimulatory domain; therefore, one interesting aspect of this clinical trial is to assess the function of T cells expressing 2 CARs with one CAR containing a CD28 costimulatory domain and the other CAR containing a 4-1BB costimulatory domain. The CAR construct is designated Hu1928-Hu20BB; it is encoded by the MSGV1 gamma-retroviral

vector. T cells expressing this anti-CD19 and anti-CD20 CAR construct can eradicate tumors in mice. We propose to conduct a phase I clinical trial of B cell lymphomas, Hodgkin lymphoma, and chronic lymphocytic leukemia. Chronic lymphocytic lymphoma patients will only be enrolled if they do not have leukemia cells in their blood at the time of protocol enrollment. 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. After the lymphocyte-depleting chemotherapy, patients will receive an infusion of autologous CAR T cells. The T cell dose will escalate with sequential cohorts of patients until a maximum tolerated dose is determined.

### 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 1. B cell malignancies have quite heterogeneous clinical manifestations and prognoses. Chronic lymphocytic leukemia (CLL) is a common disease that is incurable by chemotherapy 2. 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 reported3, but follow-up is short, and some patients receiving these agents do develop progressive CLL that in some cases has transformed to a higher grade3. 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 4. 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 need5. Follicular lymphoma is a common lymphoma with an extremely variable course 6. Patients with follicular lymphoma have a median survival of about 4.5 years after first relapse 7,8. 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 9. 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 10. However, patients with DLBCL that is refractory to 1st salvage chemotherapy have response rates to 2nd salvage therapy of only 14 to 43% 11-15. Patients with DLBCL refractory to 2nd-line chemotherapy have very poor prognoses with a reported median survival of only 4 months and a 1-year survival rate of only 4% 15. 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% and substantial chronic morbidity due to graft-versus-host disease 16,17. All in all, improved therapies for B cell malignancies are clearly needed.

### 1.2.3 Rationale for treating Hodgkin lymphoma with anti-CD19/anti-CD20 T cells

Classical Hodgkin lymphoma (cHL) is characterized pathologically by the presence of a multinucleated cell called the Reed Sternberg cell intermixed with smaller mononuclear Hodgkin

cells in an extensive background of inflammatory cells. The malignant cells in cHL typically express CD30 and most often lack expression of many B cell markers including CD19 and CD20 [18]. The malignant Hodgkin Reed Sternberg (HRS) cells make up <1-2% of the total cellular infiltrate in Hodgkin lymphoma masses because the malignant cells are surrounded by an extensive inflammatory infiltrate [19,20]. The multinucleated Reed Sternberg-like cells have been consistently shown to have reduced proliferative capacity as compared to the Hodgkin cells as demonstrated by their reduced potential to form colonies [21], incorporate bromodeoxyuridine[22], divide on time-lapse imaging[23], and inability to form tumors in nude mice [24].

The cellular origin of the HRS cells was at first difficult to determine given their lack of expression of typical B cell markers and because the malignant cells are surrounded by an extensive non-malignant inflammatory infiltrate leading to contamination. However, improvements in microdissection techniques combined with analysis of immunoglobulin rearrangements showed that the cellular origin of the HRS cells traces back to the germinal center B lymphocytes [25,26]. These initiating germinal center cells express CD19. In fact, a more recent study demonstrated that there are circulating clonotypic $CD19^{+}$ cells in the peripheral blood of Hodgkin lymphoma patients that may give rise to the HRS cells seen on pathologic examination[27].

Patients with classical Hodgkin lymphoma have an excellent response rate to front-line multi-agent chemotherapy. Unfortunately, the approximately 20% of patients who do not respond to front line treatment or relapse after salvage chemotherapy or autologous transplant have a less optimistic prognosis. The CD30 antibody-drug conjugate brentuximab vedotin (BV) has recently changed treatment algorithms for relapsed cHL. In the initial study, BV had an overall response rate of 75% and complete response rate of 34% [28]. However, long-term follow-up of these same patients shows a PFS at 5 years of 22% and many patients require additional therapy [29]. Therefore, while BV represents a large advance in the treatment of cHL, BV treatment alone is curative in only a small fraction of patients. Pembrolizumab represents another therapeutic strategy for patients with relapsed Hodgkin lymphoma and has FDA approval based on a phase II study showing an overall response rate of 69% and complete response rate of 22.4% [30] . Similarly, nivolumab has a 69% overall response rate and a 16% complete response rate[31]. Finally, anti-CD30 CAR T cells have also been investigated in patients with cHL in two published trials [32,33]. In the Ramos et al paper, a 2/7 patients achieved prolonged complete responses lasting almost 2 years or more [33]. In the Wang et al paper, 7/ 18 patients achieved a partial response but these were unfortunately short-lived with a median response duration ranging from 2-9 months with one ongoing response at 6.5 months [32]. Collectively, these multiple new agents for classical Hodgkin lymphoma represent encouraging steps forward but do not provide efficacy in all patients and often do not lead to complete responses that are durable.

We purpose that Hu1928-Hu20BB CAR T cells may represent an additional avenue for treatment of Hodgkin lymphoma. These cells may be efficacious in Hodgkin lymphoma by targeting any cases of CD20 positive disease, by depleting the CD19 positive clonotypic B lymphocytes that are the origin of the HRS cells, and by impacting non-malignant immune cells in the tumor microenvironment.

First, Hu1928-Hu20BB CAR T cells may work to target any cases of CD20 positive lymphoma. Nodular lymphocyte predominant Hodgkin lymphoma expresses CD20 in 98% of cases [34]. In contrast, only approximately 20% of cases of classical Hodgkin lymphoma express CD20 [35]. Importantly, within the CD20 expressing tumors, the median percentage of HRS cells expressing

CD20 was 55% 35. Studies regarding whether CD20 expression is associated with a worse prognosis have demonstrated inconsistent results with some demonstrating an association with better prognosis 36, others with worse prognosis 37, and still others showing no change in prognosis 38. The expression of CD20 in nodular lymphocyte predominant Hodgkin lymphoma and in some cases of classical Hodgkin lymphoma made investigation into treatment with the anti-CD20 monoclonal antibody rituximab a logical choice. In patients with nodular lymphocyte predominant Hodgkin lymphoma, overall response rate to rituximab is 100% 39. Studies of rituximab in classical Hodgkin lymphoma have been less successful. In the relapsed Hodgkin lymphoma setting, rituximab has single agent activity with an overall response rate of around 20% 40 and also may have activity when added to gemcitabine 41. Initial studies of adding rituximab in the upfront setting to ABVD were promising 42·43 but a follow-up randomized study was limited by poor accrual and did not show a benefit in either event free survival or overall survival 44. Similarly, studies of adding rituximab to escalated BEACOPP also failed to show a benefit 45·46. Rituximab may be insufficient for the treatment of Hodgkin lymphoma because it does not cause complete eradication of B cells. In particular, studies of rituximab in primates have shown it causes depletion of only 34-78% of B cells in lymph nodes 47. Similarly, in humans rituximab treatment leads to the rapid clearance of B cells from the peripheral blood 48 but with some persistence in the lymph nodes 49·50. We have observed that our anti-CD19 CAR T cells cause a more complete eradication of B cells than rituximab with many patients experiencing prolonged B cell depletion lasting over a year 51·52**.** This more complete eradication of B cells may lead to a better therapeutic response as compared to rituximab.

Second, Hu1928-Hu20BB CAR T cells may work to target the clonotypic CD19 positive cells that may serve as HRS stem cells. There is a precedent for the concept of targeting CD19 positive progenitor cells with anti-CD19 CAR T cells in diseases where the mature malignant cells do not express CD19 but may arise from CD19 positive progenitors. These studies have been performed in both myeloma and Hodgkin lymphoma. An initial case report in myeloma demonstrated a 12-month complete response in a heavily pre-treated patient who received anti-CD19 autologous CAR T cells following myeloablative chemotherapy 53. This response was despite the absence of CD19 expression as detected by flow cytometry on 99.95% of the patient's myeloma cells 53. A follow-up study using the same approach with this patient and 9 others showed that 2/10 patients undergoing anti-CD19 second autologous transplant patients experienced a longer duration of remission as compared to their first transplant. For example, 181 days with first transplant versus 479 days with second anti-CD19 autologous transplant 54. This is significant because the duration of response to second autologous transplant is generally much shorter than the response to first autologous transplant.

There has also been a phase 1 study of CD19 CAR T cells in Hodgkin lymphoma55. This study was limited because it used electroporation of non-viral RNA to deliver the CAR construct and this results in very short-lived CAR T cells. Furthermore, the study only infused 4 patients which limits evaluation of response. However, even in this small patient population and using a very transiently expressed construct, responses were seen in 2/4 infused patients and one of these responses was a complete response. This suggests that the underlying concept of targeting Hodgkin lymphoma through CD19 modulation can be effective. In our previous viral CD19 CAR studies, we have observed significantly longer CAR T persistence 56 52 and therefore expect our Hu1928-Hu20BB CAR T cells to have greater efficacy and longer term response than that seen in this non-viral CD19 CAR T study.

Finally, anti-CD19 anti-CD20 CAR T cells may cause responses by impacting the tumor microenvironment. The malignant HRS cells are surrounded by an extensive inflammatory infiltrate composed of fibrous tissue in addition to immune cells including T cells, B cells, macrophages, eosinophils, and others 18. This inflammatory cellular background appears to provide a tumor microenvironment that may support growth of the malignant cells57. In a previous study with anti-CD19 CAR T cell therapy comparing pre and post-treatment biopsies, the immune gene signature is markedly altered following treatment 58. These effects on local inflammatory cells could impact tumor bulk and response to Hu1928-Hu20BB CAR T cells.

### 1.2.4 T-cell gene therapy

In an attempt to develop effective immunotherapies for cancer that are less toxic than allogeneic stem cell transplantation, many investigators have developed T-cell gene therapy approaches to specifically target T cells to tumor-associated antigens59. T cells can be prepared for adoptive transfer by genetically modifying the T cells to express receptors that specifically recognize tumor-associated antigens.59-66 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.59,67-70 Genetically modified antigen-specific T cells can be generated from peripheral blood mononuclear cells (PBMC) in sufficient numbers for clinical treatment within 10 days.68 Genetically modifying T cells with gamma-retroviruses consistently causes high and sustained levels of expression of introduced genes without in vitro selection67,70-72, and genetic modification of mature T cells with gamma-retroviruses has a long history of safety in humans.73-75 There are two general approaches for generating antigen-specific T cells by genetic modification: introducing genes encoding natural αβ T cell receptors (TCRs) or introducing genes encoding chimeric antigen receptors (CARs).59,61,63,65 CARs are fusion proteins incorporating antigen recognition moieties and T cell activation domains.64,76-78 The antigen-binding domains of most CARs currently undergoing clinical and preclinical development are antibody variable regions.61,64,76,78 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 molecules59,61,63,76, 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.59,61,79-81

### 1.2.5 Chimeric antigen receptors preclinical background

Preclinical experiments evaluating CAR-expressing T cells as cancer therapy were initiated in 1993.82,83 These experiments led to a clinical trial of CAR-transduced T cells targeting the α-folate receptor on ovarian cancer cells; no tumor regressions were observed during this clinical trial.84 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 retroviruses67,70-73,85-87, lentiviruses68,88-91, or transposon systems92,93 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.68,85,94 Different portions of CARs including antigen-recognition moieties, extracellular structural components, costimulatory domains such as the cytoplasmic portion of the CD28 protein, and T-cell-activation moieties such as the signaling domains of the CD3ζ protein can all be important to the in vivo function of CAR-

expressing T cells, and all of these portions of CARs remain the subject of intensive investigation.[76,85,89,95-98]

Much of the preclinical work evaluating CARs has been performed with CARs targeting the B-cell antigen CD19.[85-87,92,99-101] 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.[87,96] These studies showed that adding CD28 moieties to CARs enhanced antigen-specific cytokine production and proliferation by anti-CD19 CAR T cells.[96,102,103] T cells expressing CARs with CD28 signaling moieties and CD3ζ signaling domains were more effective than T cells expressing CARs without CD28 moieties at eradicating human leukemia cells from mice.[102,103] Subsequently, CARs incorporating other signaling domains from costimulatory molecules such as 4-1BB (CD137) were developed.[88] Anti-CD19 CARs containing the signaling domains of both 4-1BB and CD3ζ were superior to CARs containing the signaling domains of CD3ζ without any costimulatory domains at eradicating human malignant cells from mice.[89,95] Similar to CD28, including 4-1BB signaling moieties in CARs led to increased CD19-specific proliferation and enhanced in vivo persistence.[89] 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.[89,95]

### 1.2.6 CD19

CD19 is commonly used as the target of CAR T cells because it is expressed on most malignant B cells [104,105], but the only normal cells that express CD19 are B cells and perhaps follicular dendritic cells [105,106]. Importantly, CD19 is not expressed on pluripotent hematopoietic stem cells [107]. 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 [108], yet patients that receive chemotherapy plus rituximab do not have an increased rate of common infections when compared to patients who receive chemotherapy alone.[109] Finally, patients can be treated with intravenous infusions of IgG if necessary to increase IgG levels.[110]

### 1.2.7 CD20

B-lymphocyte antigen CD20 is an activated-glycosylated phosphoprotein expressed on the surface of all B-cells. CD20 is encoded by the *MS4A1* gene in humans. [79].[110,111] CD20 is expressed on all stages of B cell development except the first and last, i.e., not on either early pro-B cells or plasma cells.[112] CD20 expression on normal cells is limited to B cells except for possible expression on a small subset of T cells.[113,114] [115] CD20 is also found on B-cell lymphomas, hairy cell leukemia, B-cell chronic lymphocytic leukemia, transformed mycosis fungoides, [116] and melanoma cancer stem cells.[117] The expression of CD20 is regulated by the chemokine signaling through the CXCR4/SDF1 axis, and this can be impaired by drugs interfering with microenvironmental interactions.[118]

CD20 has no known natural ligand and its function is to enable optimal B-cell immune responses, specifically against T-independent antigens.[119] CD20 may function as a calcium channel in the cell membrane. It has been shown that CD20 plays a role in the microenvironmental interactions of B cells,[118] and B-cell receptor signaling. In chronic lymphocytic leukemia, when the anti-CD20 monoclonal antibody rituximab is used in clinical therapy, it seems to primarily

eliminate the malignant B cells that express high levels of CD20 and have a high potential for activation of the B-cell receptor signaling pathway.[120] Rituximab combined with chemotherapies could down-regulate the CD20 expression, however, when CD20-negative cells were treated with 5-aza-2'-deoxycytidine in vitro, the expression of CD20 mRNA was stimulated within 3 days, resulting in the restoration of both cell surface expression of the CD20 protein and rituximab sensitivity. These findings suggest that some epigenetic mechanisms may be partly related to the down-regulation of CD20 expression after rituximab treatment.[121] Because the cell surface density of CD20 is higher than that of CD19 in normal B cells, [122] CD20 became a popular target for the monoclonal antibodies.

### 1.2.8 CD20 expression on B-cell lymphomas and treatment of lymphoma with antibodies targeting CD20

CD20 is an antigen that is expressed on mature B cells but not expressed on early B-cell progenitors or more mature plasma cells[123]. In addition, CD20 is broadly expressed in a range of B-cell malignancies[124]. Previous studies showed that CD20 is expressed on more than 90% of cases of diffuse large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, and CLL [125]. Moreover, the degree of expression of CD20 varies between the different B-cell malignancies with CLL, for example, expressing CD20 at a lower level than seen in DLBCL [125]. The variable expression of CD20 on different malignancies is believed to account, at least partially, for the different responses of individual B-cell malignancies to CD20 targeted therapy [126].

The expression of CD20 on most B-cell malignancies made CD20 an attractive therapeutic target. The first CD20 targeted monoclonal antibody, rituximab, resulted in a dramatic improvement in the prognosis of many B-cell malignancies. For example, patients with DLBCL treated with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) showed an improvement in 10 year overall survival from 27.6% in the CHOP group to 43.5% in the R-CHOP group[127]. Rituximab is also effective in a range of other B-cell malignancies. In particular, rituximab has marked efficacy in follicular lymphoma both in combination with chemotherapy [128-130] and as a single agent [131]. It is also effective in combination chemotherapy for CLL [132,133] and improves the rate of complete response in mantle cell lymphoma [134].

There are ongoing efforts to improve upon rituximab to target CD20 more effectively. Resistance to rituximab can arise through down-regulation of CD20 on the malignant cell surface by multiple different mechanisms [126]. The next generation CD20 antibodies have been developed to target CD20 more effectively even in the context of lower CD20 expression level as is seen in CLL. These antibodies include ofatumumab [135] and obinutuzumab [136], both of which are FDA approved for treatment of CLL. In follicular lymphoma, a head-to-head comparison of obinutuzumab to rituximab did show a slight improvement in progression free survival (3 year PFS 80% versus 73.3%) but at the cost of increased toxicity with obinutuzumab [137]. This suggests that further antibody modifications or alternative methods to target CD20 are needed.

### 1.2.9 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.[67,68,70-72,138-142] 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.[71] This clinical trial utilized a gamma retroviral vector to introduce an anti-CD19 CAR containing the signaling domains of the CD28 and CD3ζ molecules.[71] 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.[71] 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.[71] The bone marrow B-cell eradication was confirmed by flow cytometry, and it persisted for over 36 weeks.[71] B cells were also completely absent from the blood during this time, while T cells and other blood cells recovered rapidly.[71] Seven months after the anti-CD19 CAR T cell infusion, progressive lymphoma was detected in the patient's cervical lymph nodes. The lymphoma remained $CD19^+$, so the patient was treated a second time with anti-CD19 CAR T cells. The first and second treatment regimens were the same except the patient received a higher dose of cells with the second treatment. After the second treatment, the patient obtained a second partial remission that is ongoing over 9 years post-treatment.[70]

Seven more patients were subsequently treated with the same regimen of chemotherapy, anti-CD19 CAR T cells, and high-dose IL-2.[70] 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.[70,71] In all 4 patients with B-cell depletion, the depletion lasted for over 36 weeks. The B-cell depletion could not be attributed to the chemotherapy that was administered because blood B-cells recovered to normal levels in 8 to 19 weeks in patients receiving the same chemotherapy plus infusions of T cells targeting NY-ESO or gp100, which are antigens that are not expressed by B cells.[71] Because normal B cells express CD19, prolonged normal B-cell depletion after anti-CD19 CAR T-cell infusions demonstrated that CAR-expressing T cells had a powerful ability to eradicate $CD19^+$ cells in humans. All of the patients with long-term B-cell depletion obtained either complete or partial remissions of their malignancies, and the 4 patients with long-term B cell depletion also developed hypogammaglobulinemia. Hypogammaglobulinemia in these patients was routinely treated with infusions of intravenous immunoglobulins. Of the eight patients treated, seven patients were evaluable for malignancy response; the one patient who was not evaluable died with pneumonia caused by influenza A.[70] Six of the seven evaluable patients had remissions of their malignancies. Two of the remissions were complete remissions (CRs) of CLL.[70] Both of these CRs were confirmed by multicolor flow cytometry of bone marrow cells.[70] One of these CRs lasted 24 months, and the other is ongoing at 48 months.[70] 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.[70] All of these toxicities peaked within 10 days after the cell infusion and resolved less than 3 weeks after the cell infusion.[70] These acute toxicities correlated with serum levels of the inflammatory cytokines tumor necrosis factor and interferon-γ, and T cells producing these inflammatory cytokines in a CD19-specific manner were detected in the blood of patients after the anti-CD19 CAR T cell infusions.[70]

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[143]. In summary this paper reported treatment of 15 patients with advanced B-cell malignancies. Nine patients had diffused large B-cell lymphoma (DLBCL), 2 patients had indolent lymphomas, and 4 patients had chronic lymphocytic leukemia (CLL). Patients received a conditioning chemotherapy regimen of cyclophosphamide and fludarabine followed by a single infusion of anti-CD19-CAR T cells. Of 15 patients, 8 obtained complete remissions (CRs), 4 patients obtained partial remissions, 1 patient had stable lymphoma, and 2 patients were not evaluable for response. CRs were obtained by 4 of 7 evaluable patients with chemotherapy-refractory DLBCL; 3 of these 4 CRs are ongoing with durations ranging from 9 to 22 months. Acute toxicities including fever, hypotension, delirium, and other neurological toxicities occurred in some patients after infusion of anti-CD19-CAR T cells; these toxicities resolved within 3 weeks after cell infusion. One patient died suddenly of an unknown cause 16 days after cell infusion. CAR T cells were detected in the blood of patients at peak levels ranging from 9 to 777 $CAR^+$ T cells/μL. Elimination of exogenous IL-2 from our protocol did eliminate the toxicity that is known to occur with administration of high-dose IL-2, but cytokine-release type toxicity attributable to the CAR T cells still remained[143]

In an attempt to further reduce the overall toxicity of our anti-CD19 CAR treatment protocol, we substantially reduced the dose of the chemotherapy regimen administered before CAR T-cell infusions. We treated 9 patients with B-cell lymphoma who received a single infusion of $1x10^6$ anti-CD19-CAR-expressing T cells/kg bodyweight preceded by a low-dose chemotherapy regimen of 3 daily doses of cyclophosphamide 300 $mg/m^2$ and fludarabine 30 $mg/m^2$ administered on the same days.[56] As expected, the severity of neutropenia and thrombocytopenia was reduced with the low dose chemotherapy compared to high-dose chemotherapy. We went on to treat a total of 22 patients with either a type of diffuse large B-cell lymphoma (19 patients), follicular lymphoma (2 patients), or mantle cell lymphoma (1 patient).[56] The overall response rate was 73% with 55% complete remissions.[56] Most importantly, of 12 patients obtaining complete remissions, 11 are still in ongoing complete remissions of up to 36 months duration.[56] We also went on to show that long-term remissions of diffuse large B-cell lymphoma do not depend on persisting CAR T cells.[144] This work led to several multicenter trials of the same anti-CD19 CAR used in the other trials described in the last 4 paragraphs, and ultimately to the first FDA-approved CAR T-cell therapy for lymphoma. The multicenter trials on diffuse large B-cell lymphoma confirmed results from the original NCI clinical trials.

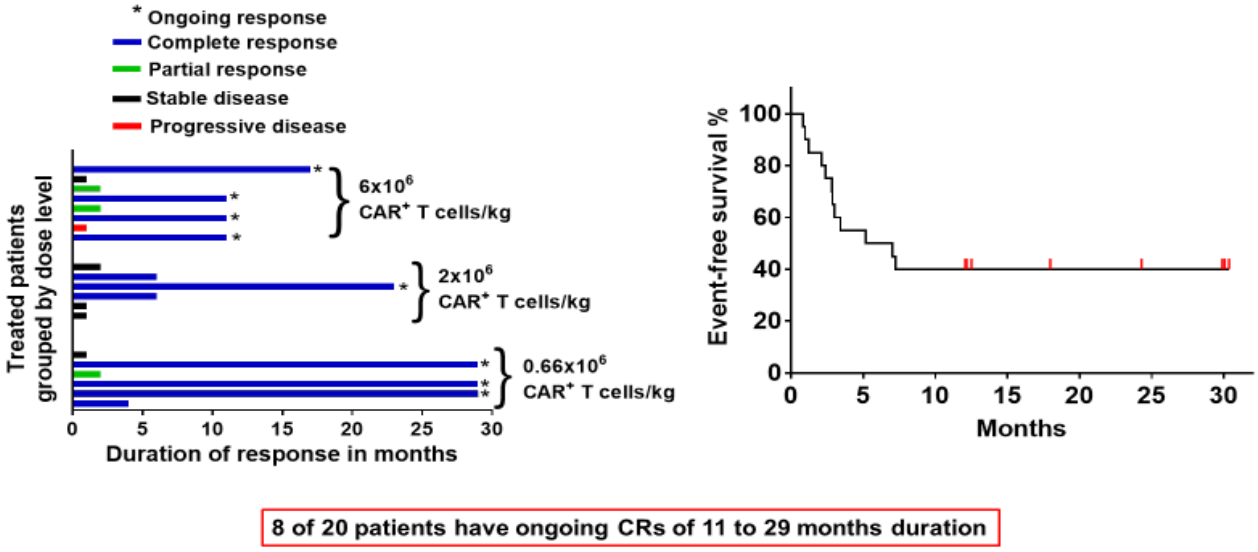
The effectiveness of anti-CD19 CAR T cells against advanced B-cell malignancies, particularly CLL and ALL has been reported by multiple groups[90,140-142,145] Multiple groups have recently demonstrated the 70% to 90% complete remission rates when anti-CD19 CAR T cells are used to treat ALL[140,142,145] 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[140,142,145]. 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[142,145]. Neurologic toxicity was the most problematic aspect of treating patients with the FMC63-28Z CAR. Fifty-five percent of patients on our final clinical trial of this CAR for lymphoma at the NCI had Grade 3 or 4 neurologic toxicity. All of this early work on anti-CD19 CAR T-cell therapies has led to multicenter trials for most types of B-cell lymphoma and FDA-approval of the first anti-CD19 CAR product for treatment of DLBCL.[146]

To further reduce toxicity of CAR T cells and to reduce the potential immunogenicity of our original anti-CD19 CAR, we designed a new anti-CD19 CAR. This CAR had a fully-human scFv to reduce immunogenicity, and it had a CD8α hinge and transmembrane domain that we hypothesized would reduce T-cell cytokine production.[98] The CD8α hinge and transmembrane domain was shown to deliver a weaker activation stimulus compared to the CD28 hinge and transmembrane domain in the FMC63-28Z CAR used in all of our prior clinical trials.[98] This weaker activation stimulus consistently led to lower levels of cytokine production by T cells expressing CARs with CD8α hinge and transmembrane domains compared with CARs containing CD28 hinge and transmembrane domains.[98]

We tested the CAR that resulted from this preclinical work on CARs with human antibody variable domains and CD8α hinge and transmembrane domains, Hu19-CD828Z in a phase I clinical trial.[147] Twenty patients were treated on this clinical trial of Hu19-CD828Z CAR T cells. The anti-lymphoma efficacy was very similar to that of FMC63-28Z CAR T cells. Fifty-five percent of patients receiving Hu19-CD828Z CAR T cells obtained complete remissions, and responses were durable with 40% of responses ongoing with durations of 5 to 29 months duration.[147] The striking difference between results of the Hu19-CD828Z trial and our earlier work with FMC63-28Z CAR T cells was the much lower rate of neurologic toxicity with Hu19-CD828Z CAR T cells.[147] Only 5% of patients had Grade 3 or 4 neurologic toxicity with Hu19-CD828Z CAR T cells compared with 55% of patients having Grade 3 or 4 neurologic toxicity with FMC63-28Z CAR T cells.[147] The anti-lymphoma response and event-free survival of patients on the trial of Hu19-CD828Z are in *Figure 1*. One issue that arose during the trial of Hu19-CD828Z was loss of CD19 expression on lymphoma cells. We documented CD19 loss on 4 of 20 treated patients on the Hu19-CD828Z trial; this finding led us to develop an approach that could target CD19 and another antigen simultaneously. Because of the proven anti-lymphoma activity and favorable toxicity profile of Hu19-CD828Z, we have chosen to include Hu19-CD828Z along with an anti-CD20 CAR in a bicistronic construct called Hu19-CD828Z.

Figure 1 **Duration of response and event-free survival from Hu19-CD828Z clinical trial.**




### 1.2.10 Anti-CD20 CARs

Clinical experience with anti-CD20 CARs is much more limited than the clinical experience with anti-CD19 CARs. Preclinical work with anti-CD20 CARs has been published and includes studies of the interaction of CARs with serum anti-CD20 antibodies and a study of antigen expression levels on the activity of CAR T cells.[148] Substantial work has been performed in developing bispecific CARs in which 1 CAR contains binding domains for both CD20 and CD19.[149-151] These bispecific CARs differ from the Hu1928-Hu20BB CAR used in this protocol. The bispecific CARs encode 1 CAR with 2 binding domains; in contrast, our bicistronic approach has 2 different CARs expressed simultaneously by 1 T cell. Three papers have been published on CAR T-cell clinical trials, but the trials contained a very small number of patients. Two papers dealt with anti-CD20 CAR trials that used electroporation for T-cell modification. One trial reported only 1 treated patient;[139] the other reported 7 patient treated of which only one obtained an objective anti-lymphoma response, a partial response (PR).[152] One possible reason for the unimpressive results is that electroporation led to only short persistence of CAR+ T cells of only 1 to 3 weeks.[152] A more recent trial used an anti-CD20 CAR with both CD28 and 4-1BB costimulatory domains.[153] This CAR was encoded by a lentivirus vector; only 3 patients have been reported.[153] Two patients maintained pre-existing complete responses (CRs), and one patient obtained a PR.[153]

### 1.2.11 Development and preclinical testing of a CAR construct encoding both an anti-CD19 CAR and an anti-CD20 CAR

In order to simultaneously target 2 lymphoma-associated antigens with 1 T cell, we designed CAR constructs that encoded 2 CARs. One CAR included in all constructs was the previously-described Hu19-CD828Z that has been shown to be save an effective in a prior phase I clinical trial. The second CAR targeted CD20 and included a 4-1BB moiety. We evaluated several different anti-

CD20 scFvs as the antigen-recognition domain of the anti-CD20 CAR before selecting a single-chain variable fragment (scFv) from the fully-human antibody 2.1.2.[154] We designated the anti-CD20 CAR containing the 2.1.2 scFv Hu20-CD8BBZ; Hu20-CD8BBZ also contained CD8α hinge and transmembrane domains, a 4-1BB domain, and the cytoplasmic part of CD3ζ.

The bicistronic construct also incorporated the anti-CD19 CAR Hu19-CD828Z that contained a single chain variable fragment (scFv) from the fully-human anti-CD19 antibody 47G4, CD8α hinge and transmembrane domains, the CD28 cytoplasmic domain, and the cytoplasmic part of CD3ζ.[155] Both the Hu19 and 2.1.2 (Hu20) antibodies were derived by immunizing mice transgenic for human immunoglobulin genes.[154,155]

The entire bicistronic construct was encoded by the MSGV1 gamma-retroviral vector backbone.[156] The Hu19-CD828Z and Hu20-CD8BBZ CARs were separated by a ribosomal skip domain that caused both CARs to be expressed as separate proteins. The concept of a T cell simultaneously expressing 2 CARs is depicted in *Figure **2***, and diagrams of the bicistronic CAR construct, Hu19-CD828Z, and Hu20-CD8BBZ are shown in *Figure **3***.

Figure **2 A bicistronic CAR construct leads to expression of 2 CARs by the same T cell.**

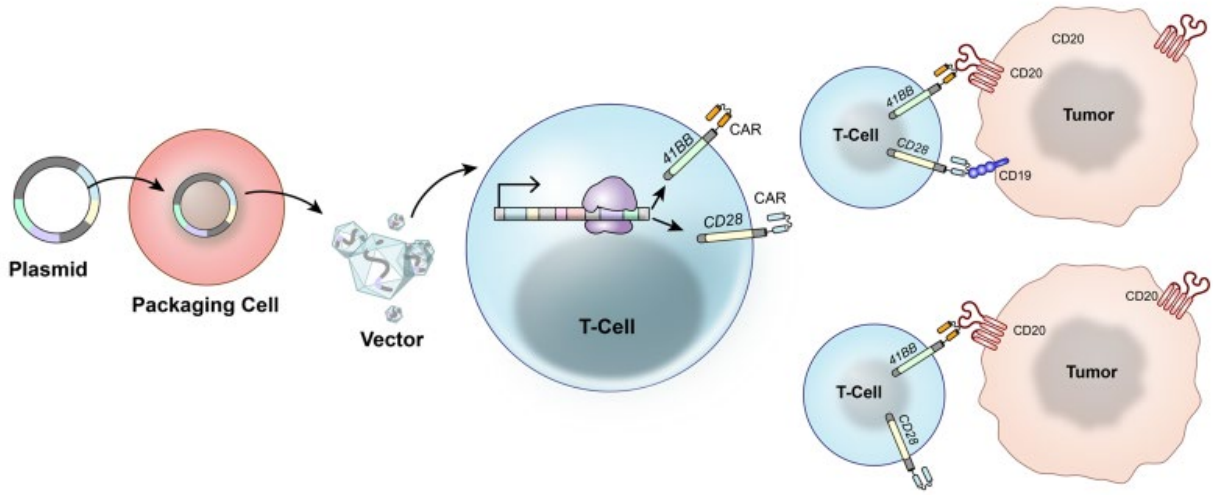
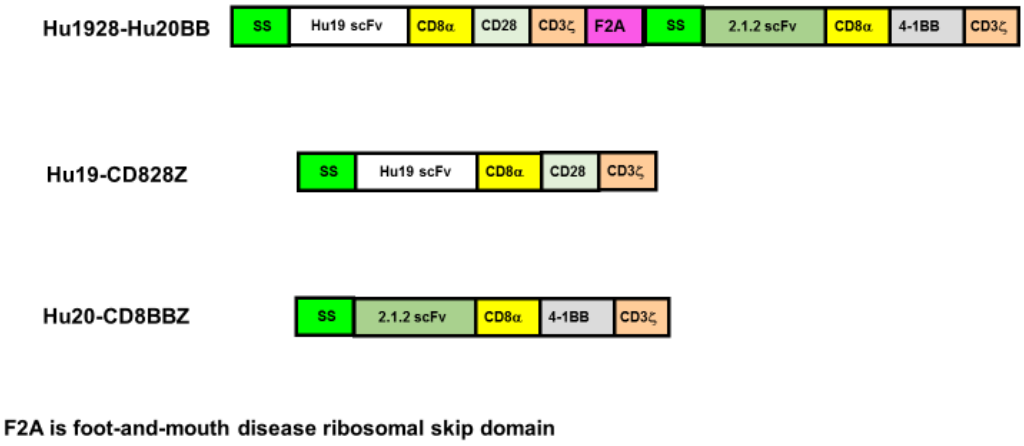



Figure **3** :**Diagrams of the CAR construct Hu1928-Hu20BB and individual CAR in the construct.**




A diagram depicting the cleavage at the ribosomal skip sequence and expression of 2 separate CAR proteins is shown in *Figure 4*. This diagram depicts the furin enzyme cleavage or the 2A (F2A in this case) from the C-terminus of the Hu19-CD828Z CAR. In this diagram, SS stands for signal sequence, and HTM stans for hinge and transmembrane domain.

Figure **4** : **Diagram of simultaneous expression of 2 CARs from Hu1928-Hu20BB construct.**

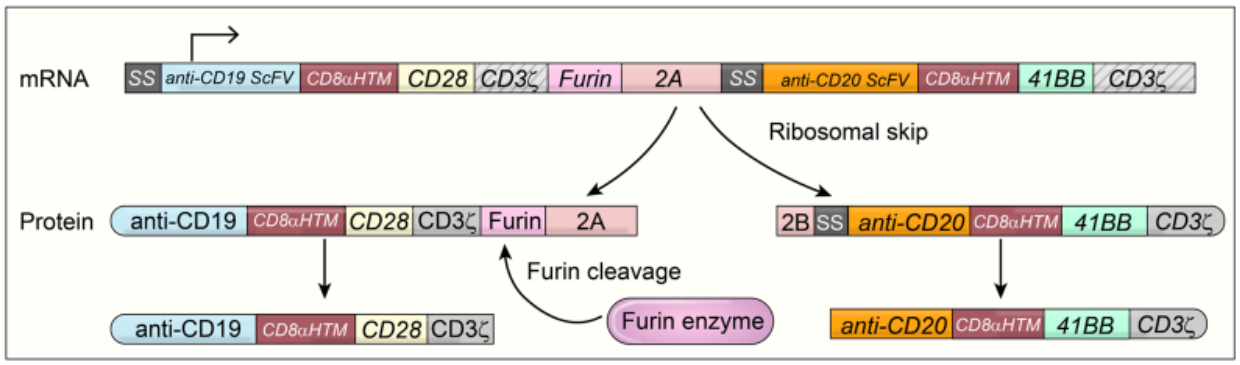



*Figure 5* shows expression of Hu19-CD828Z and Hu20-CD8BBZ on the surface of T cells five days after transduction with gamma-retroviruses encoding the Hu1928-Hu20BB CAR construct. For this experiment, whole peripheral blood mononuclear cells (PBMC) were started in culture on day 0 by stimulating with an anti-CD3 monoclonal antibody in interleukin (IL)-2-containing medium. Transductions were carried out 2 days after the cultures were started, and the T cells were assessed for CAR expression 6 days later, when cells had been in culture for a total of 8 days. The plots in Figure Figure ***5*** are gated $CD4^+$ or $CD8^+$ live, $CD3^+$ lymphocytes. The CAR staining in Figure 5 was performed with the Kip-1 antibody, which binds to the Hu19 scFv that is in Hu19-CD828Z. Kip-1 does not bind to Hu20-CD8BBZ. Note that all T cells transduced in this work were transduced in the same manner as described above.

Figure **5: CAR expression on T cells by Kip-1 antibody staining**

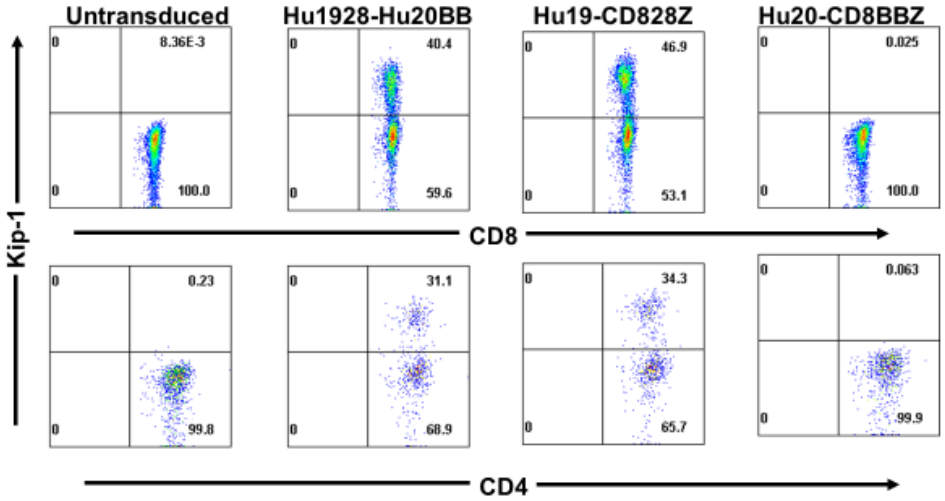
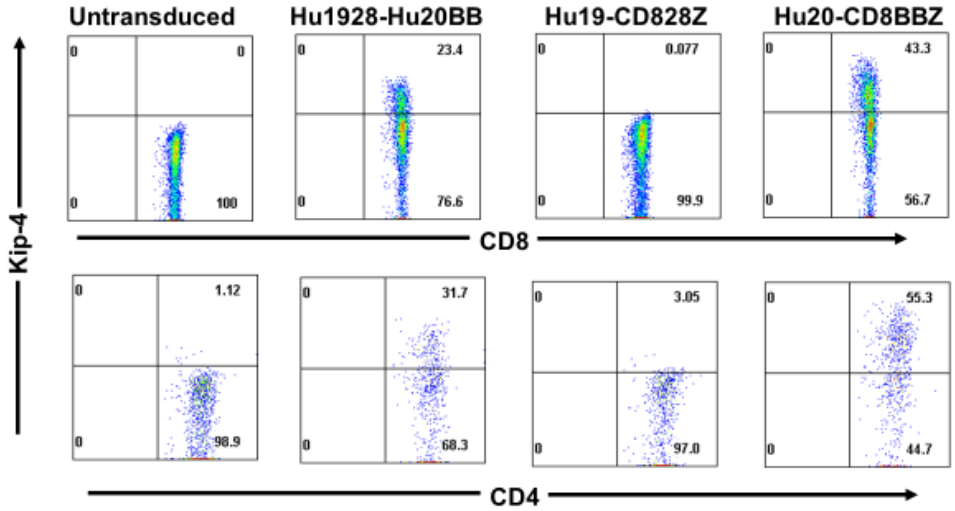



*Figure 6* shows T cells from the same cultures shown in *Figure 5*, but in *Figure 6* staining with the Kip-4 antibody is shown. Kip-4 binds to the scFv of the Hu20-CD8BBZ CAR but does not bind to the Hu19-CD8BBZ CAR. Kip-4 is not as sensitive or specific as Kip-1.

Figure **6: CAR expression on T cells by Kip-4 antibody staining**




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 7* shows upregulation of CD107a on $CD8^+$ T cells, which indicates degranulation and correlates with cytotoxicity.157 when T cells were transduced with the Hu1928-Hu20BB, the T cells could recognize $CD19^+$ CD19-K562 target cells, $CD20^+$ CD20-K562 target cells, and ST486

target cells that expressed low levels of CD19 and moderate levels of CD20. The negative-control target cells NGFR-K562 and CCRF-CEM were recognized at background levels. CD107a was not upregulated when anti-CD19-CAR-expressing T cells from Donor 1 were cultured for 4 hours with the negative control cell lines NGFR-K562 and CCRF which do not express CD19. Hu19-CD828Z-expressing T cells recognized CD19-K562 and ST486 but not CD20-K562. Hu20-CD8BBZ recognized CD20-K562 and CCRF-CEM but not CD19-K562. Untransduced T cells from Donor 1 did not upregulate CD107a when cultured with either CD19-K562 or NGFR-K562 except at background levels. The plots are gated on live $CD3^+$ lymphocytes.

Figure **7** : **Degranulation in an antigen-specific manner by CAR T cells**

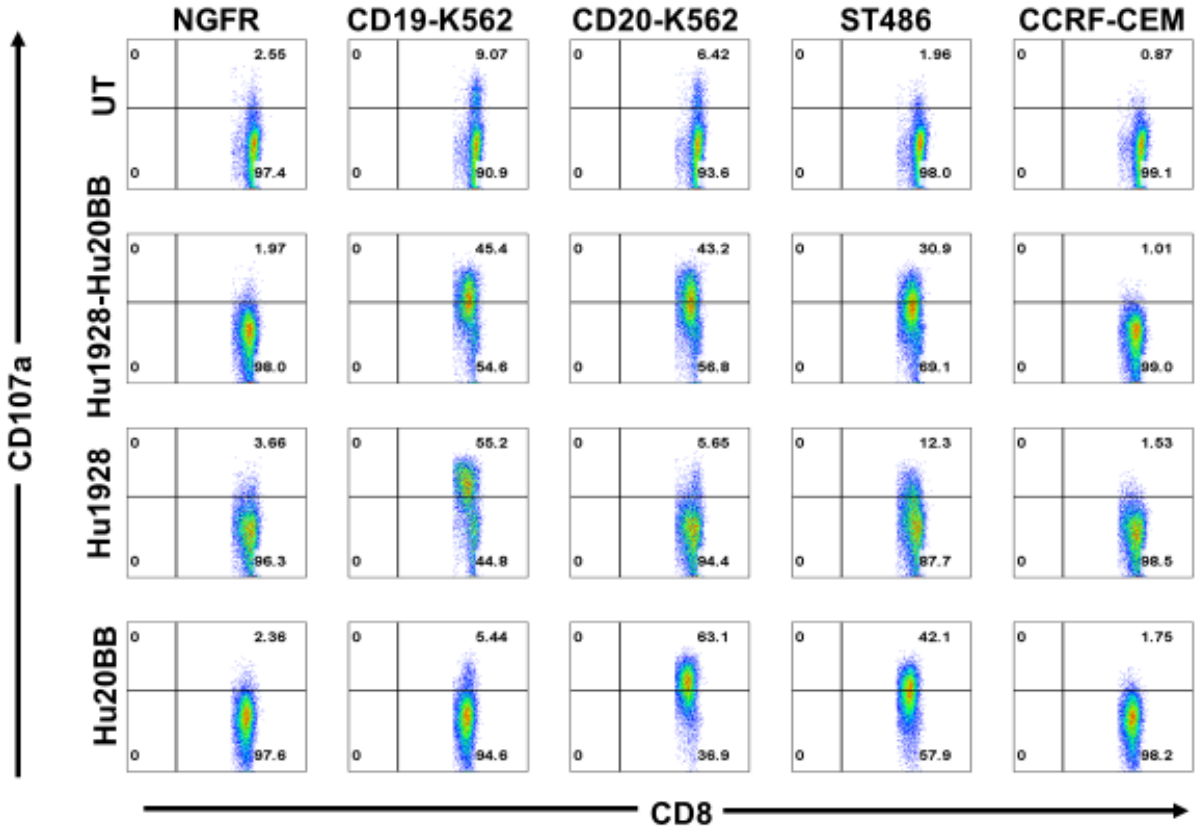



*Figure 8* shows a 4-hour cytotoxicity assay in which "Toledo" $CD19^+/CD20^+$ target cells were incubated with T cells transduced with Hu1928-Hu20BB, Hu19-CD828Z, or Hu20-CD8BBZ. All 3 CARs could kill the ST486 cells that express both CD19 and CD20.

Figure **8 : Cytotoxicity of a lymphoma cell line by CAR-expressing T cells**

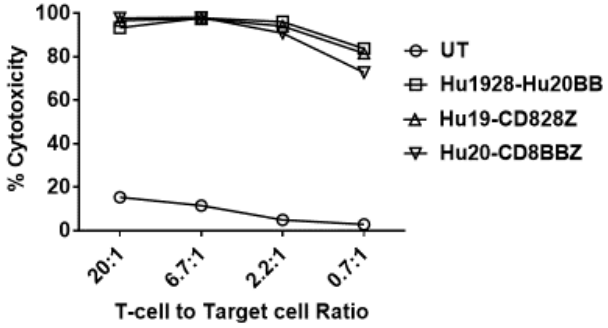
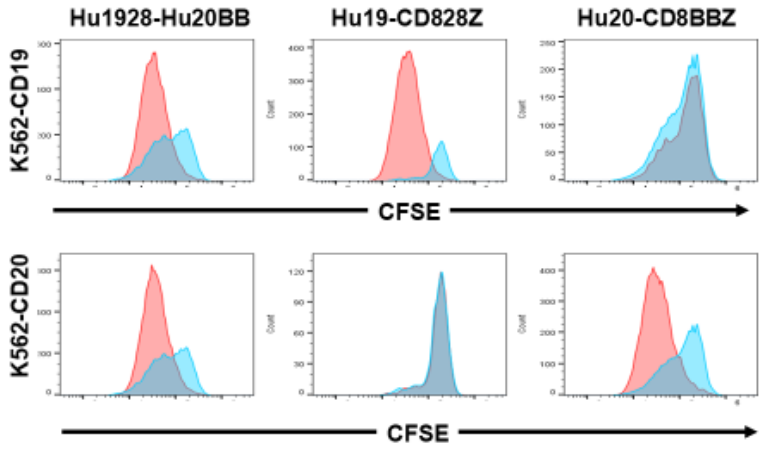



*Figure 9* shows a carboxyfluorescein diacetate,succinimidyl ester (CFSE) proliferation assay in which CAR-transduced T cells were cultured for 4 days with either CD19-K562 cells, CD20-K562 cells, or CD19-negative NGFR-K562 cells. CFSE was diluted to a greater degree, when CAR-expressing T cells were cultured with target cells expressing the antigen targeted by the CAR(s) expressed by each T-cell population. Hu1928-Hu20BB-expressing CARs proliferated in response to both CD19-K562 cells and CD20-K562 cells. Hu19-CD828Z T cells proliferated only in response to CD19-K562 cells, and Hu20-CD8BBZ T cells proliferated strongly in response to CD20-K562 cells with some background proliferation that is common with 4-1BB-containing CARs. The assay was conducted as described previously.[158]

Figure 9: **Degranulation in an antigen-specific manner by CAR T cells**




It is critical to test any new CAR for specificity in order to avoid destruction of normal tissues. The Hu1928-Hu20BB CAR construct encodes 2 CARs, Hu19-CD828Z and Hu20-CD8BBZ. Hu19-CD828Z has been tested in a phase I trial and found to have an excellent safety profile with significantly less neurological toxicity than an earlier anti-CD19 CAR, FMC63-28Z; therefore, the Hu19-CD828Z CAR has substantial safety data in humans to justify its use. Hu20-CD8BBZ has not been tested before in humans, so we decided to use a new approach to assess its specificity.[159] A company called Retrogenix™ has developed a process in which HEK293 cells are transfected to express 5647 human plasma membrane proteins. This allows screening against this very large

number of human proteins for reactivity of antibody-based reagents. This approach can be used with whole intact CAR T cells that are fluorescently labeled. We provided Retrogenix™ with untransduced human T cells and T cells from the same donor that expressed Hu20-CD8BBZ. The Hu20-CD8BBZ T cells were labeled and used to screen the 5647 human plasma membrane proteins. The only differences in binding between Hu20-CD8BBZ T cells and untransduced T cells was for CD20, which was expected for this anti-CD20 CAR, and CD27. CD27 binding was very weak and inconsistent, but nonetheless, we transduced 293T cells in my lab and assessed for reactivity against Hu20-CD8BBZ T cells in an IFNγ enzyme-linked immunosorbent assay (ELISA). We found no release above background when Hu20-CD8BBZ T cells were exposed to $CD27^+$ target cells; therefore, we concluded that the Hu20-CD8BBZ CAR does not functionally recognize CD27.

We also performed our usual method of specificity staining in my lab of assessing the ability of CAR-expressing T cells to recognize primary human cells and human cell lines. We cultured CAR-expressing T cells or untransduced T cells from the same patient with target cells overnight, and then performed a standard IFNγ ELISA to see if T cells are activated, as indicated by IFNγ release, when the T cells are cultured with target cells (Table 1-3). Ideally, the anti-CD19/anti-CD20 Hu1928-Hu20BB CAR T cells should only react with $CD19^+$ and/or $CD20^+$ target cells.

We used the target cell lines and human primary cells as described in this paragraph. We previously transduced K562 cells to express CD19 (CD19-K562) or low-affinity nerve growth factor (NFGR-K562).[85] We also transduced K562 cells to express CD20. All of these genes were transferred to K562 cells by standard methods with the MSGV gamma-retroviral vector. The NGFR-K562 cells served as CD19-negative control cells. The following cells $CD19^+$ and or $CD20^+$ cell lines were used: NALM6 cells are $CD19^+$ and CD20-negative by flow cytometry (acute lymphoid leukemia from DSMZ, Braunschweig, Germany). ST486, and Toledo were obtained from ATCC; both of these cells lines express both CD20 and CD19, but CD19 expression is very weak on ST486 cells. ST486 -/- cells had CD19 eliminated by CRISPR/Cas9 in our lab.

The CD19-negative cell lines were used: melanoma cell line 624, the leukemia cell line NGFR-K562, the T-cell leukemia cell line CCRF-CEM; A549 (a lung carcinoma cell line); MDA-MB231 (a breast cancer 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). Reactivity of CAR T cells with human primary cells was also assessed (Table 3). Fibroblasts were primary human skin fibroblasts; endothelial cells were primary human endothelial cells. The primary microvascular endothelial cells and primary hepatocytes were from Lonza. The primary human cardiac myocytes were from Dr. Yongshun Lin, NHLBI, NIH In each experiment, the result for effector T cell cultured alone was also given.

T cells transduced with the anti-CD19 CAR produced large amounts of IFNγ when they were cultured overnight with the CD19-expressing cell lines but only small amounts of IFNγ when cultured with CD19-negative cell lines or CD19-negative primary human cells (Table 1). All cytokine values in Tables 1-3 are IFNγ levels in picograms/mL. The % CAR columns show the percentage of T cells in the ELISA assay that expressed the indicated CAR.

*Table* **1** *: IFNγ ELISA with Hu1928-Hu20BB and its constituent CARs shows antigen-specific recognition.*

| | CD19-K562 | CD20-K562 | Nalm6 | ST486 | ST486-CD19-/- | NGFR-K562 | CEM | T-cells Alone | % CAR+ |
|---|---|---|---|---|---|---|---|---|---|
| Untransduced | 441 | 368 | 18 | 390 | 197 | 530 | 12 | 7 | 0 |
| Hu1928-Hu20BB | 10103 | 21162 | 6685 | 7970 | 8317 | 781 | 88 | 52 | 44 |
| Hu19-CD828Z | 13280 | 808 | 6524 | 1433 | 641 | 918 | 39 | 20 | 36 |
| Hu20-CD8BBZ | 171 | 4014 | 1647 | 4620 | 4506 | 115 | 137 | 128 | 48 |

Table 2: **IFNγ ELISA shows lack of reactivity of Hu1928-Hu20BB T cells with a panel of human tumor cell lines.**

| T cells | K562-CD19 | K562-CD20 | A431-H9 | Panc 10.05 | U251 | Colo 205 | Hep G2 | MDA231 | A549 | Tc71 | 624 | T-cells Alone | %CAR+ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Untransduced | <12 | <12 | <12 | <12 | 76 | <12 | <12 | 873 | 66 | 15 | <12 | <12 | 0.1 |
| Hu1928-Hu20BB | 9343 | 6127 | 79 | 88 | 124 | 27 | 90 | 459 | 75 | 97 | 104 | 74 | 51 |

All values are IFNγ in pg/mL except the last column, which is the percentage of each culture that expressed the Indicated CAR by flow cytometry.

T cells were cultured with the indicated target cells overnight, and an IFNγ ELISA was performed.

CD19-K562 expresses CD19 and CD20-K562 expresses CD20

All other targets listed lack both CD19 and CD20

*T*able **3** : **IFN$\gamma$ ELISA shows lack of reactivity of Hu1928-Hu20BB T cells with a panel of human tumor cell lines.**

| T cells | CD19-K562 | CD20-K562 | NGFR-K562 | Proximal tubular cells | Skeletal muscle | Hepatic cells | Renal cortical eptih. | Mammary epith. cells | T-cells Alone | % CAR+ |
|---|---|---|---|---|---|---|---|---|---|---|
| Untransduced | 56 | 31 | 40 | 54 | 15 | 36 | 108 | <12 | <12 | 0.21 |
| Hu1928-Hu20BB | 5536 | 10929 | 109 | 128 | 79 | 35 | 174 | 144 | 106 | 84.7 |
| Hu19-CD828 | 13946 | 118 | 85 | 38 | 27 | 20 | 107 | 19 | 15 | 81.0 |
| Hu20-CD8BB | 255 | 10197 | 280 | 268 | 146 | 48 | 374 | 251 | 224 | 90.9 |

All values are IFN$\gamma$ in pg/mL except the last column, which is the percentage of each culture that expressed the Indicated CAR by flow cytometry.

T cells were cultured with the indicated primary human target cells overnight, and an IFN$\gamma$ ELISA was performed.

CD19-K562 expresses CD19 and CD20-K562 expresses CD20

All other targets listed lack both CD19 and CD20

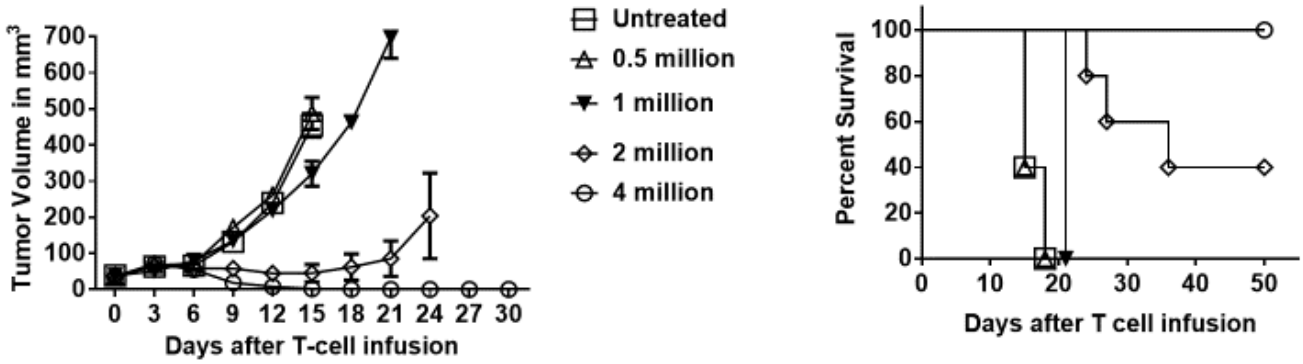
One potential problem with anti-CD20 CARs is possible blocking by serum anti-CD20 antibodies that many patients enrolled on this trial will have received. Anti-CD20 monoclonal antibodies such as Rituximab might block binding of CAR T cells to lymphoma cells, or they could cause nonspecific activation of CAR T cells. Prior reports have assessed rituximab levels in the serum of patients and found that the median serum rituximab concentration to be 38.3 μg/mL in patients who had received rituximab in the past 4 months. We assessed the impact that soluble rituximab might have on anti-CD20 CAR T cells by doing ELISA assays in which CAR T cells, CD20+ target cells, and graded concentrations of rituximimab were added together in overnight cultures. After the cultures IFNg ELISAs were performed on the culture supernatant. We found that rituximab did decrease IFNg release in a dose-dependent manner, but it never eliminated the ability of the CAR T cells to recognize lymphoma. We also found that rituximab did not cause non-specific CAR activation (Table 4). All cytokine values in Table 4 are pg//mL. rituximab concentrations are at the top of the table. Target cells use were ST486 -/- cells that express CD20 but not CD19. Human IgG was added to some wells as a control.

Table 4 : **Impact of rituximab on Hu1928-Hu20BB CAR T cells.**

| Average pg/mL IFNg | 100 ug/ml | 50 ug/ml | 25 ug/ml | 12.5 ug/ml | 6.2 ug/ml | 0 ug/ml | %CAR+ |
|---|---|---|---|---|---|---|---|
| Human IgG | 5921 | 6514 | 5470 | 5591 | 5841 | 6490 | 57.3 |
| Rituximab | 1661 | 2925 | 4165 | 5309 | 6165 | 6388 | 57.3 |

Next, we performed murine experiments to test Hu1928-Hu20BB and its constituent CARs. In the experiment depicted in *Figure 10*, immunocompromised Nod-Scid common γ-chain knockout (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 graded doses of Hu1928-Hu20BB T cells as shown in *Figure 10*. Tumor eradication was dose-dependent, and doses of 2 and 4 million CAR T cells had clear anti-tumor activity (5 mice per group). In this experiment, 4 million ST486 cells were injected 6 days to establish palpable intradermal tumors prior to CAR T cell infusion.

Figure 10




We also compared anti-tumor activity of T cells expressing H1928-Hu20BB and its constituent CARs against the st486 null (-/-) cell line that has CD19 expression knocked out by CRISPR/Cas9. In this model, Hu1928-Hu20BB and Hu20-CD8BBZ were much more effective than Hu19-CD828Z. We hypothesize that Hu19-CD828Z was reacting against some residual CD19 that was expressed despite the attempt at CD19 abrogation. In this experiment, 4 million ST486 (-/-) cells were injected 6 days to establish palpable intradermal tumors prior to CAR T cell infusion. There were 5 mice/group. In all mouse experiments, mice received only 1 infusion of CAR T cells and

no other interventions. Mice were sacrificed when tumors reached 1.5 cm in longest dimension. Note that mice did not exhibit any toxicity attributable to CAR T cells in any of our murine experiments.

Figure **11**


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 CAR-expressing T cells because substantial evidence demonstrates an enhancement of the anti-malignancy activity of adoptively-transferred T cells when chemotherapy or radiotherapy are administered before the T cell infusions.[160-162] 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.[59,63,160-163] 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.[160,161,163,164] 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.[160] Experiments in a murine xenograft model showed that regulatory T cells could impair the anti-tumor efficacy of anti-CD19 CAR T cells.[165] Myeloid suppressor cells have been shown to inhibit anti-tumor responses.[164] 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.[101] 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.[101]

Strong suggestive evidence of enhancement of the activity of adoptively-transferred T cells has been generated in humans.[67,166,167] 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.[67,72] 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.[68,70,71,90] 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.[68-71,166,167] Both cyclophosphamide and fludarabine are highly effective at depleting lymphocytes.[166,167] One well-characterized and commonly used regimen is the combination of 300 mg/m$^2$ of cyclophosphamide administered daily for 3 days and fludarabine 30 mg/m$^2$ administered daily for three days on the same days as the cyclophosphamide.[168] Multiple cycles of this regimen can be tolerated by heavily pretreated leukemia and lymphoma patients.[168]

### 1.2.13 Rationale for dose-escalation

The clinical trial described in this protocol is planned as a dose escalation in which the number of Hu1928-Hu20BB CAR T cells administered to patients will be increased with sequential dose levels. The rationale for conducting a dose-escalation trial of a cellular therapy is based on two main sources of evidence. First, the anti-tumor efficacy of adoptively-transferred T cell treatments increases as the dose of T cells administered to mice increases.[169-171] Second, in the setting of allogeneic transplantation, relapsed malignancy is often treated with infusions of allogeneic donor lymphocytes (DCIs).[172,173] 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'.[172,173]

### 1.2.14 Rationale for fully-human CARs

Immune responses against genetically-modified T cells have previously been reported [139,174,175]. Because treatment with Hu1928-Hu20BB CAR T cells is expected to eliminate recipient B cells, the most concerning type of immune response against 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 [174]. 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 [139,175]. Riddell and coworkers have reported cytotoxic T-cell responses against the FMC63 scFv (Riddell, Presentation at the American Society of Hematology Annual Meeting, 2013). Because this protocol intends to give patients multiple doses of CAR-modified T cells, the risk of developing anti-CAR immune responses is possibly even higher than in most previous clinical trials that only administered a single infusion of CAR T cells. All in all, use of a fully-human CAR will decrease the risk of anti-CAR T-cell responses that we hypothesize could interfere with CAR T-cell efficacy especially when multiple infusions of T cells are given.

The use of fully-human CARs might increase persistence of CAR T cells after a first dose of CAR T cells. In our prior trials of murine anti-CD19 CARs, persistence of CAR T cells in the

blood has been limited (*Figure 10*)[143], we hope that the fully-human anti-CD19 CAR will have longer persistence. The graphs shown in *Figure 10* are absolute numbers of CAR T cells in the blood of 5 patients as determined by a quantitative PCR assay that is specific for the CAR. These results show the usual pattern of T-cell persistence with the current anti-CD19 CAR used in ETIB CAR trials. The CAR is called FMC63-28Z, and it contains a murine scFv. After infusion, CAR T cells rapidly rise to a peak. The cells do not persist in the blood long-term. Cells were undetectable in the blood by approximately 2 months after infusion.

1.2.15 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 [13,15,176]. 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 5 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.[70,141] 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 and anti-CD20 CAR with proteins other than CD19 and CD20 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.[70,71] Hypogammaglobulinemia in these patients was routinely treated with infusions of intravenous immunoglobulins.[70]

The potential benefits to subjects enrolling on this trial include the possibility that the Hu1928-Hu20BBCAR 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[68,70,177], so there is a chance that recipients of the Hu1928-Hu20BBCAR 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 CAR T-cell therapies is needed.

# 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

Note: As of approval of Amendment A, no patients with Hodgkin lymphoma can be enrolled until at least 6 patients with B-cell malignancies are treated without incidence of Guillain-Bare syndrome

- Patients must have any B-cell lymphoma, or CLL/SLL, Gray-zone lymphoma, nodular lymphocyte-predominant Hodgkin lymphoma, or classical Hodgkin lymphoma with any CD19 or CD20 expression on Reed-Sternberg cells. Lower grade lymphomas or CLL transformed to DLBCL are potentially eligible as is primary mediastinal B-cell lymphoma and all other subtypes of DLBCL. Burkitt and mantle cell lymphoma are potentially eligible.
- For classical Hodgkin lymphoma only, a biopsy from any time from any institution that shows any CD19 or CD20 expression on Reed-Sternberg cells is adequate for eligibility. CD19 or CD20 expression on the Reed-Sternberg cells that is weak or only present on some Reed-Sternberg cells by immunohistochemistry is compatible with protocol eligibility.
- For all lymphoma types except for classical Hodgkin lymphoma, either CD19 or CD20 expression must be "uniform". "Uniform" CD19 or CD20 expression is defined as no obvious lymphoma population lacking antigen expression is present. Antigen expression can be assessed by either immunohistochemistry or flow cytometry.
- Only when insufficient biopsy material is available to allow CD19 and CD20 expression assessment at the NIH, CD19 and/or CD20 staining performed at another institution can be used
- 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 B-cell 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 inhibitorand venetoclax.
- Hodgkin lymphoma patients must have:
    - had at least 3 prior lines of therapy.
    - had at least 1 prior cytotoxic chemotherapy-containing regimen.
    - had prior exposure to brentuximab vedotin.

- had undergone autologous stem cell transplant or been transplant ineligible or refused autologous transplantation

- Eligibility will be expanded to include CD19 and CD20-negative classical Hodgkin lymphoma if any 2 patients with classical Hodgkin lymphoma and CD19/CD20 expression on RS cells have durations of response 6 months or greater (responses could be PRs or CRs) or a CR of 3 months or greater.
- All 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 or equal to 10.0 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. CLL masses do not need to have increased activity on 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. Note that leukemia cells must make up 1% or less of peripheral blood lymphocytes in CLL patients for these patients to be eligible.

2.1.1.2 Other inclusion criteria:

- Greater than or equal to 18 years of age.
- 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 sexes must be willing to practice birth control from the time of enrollment on this study and for four months after receiving the protocol treatment.
- A patient with a negative blood PCR test for hepatitis B DNA test can be enrolled. If hepatitis B DNA (PCR) testing is not available, patients with a negative hepatitis B surface antigen and negative hepatitis B core antibody can be enrolled.
- Patients must be tested for the presence of Hepatitis C antigen by PCR and be HCV RNA negative in order to be eligible. Only if Hepatitis C PCR testing is not available in a timely manner, patients who are Hepatitis C antibody-negative can be enrolled.
- Absolute neutrophil count greater than or equal to 1000/$mm^3$ without the support of filgrastim or other growth factors.
- Platelet count greater than or equal to 50,000/$mm^3$ without transfusion support
- Hemoglobin greater than 8.0 g/dl.

- For CLL only, less than or equal to 1% malignant cells in the peripheral blood lymphocytes must be documented by flow cytometry of blood within 2 weeks of protocol enrollment.
- 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 1.5 mg/dl.
- Total bilirubin less than or equal to 2.0 mg/dl.
- Normal cardiac ejection fraction (greater than or equal to 50% by echocardiography) and no evidence of hemodynamically significant pericardial effusion as determined by an echocardiogram within 4 weeks of treatment start.
- 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 must be able to understand and be willing to sign a written informed consent.
- Patients who have either been previously treated on protocols of genetically-modified T cells on a clinical trial at the NCI or received T cells modified with the MSGV or MSGV1 gamma-retroviral vectors at any institution are potentially eligible under these conditions:
  - At least 3 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 protocol Principal Investigator), and persisting genetically-modified T cells are either not detectable in the patient's blood or detectable at levels less than or equal to 0.2% of blood T cells as measured by flow cytometry using the Kip-1 antibody in the flow cytometry lab of the NCI Laboratory of Pathology (Maryalice Stetler-Stevenson's lab).

2.1.2 Exclusion criteria:

- Patients that require urgent therapy due to tumor mass effects or spinal cord compression.
- Patients must not have received any anti-CD20 or anti-CD19 antibody products in the past 30 days prior to CAR T-cell infusion.
- Any history of receiving PD-1 or PD-L1 inhibitors.
- Patients that have active hemolytic anemia.
- HIV-positive patients are excluded because HIV causes complicated immune deficiency and study treatment can pose more risks for these patients.
- 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 3 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.
- Pregnant women are excluded from this study because study therapy can cause fetal harm. Because there is potential risk for adverse events in nursing infants secondary to treatment of the mother with study therapy, breastfeeding should be discontinued if the mother is treated with study drugs.
- 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.
- Hospitalization within the 7 days prior to enrollment.
- Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).
- Prior allogeneic stem cell transplant
- Systemic lymphoma treatment of any type and corticosteroid steroid therapy of any dose greater than 5 mg/day or more of prednisone or equivalent is not allowed within 14 days prior to the required leukapheresis, or the initiation of the conditioning chemotherapy regimen. Corticosteroid creams, ointments, and eye drops are allowed.
- History of severe immediate hypersensitivity reaction to any of the agents used in this study.
- Patients on systemic anticoagulant therapy except aspirin.
- Active central nervous system metastases or cerebrospinal fluid malignancy. Patients with known brain metastases will be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.
- Any current neurologic disorders except migraine headaches.

2.1.3 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on the NIH websites and on NIH social media platforms. Participants will be recruited from the current patient population at NIH, and local community physicians.

## 2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes.

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

The following activities will be performed only after the subject has signed the consent for study #01-C-0129 on which screening activities will be performed. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent.

The following assessments must be completed within **30 days** prior to enrollment unless otherwise noted below:

- 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 for B-cell malignancies of uniform CD19 and/or CD20 expression on the malignant cells from either bone marrow or a lymphoma mass by flow cytometry or immunohistochemistry. The sample used for this expression analysis can come from any time prior to enrollment on the protocol. The sample can be a fresh biopsy or paraffin-fixed slides. For all types of Hodgkin lymphoma any level of documented CD19 or CD20 expression at any time is adequate for enrollment; this requirement might be dropped later in the trial.
- Electrocardiogram (ECG)
- MRI of the brain
- PET only if necessary, to document measurable malignancy. If possible, PET scans previously performed after the most recent lymphoma treatment will be obtained, so that a new PET at NIH can be avoided.

- Cardiac echocardiogram
- CT scan of neck, chest, abdomen, and pelvis only if necessary, to document measurable malignancy. If possible, CT scans previously performed after the most recent lymphoma treatment will be obtained, so that new CTs at NIH can be avoided.
- Bone marrow biopsy with flow only if necessary, to document measurable $CD19^+$ and/or $CD20^+$ malignancy for enrollment purposes. If not necessary for enrollment, the bone marrow biopsy can be delayed until the baseline evaluation.
- A biopsy, possibly CT-guided might be needed for confirmation of lymphoma diagnosis and to confirm antigen expression for eligibility purposes. This biopsy will not be performed if biopsy material can be obtained from a prior biopsy conducted before protocol enrollment.
- Lumbar Puncture is required for patients with Burkitt's lymphoma only.
- Venous assessment for Apheresis (will be performed within 6 months before apheresis. Does not need to be repeated if no further apheresis needs to take place.)
- Laboratory Evaluation
    - Antibody screen for Hepatitis B and C; HIV, HTLV-I/II, T. cruzi (Chagas agent), West Nile, and syphilis (RPR)
    - Chemistries: (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)
    - Hematological profile: CBC with differential
    - PT/PTT
    - Glucose-6-phosphate dehydrogenase (G6PD) deficiency screening
    - Thyroid Stimulating Hormone, T3**, T4**
    - Serum cortisol
    - Serum or urine pregnancy test for female participants of childbearing age (in the absence of prior hysterectomy).

## 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 (FOR REGISTRATION PURPOSES ONLY):

**Cohorts:**

| Number | Name | Description |
|---|---|---|
| 1 | Cohort 1 | Phase I dose escalation cohort for patients with B-cell lymphoma or CLL |
| 2 | Cohort 2 | Phase I dose escalation cohort for patients with Hodgkin lymphoma |
| 3 | Cohort 3 | Patients enrolled at the MTD or maximum administered dose of CAR-T cell after MTD of CAR-T cell is established |

**Arms:**

| Number | Name | Description |
|---|---|---|
| 1 | Conditioning chemotherapy plus CAR T-cells dose escalation | All patients will be receiving escalating dose of Anti-CD19 and anti-CD20 CAR T cells/kg + conditioning chemotherapy |
| 2 | Conditioning chemotherapy plus CAR T-cells expansion phase | MTD dose or maximum administered dose of Anti-CD19 and anti-CD20 CAR T cells/kg + Conditioning chemotherapy |

**Arm Assignment:**

**Note:** As of approval of Amendment A, Cohort 2 will open only after at least 6 patients with B-cell malignancies are treated without incidence of Guillain-Bare syndrome

Patients in cohort 1 and cohort 2 will be directly assigned to arm 1, each with its own dose escalation conducted separately.

Patients in cohort 3 will be directly assigned to arm 3

# 3 STUDY IMPLEMENTATION

## 3.1 STUDY DESIGN

### 3.1.1 General study plan

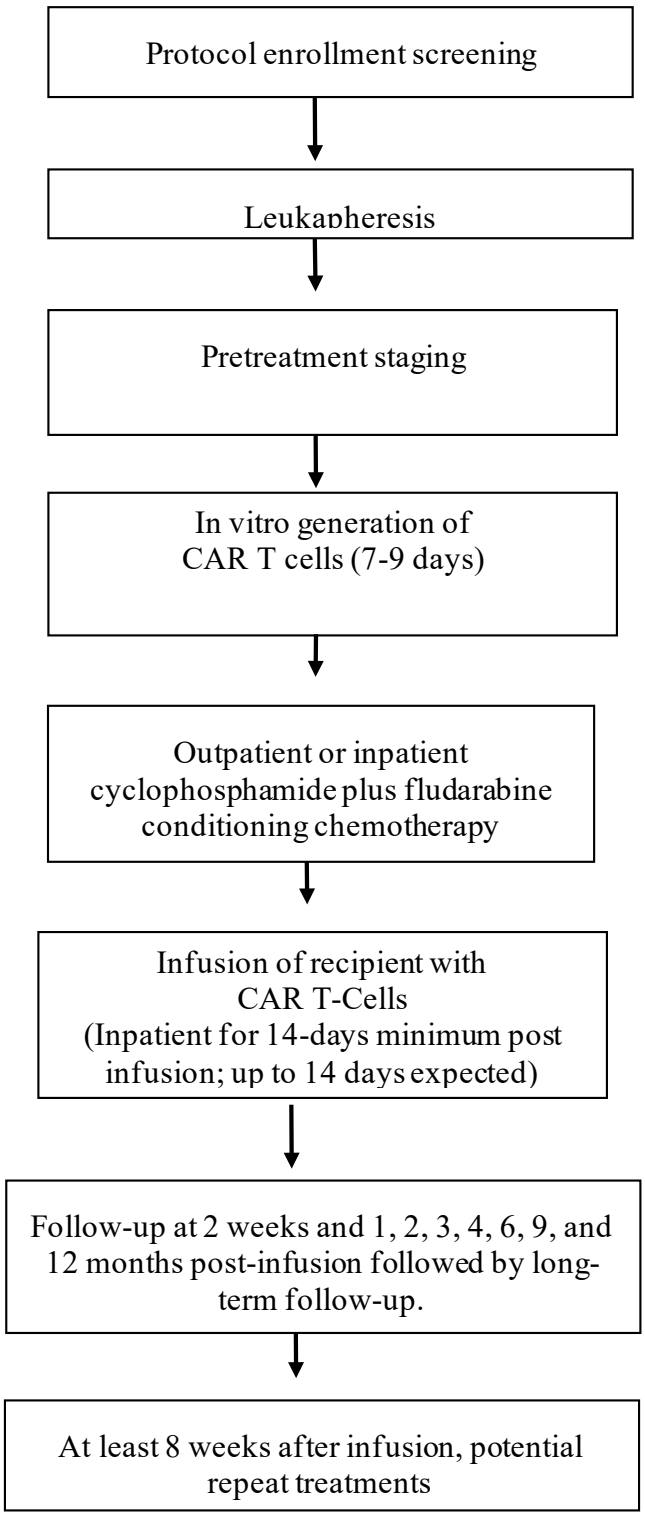
This protocol is a phase I dose-escalation study of autologous T cells that are genetically modified to express a bicistronic construct that leads to expression of a fully-human anti-CD19 CAR and a fully-human anti-CD20 CAR by the same T cells. The cell dose will be escalated unless occurrence of dose-limiting toxicities limits further escalation or until the maximum tolerated dose level is reached.

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 and CD20 expression will be an important part of the eligibility screening. Patients enrolled on the study will undergo leukapheresis, and CAR-expressing T cells will be generated by transducing the patient's T cells with a lentivirus encoding the CAR construct. Patients will receive a conditioning chemotherapy regimen of cyclophosphamide 500 mg/m$^2$ daily for 3 days and fludarabine 30 mg/m$^2$ IV daily for 3 days on the same days. This is an extensively-used chemotherapy regimen that can be easily administered on an outpatient basis. Three days after the end of the conditioning chemotherapy, patients will receive a single infusion of CAR-expressing T cells in the inpatient setting. Patients will then be evaluated for toxicity, and malignancy will be staged at 1, 2, 3, 4, 6, 9, and 12 months after the infusion.

A small number of subjects may be eligible for re-enrollment if a patient is removed from the protocol BEFORE completing protocol therapy or if a patient is eligible for re-treatment.Re-treatment will only be administered after dose finding is complete and preliminary safety and efficacy of the product has been established. At that point, re-treatment can be considered on a case-by-case basis. Patients for re-enrollment 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 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 Protocol schema



### 3.1.3 Dose Limiting Toxicity

Dose-limiting toxicities are defined as the following toxicities that are possibly, probably, or definitely attributable to protocol interventions and occurring between the first protocol treatment through 28 days after the CAR T-cell infusion.

- Grade 3 toxicities possibly or probably related to Hu1928-Hu20BB CAR T cells and lasting more than 9 days, with the exceptions listed below. Symptomatic seizure activity is included as a grade 3 toxicity. This must be deemed by a neurologist to be true seizure activity. All suspected seizures must be evaluated by a neurologist.
- Grade 4 toxicities possibly or probably related to the study interventions with the exceptions listed below

#### 3.1.3.1 Exceptions

- The following specific toxicities will <u>not</u> be dose-limiting toxicities:
  - Neutropenia (ANC<500/μL) lasting continuously 9 days or less
  - Anemia (Hgb<8 g/dL) lasting continuously 9 days or less
  - Thrombocytopenia that is not transfusion-dependent
  - Grade 3 thrombocytopenia
  - Transfusion-dependent thrombocytopenia lasting 30 days or less
  - All cytopenias except neutropenia, anemia, or thrombocytopenia
  - Hypotension (defined as systolic blood pressure <90 mm Hg) requiring treatment with vasopressors (norepinephrine dose of >3 mcg/minute or equivalent, doses less than or equal to 3 mcg/minute are not a DLTs) for 72 continuous hours or less.
  - Fever
  - Hypophosphatemia
  - Grade 4 elevation in alanine aminotransferase, aspartate aminotransferase, or bilirubin that resolves to Grade 3 or less within 3 days or less
  - QT interval increases not associated with ventricular arrythmias
  - Grade 4 creatinine kinase elevation that resolves to Grade 3 or less within 4 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

3.1.4 Dose Escalation

The trial will be a dose-escalation with 4 dose levels based on the patient's **actual** bodyweight.

For the following dose escalation plan, 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 Hu1928-Hu20BB CAR-expressing T cells transferred for each dose level will be as follows:

**Table *5:*Dose escalation plans for each cohort**

| **Dose Escalation Plan-Cohort 1 B-cell lymphoma or CLL or lymphocyte-predominant Hodgkin lymphoma or Gray-zone lymphoma** | |
|---|---|
| **Dose Level** | Dose of anti-CD19 and anti-CD20 CAR T cells |
| Level -1 | $0.3x10^6$ CAR+ T cells per kg of recipient bodyweight |
| Level 1 | $0.66x10^6$ CAR+ T cells per kg of recipient bodyweight |
| Level 2 | $2.0x10^6$ CAR+ T cells per kg of recipient bodyweight |
| Level 3 | $6.0x10^6$ CAR+ T cells per kg of recipient bodyweight |
| Level 4 | $10.0x10^6$ CAR+ T cells per kg of recipient bodyweight |

| **Dose Escalation Plan-Cohort 2 Classical Hodgkin lymphoma** | |
|---|---|
| **Dose Level** | Dose of anti-CD19 and anti-CD20 CAR T cells |
| Level -1 | $0.3x10^6$ CAR+ T cells per kg of recipient bodyweight |
| Level 1 | $0.66x10^6$ CAR+ T cells per kg of recipient bodyweight |
| Level 2 | $2.0x10^6$ CAR+ T cells per kg of recipient bodyweight |
| Level 3 | $6.0x10^6$ CAR+ T cells per kg of recipient bodyweight |
| Level 4 | $10.0x10^6$ CAR+ T cells per kg of recipient bodyweight |

The trial consists of 2 cohorts, one for B-cell malignancies and the other for Hodgkin lymphoma. Each cohort has a separate but identical dose escalation scheme because toxicities for patients with B-cell malignancies might be significantly different than toxicities for Hodgkin lymphoma patients. One factor that is different is that Hodgkin lymphoma masses are made up of cells that are in large part negative for CD19; in contrast, B-cell lymphoma masses usually have CD19 expression on all or almost all cells. Dose escalation will proceed independently for each cohort. The delay between patients treated and the decision to escalate to a higher dose level is specific for each cohort. For example, if a patient is treated on Cohort 1, a different patient could be treated on Cohort 2 immediately. Also, if criteria have been met for escalation from Dose Level 1 to Dose Level 2 on Cohort 1, Cohort 2 cannot escalate to Dose Level 2 until criteria for escalating to Dose Level 2 are met specifically by patients enrolled on Cohort 2.

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 16 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 minimum 21-day delay between sequential CAR T-cell infusions. **The only exception to this rule is that we will wait at least 28 days between treating the first and second patients on each dose level treated on this protocol <u>for each cohort</u>**. There will also be a 28-day 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.

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 for a cohort, additional patients can be treated on this cohort. Up to 10 total additional recipients can be treated with the MTD after an MTD is established; therefore, the maximum number of patients treated on this protocol is 68. In addition, we will allow 20 additional patients to allow the trial to be completed as planned if some patients who enroll are not treated due to reasons such as rapid clinical deterioration requiring cancellation of protocol participation, so total allowed enrollment will be 88 subjects.

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 or a maximum tolerated dose is not reached due to DLTs not occurring, the highest achievable dose level will be declared the maximum administered dose, and up to 10 additional patients will be treated with the maximum administered dose.

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

**Table** 6

| **Number of Patients with DLT at a Given Dose Level** | **Escalation Decision Rule** |
|---|---|
| 0 out of 3 | Enter up to 3 patients at the next dose level |
| $\geq$ 2 | Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Up to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose. |
| 1 out of 3 | Enter up to 3 more patients at this dose level.<br>• If 0 of these 3 patients experience DLT, proceed to the next dose level.<br>• If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Up to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose. |
| $\leq$1 out of 6 at highest dose level below the maximally administered dose | This is the MTD and is generally the recommended phase 2 dose unless $\leq$1 out of 6 DLTs occurred at the maximally administered dose, in which case, the maximally administered dose will be the recommended phase 2 dose |

## 3.2 PRE-TREATMENT PHASE

### 3.2.1 Leukapheresis

The patient will undergo a 15 to 20-liter leukapheresis (generally, 15 liters will be processed to target a yield of 6-10 x$10^9$ 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.

### 3.2.2 Hu1928-Hu20BB CAR-expressing T-cell preparation

After cells are obtained by apheresis, further cell processing to generate 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. In addition, as stated in eligibility criteria, cryopreserved cells stored continuously in the Center for Cellular Engineering can be used to prepare autologous clinical cell products on other NCI cell therapy protocols, if the future cell therapy is conducted under a different NCI protocol. Peripheral blood mononuclear cells will be isolated. Sufficient cells for the initial cell production and 1 complete back-up cell production will be retained in the Department of Transfusion Medicine. The excess cells will be sent to the Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755 for cryopreservation at 200 to 300 million PBMC per vial. The anti-CD3 monoclonal antibody OKT3 will be used to stimulate T-cell proliferation. The cells will be transduced by exposing them to replication-incompetent gamma-retroviruses encoding the CAR by using DTM SOPs. The cells will continue to proliferate in culture. CAR T cells will be cryopreserved between day 7 and day 9 of culture. $CAR^+$ T cells will be determined by flow cytometry 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 10x$10^6$ $CAR^+$ T cells/kg) than the first dose. Cryopreserved cells will be used for all infusions.

Ten vials of the infused cells will be cryopreserved for research use and stored in the Surgery Branch Cell Production Facility SB-CPF. Each vial will contain 20 million cells.

Before cryopreservation, the percentage of T cells expressing the CAR will be determined by flow cytometry, and this percentage of $CAR^+$ T cells will be used in calculating the total number of cells to be cryopreserved in a single-infusion bag to meet the dose requirements of the dose-escalation plan described in Section **3.1.4** As noted above, cells for potential 2nd doses will also be cryopreserved. For the first 2 dose levels, third infusions will also be cryopreserved at this time. In addition to flow cytometry, further testing of the cells will take place prior to infusion to evaluate for microbial contamination, replication-competent lentiviruses, and viability. Details of this testing can be found in the appropriate DTM SOPs. When a patient is no longer eligible for retreatment on this protocol due to meeting any of the off-study criteria listed in section **3.11.2**, any remaining cryopreserved cells from this protocol will be coded-linked and used for research or discarded after approval of the Principal Investigator of this protocol.

## 3.3 DRUG ADMINISTRATION

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

3.3.1 Overall summary of the treatment plan

**Table 4**

| Drug | Dose | Days |
|---|---|---|
| Cyclophosphamide | 500 mg/m$^2$ IV infusion over 30 minutes | Daily x 3 doses on days -5, -4, -3 |
| Fludarabine | 30 mg/m$^2$ IV infusion over 30 minutes administered immediately following the cyclophosphamide on day -5, -4, -3 | Daily x 3 doses on days -5, -4, -3 |
| CAR T cells | Variable. | Infuse on day 0 |

3.3.1.1 Detailed treatment plan

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

Patients will receive anti-emetics following NIH Clinical Center guidelines, but **dexamethasone will not be administered**. One suggested regimen is ondansetron 16 to 24 mg orally on days -5, -4, and -3 one hour before chemotherapy (I.V. ondansetron can be substituted). Consider aprepitant in addition to ondansetron for nausea prophylaxis. 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 500 mg/m$^2$ I.V. will be diluted in 100 ml 5% dextrose solution and infused over 30 minutes. After the cyclophosphamide on days -5, -4, and -3, patients will receive 30 mg/m$^2$ I.V. fludarabine in 100 mL 0.9% sodium chloride over 30 minutes. **Note: in patients with a creatinine clearance calculated by the CKD-EPI equation less than 80 ml/minute/1.73 m$^2$of body surface are, the daily dose of fludarabine will be reduced by 20%.**

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

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

Day 0: 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 for nursing are included in Appendix Appendix D: Infusion Instructions**

Days 1 to 14: 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 16 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.

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 2000/microliter.

### 3.4 DOSE MODIFICATIONS/DELAY

- Patients may have a delay in planned treatment if they have active infections defined as infections causing fevers or infections requiring intravenous anti-microbial therapy that arise while patients are on-study but before the CAR T-cell infusion; however, such patients are eligible for treatment if they meet all eligibility criteria after the infection resolves.
- If a patient experiences a grade 3 or greater toxicity (with the exception of cytopenias including neutropenia, lymphopenia, anemia, or thrombocytopenia) 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. Exceptions to this would be if the grade 3 toxicity was present at baseline or related to progressing disease.

### 3.5 POTENTIAL REPEAT TREATMENT

- **No repeat treatments will be administered until dose-finding is complete and preliminary safety and efficacy of the product has been identified. At that point, re-treatment can be considered on a case-by-case basis after consultation with the FDA.**
- The chemotherapy component of repeat treatments will be initiated a minimum of 8 weeks after the most recent prior CAR T-cell infusion. There is no maximum time interval between treatments.
- Patients with any response except progressive disease or continuing complete remission after an initial or second CAR T-cell infusion are potentially eligible for a repeat treatment consisting of conditioning chemotherapy followed by an infusion of CAR T cells.
- Patients experiencing a DLT with any prior treatment on this protocol will not be eligible for re-treatment.
- To be eligible for repeat treatments, patients must meet the same eligibility requirements listed in Section **2.1**.
    - Uniform CD19 or CD20 expression or uniform expression of both CD19 and CD20 needs to be documented on the malignant cells after previous CAR T-cell infusions.
    - 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 2 total treatments can be administered to any one patient, and at least 8 weeks must elapse between each cell infusion.

- Patients with Grade 2 or worse neurologic toxicity will not be retreated until neurologic toxicity improves to Grade 0 or 1.

- The dose of CAR T cells administered during repeat treatments will be the maximum tolerated or maximum administered dose that will be determined by the dose escalation schedule. No patients will be retreated until the maximum tolerated or administered dose is determined.

## 3.6 PROTOCOL STOPPING RULES

- 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 (**3.1.4**).
- A death on study not attributable to progressive malignancy within 30 days of a cell infusion will be a cause for a pause to accrual pending amendment of the protocol approved/reviewed by the FDA and IRB.
- If 2 or more subjects during the dose escalation phase of a given cohort experience a Grade 4 dose limiting toxicity possibly or probably or definitely attributable to CAR T cells within 30 days of cell infusion, this will be a cause for a pause to accrual to reassess the safety of the product pending amendment of the protocol approved/reviewed by the FDA and IRB.
- After 3 patients have been accrued to Cohort 2, enrollment to Cohort 2 only must be temporarily halted while a safety assessment by the Safety Monitoring Committee and the IRB. This is described in Section **7.5.1**.
- If by the time 3 patients have been treated on the highest dose level, no patient on Cohort 2 has had either a complete remission lasting at least 6 months or progression-free survival lasting at least 9 months after CAR T-cell infusion, enrollment on Cohort 2 will cease.
- If at any time more than 1 total DLT attributable to CAR T cells in the first 30 days after infusion occur on Cohort 2, and no complete remissions have occurred on Cohort 2, accrual to Cohort 2 will halt to reassess the safety of the product pending amendment of the protocol approved/reviewed by the FDA and IRB.
- Aside from the 3 points above, continued accrual on Cohort 2 will be determined by the dose-escalation scheme in Section **3.1.4**.

## 3.7 PROTOCOL EVALUATION

### 3.7.1 Baseline Evaluations

If results of these tests do not satisfy eligibility criteria, per PI discretion patients' treatment can be delayed or discontinued. An exception to this is that a platelet count of 50,000 or more is considered adequate to start chemotherapy

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 another device. Non-valved PICC lines are greatly preferred over valved PICC lines for this protocol.
- Physical exam with vital signs and oxygen saturation
- CT scan of neck, chest, abdomen, and pelvis
- PET of the torso for all patients except patients with CLL
- Bone marrow aspirate and biopsy: specifically ask for CD19 and CD20 immunohistochemistry staining of the bone marrow biopsy. Flow cytometry must be performed. The bone marrow biopsy must take place at some time after the patient's most recent malignancy treatment. If a bone marrow biopsy was performed at the NIH as part of protocol screening within 4 weeks of the start of treatment, it does not have to be repeated unless necessary for staging.
- Peripheral blood flow cytometry for patients with CLL
- G-banding cytogenetics on the bone marrow for all patients.
- 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 RCR 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
- In addition to the CPT tubes, 16 mL of blood will be collected within 3 days prior to the start of the chemotherapy.to obtain serum for research purposes (2 SST tubes, 8 mL per tube) Send to Figg lab. For pick-up page 102-11964. For help call 240-760-6180.**The following tests must be completed within 7 days of the start of the conditioning chemotherapy regimen:**
- TBNK (T, B, and NK cell)
- Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid (to be repeated on the first day of the chemotherapy at the discretion of the PI)

- Serum quantitative immunoglobulins
- ABO typing
- CBC with differential (to be repeated on the first day of the chemotherapy at the discretion of the PI)
- PT/PTT
- Urinalysis; if results are abnormal, send for urine culture
- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential
- C-reactive protein (CRP)
- Fibrinogen

3.7.2 Studies to be performed on Day 0 and during the mandatory 14-day inpatient admission after cell infusion

- Vital signs including pulse oximetry will be monitored q1h x 4 hours (+/- 15 minutes) after completion of the CAR T cell infusion and then approximately every 4 hours 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. (In the case of a later day infusion or early discharge, this may be only once a day.)
- C-reactive protein (CRP) 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, chemistries once daily until discharge: (Sodium (Na), Potassium (K), Chloride (Cl), Total $CO_2$ (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Uric Acid, creatine kinase) (In the case of a later day infusion or early discharge, this may be only once a day.)
- PT/PTT and fibrinogen daily while hospitalized after the infusion
- Other tests will be performed, as clinically indicated.
- **Day 0 Research Blood:** 1 SST tube will be drawn on the morning of CAR cell infusion prior to infusion of CAR T cells.
- **Post Infusion Research blood:** Every Monday, Wednesday, and Friday during hospitalization. This starts 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).

- **Additional Post-infusion research blood:** 1 SST tube will be drawn on the first Sunday after CAR T-cell infusion. This tube may be stored refrigerated on the nursing unit and processed first thing Monday morning at the latest. If the Monday is a holiday, Sunday research blood will not be drawn.
- Research blood CPT tubes need to be sent to the Surgery Branch Cell Production Facility. Research blood SST tubes need to go to Figg lab. Page 102-11964 for pick-up. Call 240-760-6180 for immediate pick-up.

**Note regarding research blood collection:** If any of the above time points fall on federal holiday then, we may collect research blood on the next business day.

3.7.3 Post-infusion outpatient evaluation

Patients will be seen at the NIH in follow-up to evaluate disease status and late problems related to CAR T-cell infusion at the following time-points: +14 (+/- 1 day), +30 (+/- 5 days), +60 (+/- 7 days), +90 (+/- 7 days), +120 (+/- 7 days), and at 180 (+/- 14 days), 270 (+/- 14 days), and 365 (+/- 30 days) after CAR T-cell infusion. After 12 months, the patient will be seen approximately every 6 months (+/- 30 days) up to three years; subsequently, patients will be seen annually (+/- 30 days) up to 5 years post infusion.

In addition, follow-up visits are required at the NIH Clinical Center on days 16 and 21 after infusion for neurologic checks.

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. CPT tubes need to be sent to the Surgery Branch Cell Productin Facility.
- 1 SST tube (8 mL) of Research Blood will be obtained for serum collection. Research blood SST tubes need to go to Figg lab. Page 102-11964 for pick-up. Call 240-760-6180 for immediate pick-up. Note: **after the first year of follow-up, research blood will be reduced to 4 CPT tubes (32 mL total) during required protocol visits, and these CPT tubes should be sent to the Surgery Branch Cell Production Facility**. 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 scans at 1 month after infusion. PET scans should continue at each outpatient scheduled evaluation, except the day 120 (4-month) evaluation until CR is obtained. When CR is obtained, PET scans should cease. CLL patients should not get PET scans only CT scans at each outpatient visit in the study calendar (Table 3.8 except the day 120 (4-month) follow-up visit.
- For CLL, obtain peripheral blood flow cytometry at all outpatient follow-up visits.
- Patients may need 1 biopsy to confirm lymphoma progression. Patients may also undergo a maximum of 1 biopsy after CAR T-cell infusion to obtain material to study interactions

between CAR T cell and the lymphoma microenvironment. This biopsy could occur either during the inpatient stay or during the outpatient phase. CT-guidance might be needed for these biopsies.

- 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 and CD20 staining must be requested for the flow cytometry. CD19 and CD20 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
- Serum C-reactive protein if clinically indicated
- Gene-therapy-specific follow-up must be carried out as described in section **3.9**

## 3.8 STUDY CALENDAR

| | | | Inpatient | | Follow up | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Procedures [a] | Screening | Baseline | Day 0 | Post Cells while inpt | Day+14 (+/- 1 day) | Day+30 (+/- 5 days) | Day[q] +60 (+/- 7 days) | Day +90 (+/- 7 days) | Day +120 (+/- 7 days) | Day +180 (+/- 14 days) | Day +270 (+/- 14 days) | Day +365 (+/- 30 days) | Every 6 months after day 365(+/- 30 days) up to 3 years then annually (+/- 30 days) up to 5 years post CAR cells. |
| Clinical Assessments | | | | | | | | | | | | | |
| *History and PE* | X | X[s] | X | X[k] | X | X | X | X | X | X | X | X | X |
| *Vital signs, $O_2$ saturation*[r] | X | X | X | X[k] | X | X | X | X | X | X | X | X | X |
| *Height, Weight* | X | | | | | | | | | | | | |
| *Performance Score (ECOG)* | X | | | | | | | | | | | | |
| *Lesion Location (If applicable)* | X | | | | | | | | | | | | |
| *Pathology Confirmation* | X | | | | | | | | | | | | |
| Laboratory Assessments | | | | | | | | | | | | | |
| *CBC with differential* | X | X | X | X[k] | X | X | X | X | X | X | X | X | X |
| *Sodium, Potassium Chloride, CO2, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Phosphorus, Alkaline Phosphatase, ALT, AST, T. Bilirubin, D. Bilirubin, LDH, Total Protein, CK, Uric Acid* | X | X | X | X[k] | X | X | X | X | X | X | X | X | X |
| *Serum C-reactive Protein* | | X | X | X[k] | X[d] | X[d] | X[d] | X[d] | X[d] | X[d] | X[d] | X[d] | X[d] |
| *PTT/PT* | X | X | X | X[k] | X | X | X | X | X | X | X | X | X |
| *Fibrinogen* | | X | X | X[k] | | | | | | | | | |
| *TBNK* | | X | X | X (Day 7 only) | X | X | X | X | X | X | X | X | X |
| *Quantitative immunoglobulins* | | X | | | | X | X | X | X | X | X | X | X |

| | | | Inpatient | | Follow up | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Procedures [a] | Screening | Baseline | Day 0 | Post Cells while inpt | Day+14 (+/- 1 day) | Day+30 (+/- 5 days) | Day[q] +60 (+/- 7 days) | Day +90 (+/- 7 days) | Day +120 (+/- 7 days) | Day +180 (+/- 14 days) | Day +270 (+/- 14 days) | Day +365 (+/- 30 days) | Every 6 months after day 365(+/- 30 days) up to 3 years then annually (+/- 30 days) up to 5 years post CAR cells. |
| *Thyroid stimulating Hormone, T3* | X | | | | | | | | | | | | |
| *Anti-CMV, HSV, EBV, t.cruzi, toxoplasma* | X | X[f] | | | | | | | | | | | |
| *Antibody screen for Hepatitis B and C; HIV, HTLV-I/II, T. cruzi (Chagas agent), West Nile, and syphilis (RPR)* | X | X[f] | | | | | | | | | | | |
| *Serum Cortisol* | X | | | | | | | | | | | | |
| *G6PD* | X | | | | | | | | | | | | |
| *Urinalysis (culture prn)* | X | X | | | | | | | | | | | |
| *ABO typing* | X | X | | | | | | | | | | | |
| *B2-HCG (serum or urine in women of childbearing potential)* | X | X | | | | | | | | | | | |
| Disease Assessments | | | | | | | | | | | | | |
| *CT scan of neck chest, abdomen, pelvis* | X[C] | X[g] | | | | X[g] | X[g] | X[g] | | X[g] | X[g] | X[g] | X[g] |
| *PET Scan* | X[C] | X[h] | | | | X[h] | X[h] | X[h] | | X[h] | X[h] | X[h] | X[h] |
| *Bone marrow aspirate/biopsy with flow* | X[C] | X[i] | | | | X[i] | X[i] | X[i] | | X[i] | X[i] | X[i] | X[i] |
| *Peripheral blood flow cytometry* | X[C] | X[l] | | | | X[l] | X[l] | X[l] | | X[l] | X[l] | X[l] | X[l] |
| *Lumbar Puncture (Burkitts only)* | X[e] | | | | | | | | | | | | |
| Other Specific Assessments | | | | | | | | | | | | | |
| *Documentation of CD19 and/or CD20 expression by malignancy* | X | | | | | | | | | | | | |

| | | | Inpatient | | Follow up | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Procedures [a] | Screening | Baseline | Day 0 | Post Cells while inpt | Day+14 (+/- 1 day) | Day+30 (+/- 5 days) | Day[q] +60 (+/- 7 days) | Day +90 (+/- 7 days) | Day +120 (+/- 7 days) | Day +180 (+/- 14 days) | Day +270 (+/- 14 days) | Day +365 (+/- 30 days) | Every 6 months after day 365(+/- 30 days) up to 3 years then annually (+/- 30 days) up to 5 years post CAR cells. |
| *Brain MRI* | X | | | | | | | | | | | | |
| *250 microgram cosyntropin test* | | X[d] | | | | | | | | | | | |
| *EKG, echocardiogram* | X | | | | | | | | | | | | |
| *Central Venous catheter placement* | | X | | | | | | | | | | | |
| *Venous Assessment for Apheresis* | X[p] | | | | | | | | | | | | |
| Ongoing Evaluation | | | | | | | | | | | | | |
| *RCR (replication competent retrovirus)* | | X | | | | | | X | | X | | X | X[j] |
| *Research Blood* | | X[m] | X[n] | X[n] | X[o] | X[o] | X[o] | X[o] | X[o] | X[o] | X[o] | X[o] | X[o] |
| *Adverse Events* | | | X | X | X | X | X | X | X | X | X | X | X |

Note: In addition, follow-up visits are required at the NIH clinical center on days 16 and 21 after infusion for neurologic checks.

a. see section **2.2** and section **3.7** for details
b. see section **3.3** for details of testing during hospitalization
c. PET or CT (not both) performed as disease appropriate only if needed to establish measurable disease; in addition, bone marrow biopsy, lumbar puncture, and peripheral blood flow cytometry will only be performed if needed to document measurable malignancy. If possible, imaging studies should be obtained from outside facilities from time-points after the patient's last course of lymphoma therapy prior to protocol enrollment.
d. If clinically indicated
e. Lumbar punctures will also need to be repeated after treatment if clinically indicated
f. Patients who are known to be positive for any of these tests do not need to be retested; may be performed within 3 months of chemotherapy start date
g. CT scan for lymphoma and CLL patients at the indicated time-points including within 14 days of chemo start (before treatment start)

h. PET for all patients at baseline except for CLL patients (With 14 days of start of chemo). at indicated time-points including within 14 days of chemo start (before treatment start) for all lymphoma patients. No PET scans should be done for CLL patients unless CLL is transformed to DLBCL. **As soon as CR is established, PET scans should cease.**

i. **All patients need a pre-treatment baseline bone marrow biopsy (G-banding cytogenetics included).** 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.

j. RCR blood collection will only continue after the 1 year time-point if a previous RCR test has been positive, see section **3.9** for details

k. See section **3.7.2** for details on labs during inpatient

l. Only CLL

m. Baseline Research Blood (See section **3.7.1**)

n. Inpatient Research Blood. One SST tube of research blood is to be collected on the morning of cell infusion. One SST tube of research blood is to be drawn on the first Sunday after CAR T-cell infusion. Research blood is also drawn on Mon-Wed-Fri up to Day 14 after CAR T-cell infusion while inpatient. (See section **3.7.2**)

o. Follow up Research Blood (See section **3.7.3**)

p. Valid for 6-month before apheresis collection. Does not have to be performed is cells were previously collected.

q. Assessment may be performed remotely per PI discretion (outside labs will be accepted and symptoms assessment may be performed via telephone)

r. +/- 15 minutes

s. This assessment may include a neurology consult if clinically indicated.

## 3.9 GENE-THERAPY-SPECIFIC FOLLOW-UP

### 3.9.1 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 another 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 SB. A complete blood count should be done at these physician visits for the first 5 years after infusion. After 5 years, patients who are still on this study will be transferred to the long-term gene therapy protocol NCI protocol 15-C-0141 to complete long-term gene-therapy follow-up.

### 3.9.2 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 detectable at levels <0.10% of PBMC 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.

### 3.9.3 Replication competent retrovirus (RCR) testing

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

- In case of detection of replication-competent retrovirus, the following actions will be taken:
    a. Immediately report the finding of RCR to the FDA, the NCI IRB, the NIH Institutional Biosafety Committee, and the Indiana University Vector Production Facility (where the vector was made).
    b. Repeat the S+L- and RCR 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 RCR PCR, perform standard HIV and HTLV-I 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 RCR PCR tests on the blood.

## 3.10 COST AND COMPENSATION

3.10.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center.

If some tests and procedures performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company.

Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.10.2 Compensation

Subjects will not receive compensation for participation in this study.

3.10.3 Reimbursement

This study offers subject reimbursement or payment for travel, lodging and/or meals while participating in the research. The amount, if any, is guided by NIH policies and guidelines.

On this study, the NCI will cover the cost for some of the expenses. Some of the costs may be paid directly by the NIH and some may be reimbursed to the subject. Someone will work with subjects to provide more information.

## 3.11 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

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

- If no CAR-T cells administered: visit should occur approximately 30 days after the last dose of study chemotherapy treatment.
- If CAR-T cells given: visit should occur approximately 60 days after the last dose of CAR-T-cells.

3.11.1 Criteria for removal from protocol therapy

Note that the treatment consists of a conditioning chemotherapy regimen followed by a T-cell infusion. Off-treatment criteria applies to eligibility for potential repeat treatments; and also, cancellations of treatment for toxicity arising any time before the cell infusion.

Patients will be taken off treatment for the following:

- Any DLT that makes patients 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.
- Participant requests to be withdrawn from active therapy
- Investigator discretion
- Positive pregnancy test

### 3.11.2 Off-study Criteria

Patients will be taken off study for the following (all patients coming off study must enroll on the long-term gene therapy follow-up protocol 15-c-0141):

- The patient completes the study upon reaching 5 years after CAR T-cell infusion.
- The patient completes a safety visit 30 day after last administration of study treatment (only for patients who have received chemotherapy but will not receive a cell infusion)
- The patient requests to be withdrawn from the study
- There is significant patient noncompliance
- Death
- Investigator discretion
- 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 (Except to treat CAR cell related toxicities) after CAR T-cell infusion at a dose higher than 5 mg/day of prednisone or equivalent dose of another corticosteroid.
- PI decision to end this study
- The patient no longer meets the eligibility criteria for the protocol after enrolling but before start of the chemotherapy conditioning regimen. In this case, treatment will be delayed until the patient meets eligibility criteria, or if the patient will never likely meet eligibility criteria, the patient will be removed from the study. An exception to this is that a platelet count of 50,000 or more is considered adequate to start chemotherapy. This platelet level is slightly lower than that required for initial protocol enrollment.

## 4 CONCOMITANT MEDICATIONS/MEASURES

All routine and appropriate supportive care (including blood products) will be provided during this study, as clinically indicated, and in accordance with the standard of care practices. Clinical judgment should be utilized in the treatment of any adverse event experienced by the patient.

### 4.1 ANTIBIOTIC PROPHYLAXIS

- Patients with a CD4 T-cell count less than 200 will be maintained on pneumocystis prophylaxis with atovaquone or inhaled pentamidine. Patients with a CD4 T-cell count less than 200 will also be maintained on acyclovir or valacyclovir.

  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 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 clear evidence of sepsis.

### 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 >10,000/mm3. All blood products with the exception of the CAR-transduced T cells will be irradiated.

### 4.3 ANTI-EMETICS

Anti-emetics will follow current literature such as ASCO and/or NCCN (except that corticosteroids will be avoided).

### 4.4 GRANULOCYTE COLONY-STIMULATING FACTOR

A CBC will be obtained 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. Filgrastim will be discontinued as soon as the absolute neutrophil count recovers to 2000/microliter.

### 4.5 AVOIDANCE OF CORTICOSTEROIDS

Patients should not take corticosteroids including prednisone, dexamethasone or any other corticosteroid at a dose equivalent to 5 mg/day or more of prednisone for any purpose without approval of the Principal Investigator.

### 4.6 EXCLUDED MEDICATIONS

Anticoagulation (e.g. warfarin) or anti-platelet therapy (e.g. aspirin > 325 mg/day or clopidogrel)

### 4.7 TOXICITY MANAGEMENT GUIDELINES

See **Appendix C**. .

### 4.8 MANAGEMENT OF PATIENTS WITH BURKITT AND OTHER HIGH-GRADE LYMPHOMAS

Patients with Burkitt lymphoma and other high-grade lymphomas are at increased risk for tumor lysis syndrome. The following guidelines will apply for all patients with Burkitt or other high-grade lymphomas:

- All patients with high-grade lymphomas will have the following laboratories checked every 6 hours for the first 72 hours or longer if evidence of tumor lysis syndrome is present after receiving CAR T cells: sodium, potassium, magnesium, phosphorus, creatinine, BUN, uric acid, calcium. Electrolyte abnormalities will be promptly treated as indicated.
- All patients with high-grade lymphomas will have a normal saline IV infusion maintained at a minimum of 100 mL/hr from the time of CAR T cell infusion until at least 24 hours after CAR T-cell infusion. If significant tumor lysis syndrome is observed, the rate should be increased as needed up to 250 cc/hour or other rates as appropriate.
- All patients with high-grade lymphoma will start allopurinol 300 mg every 12 hours on the day before protocol conditioning chemotherapy starts
- Patients with an LDH ≥2X the upper limit of normal will receive rasburicase 0.2 mg/kg on the day of start of conditioning chemotherapy
- Patients with a uric acid of 8 mg/dL at any time after the start of protocol treatment (including first day of chemotherapy) will receive rasburicase 0.2 mg/kg

## 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

**Note regarding research Biospecimen collection:** If any of the below time points fall on a federal holiday then, we may collect research blood on the next business day.

| Test/assay | Volume (approx.) | Type of Tube | Collection Point (+/- 48 hrs) | Location of Specimen Processing/ Storage |
|---|---|---|---|---|
| Immunological and RCR Assays | Blood | CPT tube | Within the 3 days prior to the start of | Surgery Branch Cell Production Cell |

| Test/assay | Volume (approx.) | Type of Tube | Collection Point (+/- 48 hrs) | Location of Specimen Processing/ Storage |
|---|---|---|---|---|
| | 96 mL | (12 tubes x 8 mL each) | conditioning chemotherapy<br>(can be collected on different days within the 3-day range) | Production Facility SB-CPF<br>Bldg. 10 3W-3808. Phone: 240-858-3755 |
| Immunological Assays (Serum cytokine levels) | Blood<br>16 mL | SST tubes<br>(2 tubes x 8 mL each) | Within 3 days prior to the start of the chemotherapy | Figg Lab |
| Apheresis cells to prepare the CAR T cells | Apheresis cells | NA | Prior to the start of conditioning chemotherapy | DTM CCE |
| Immunological Assays | Apheresis cells left over after CCE has used all necessary cells. | Cell culture bag or tube. | Prior to the start of conditioning chemotherapy | Cell Production Facility SB-CPF<br>Bldg. 10 3W-3808. Phone: 240-858-3755 |
| Sample of CAR T cells from Culture | CAR T cells | NA | End of CAR T cells culture | Cell Production Facility SB-CPF<br>Bldg. 10 3W-3888. Phone: 240-858-3755 |
| Immunological Assays (Serum cytokine levels) | Blood<br>8 mL | SST tube<br>(1 tube x 8 mL) | Prior to CAR T cell infusion on Day 0 | Figg Lab |
| Immunological Assays | Blood<br>48 mL | CPT tubes<br>(6 tubes x 8 mL each) | Every Monday, Wednesday, and Friday during hospitalization, starting on the first Monday, Wednesday, or Friday after the | Surgery Branch Cell Production Facility SB-CPF (CPT tubes only)<br>Bldg. 10 3W-3808. Phone: 240-858-3755 |

| Test/assay | Volume (approx.) | Type of Tube | Collection Point (+/- 48 hrs) | Location of Specimen Processing/ Storage |
|---|---|---|---|---|
| Immunological Assays (Serum cytokine levels) | Blood 8 mL | SST tube (1 tube x 8 mL) | CAR T-cell infusion and lasting up until 14 days after infusion of Hu1928-Hu20BB CAR-transduced T cells | Figg Lab (SST tubes only) |
| Immunological Assays (Serum cytokine levels) | Blood 8 mL | SST tube (1 tube x 8 mL) | On the first Sunday after CAR T-cell infusion (If the Monday is a holiday, Sunday research blood will not be drawn). | Figg Lab (tube may be stored refrigerated on the nursing unit and processed first thing Monday morning at the latest) |
| Immunological Assays (Used for RCR on 3MO, 6MO, 12MO) | Blood 48 mL | CPT tubes (6 tubes x 8 mL each) | Outpatient follow-up clinic visits 2 weeks, 1, 2, 3, 6, 9 and 12 months after CAR T-cell infusion | Surgery Branch Cell Production Facility (CPT tubes only) Bldg. 10 3W-3808. Phone: 240-858-3755 |
| Immunological Assays (Serum cytokine levels) | Blood 8 mL | SST tube (1 tube x 8 mL) | | Figg Lab (SST tubes only) |
| Immunological Assays | Bone marrow aspirate 2-2.5 mL | Heparinized syringe | Outpatient follow-up clinic visits (2 weeks, 2 and 6 months after CAR T-cell infusion) | Surgery Branch Cell Production Facility Bldg. 10 3W-3808. Phone: 240-858-3755 |

5.1.1 Immunological Testing

- T-cell assays: Direct immunological monitoring will consist of quantifying CD3+ T cells that express the CAR by quantitative PCR, and/or by flow cytometry. These assays will be performed to measure the persistence and estimate the proliferation of the infused CAR+ T cells. A quantitative PCR assay or a flow cytometry assay will be used to

quantitate CAR+ T cells at all post-infusion time-points up to at least 2 months after infusion, and CAR+ T cell analysis will continue until the CAR+ T cell level drops to undetectable levels unless a stable low level of CAR+ T cells is present at more than 3 years after infusion. The absolute number of CAR+ PBMC will be estimated by multiplying the percentage of CAR+ PBMC by the absolute number of lymphocytes plus monoctyes per microliter of blood. Ex vivo immunological assays might be used to measure the antigen-specific functional activity of the CAR+ T cells and will consist of assays such as enzyme-linked immunosorbent assays (ELISAs), intracellular cytokine staining, and anti-CD107a degranulation assays. Immunological assays will be standardized by the inclusion of pre-infusion recipient PBMC and in some cases an aliquot of the engineered T cells cryopreserved at the time of infusion.

- Serum cytokine levels will also be measured by enzyme-linked immunosorbent assays (ELISAs) or similar assays.
- Gene expression studies will be performed on patient lymphoma cells and on the infusion CAR T cells of each patient. Methods used will be either Nanostring and/or RNAseq (RNA sequencing).
- Patients' blood samples will be obtained 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. Monitoring for RCR will be discontinued if all patient samples have been negative for RCR at the 12 month time-point during this trial, efforts will be made to assay a biopsy sample for RCL. If any post-treatment samples are positive, further analysis of the RCL and more extensive patient follow-up will be undertaken, in consultation with the FDA. RCL PCR assays are performed by the National Gene Vector Laboratory at Indiana University The results of these tests are maintained by the National Gene Vector Laboratory at Indiana University and by the Surgery Branch research team.
- Due to nature of these studies, it is expected that expansion of specific T-cell clones will be observed as T-cell proliferate in response to the targeted antigen. Therefore, care will be taken to track T-cell persistence, but presence of an oligoclonal T cell population does not indicate an insertional mutagenesis event. If any patient shows an increasing population of CAR gene transduced T cells at month 6 or later (by FACS staining or qPCR), the previously archived samples will be subjected to techniques that would allow the identification of predominant clonal populations of transduced cells. Such techniques may include T cell cloning or LAM-PCR. If a predominant or monoclonal T cell clone derived from CAR gene transduced cells is identified during the follow-up, the integration site and sequence will be identified and subsequently analyzed against human genome database to determine whether the sequences are associated with any known human cancers. If a predominant integration site is observed, the T cell cloning, or LAM-PCR test will be used at an interval of no more than three months after the first observation to see if the clone persists or is transient. In all instances where monoclonality is persistent and particularly in instances where there is expansion of the clone, regardless of whether or not the sequence is known to be associated with a known

human cancer, the subject should be monitored closely for signs of malignancy, so that treatment, if available, may be initiated early.

5.1.2 Additional biopsies and additional blood draws

Patients might be asked to undergo biopsies or additional blood draws as as clinically indicated. 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. Remainder material from such 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. Standard techniques will be used for biopsies which may include CT and/or ultrasound guided biopsy. These biopsies will only be performed if minimal morbidity is expected based on the procedure performed and the granulocyte and platelet count. Biopsy tissue will be processed in the NIH Department of Pathology. Studies will be performed to evaluate the antigen expression by the tumor and to evaluate the presence of transduced cells.

5.1.3 Future studies

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

5.1.4 Samples Sent to Figg Lab

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

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

## 5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

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

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

#### 5.2.1.1 BPC Contact Information

Please e-mail 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.

#### 5.2.1.2 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 Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

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

#### 5.2.1.3 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.

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

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

### 5.2.3 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. The PI will report any loss or unanticipated destruction of samples per Section **7.2.1**.

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.

Freezer problems, lost samples, or other problems associated with samples that meet expedited reporting requirements (see Section **7.2.1**) will be reported.

5.2.4 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.

5.2.4.1 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.

# 6 DATA COLLECTION AND EVALUATION

## 6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system, C3D and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All ongoing AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

- If no CAR-T cells administered: Document AEs from the first day of study chemotherapy through 30 days after last dose of study chemotherapy or until off study, whichever comes first. Beyond 30 days after the last dose of chemotherapy, only adverse events which are serious and related to the study intervention need to be recorded.
- If CAR-T cells given: Document AEs from the first infusion of CAR-T-cells through 60 days after the subject receives the last administration of CAR-T-cells. Beyond 60 days after the last CAR-T cell infusion, only adverse events which are serious and related to the study intervention need to be recorded.

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

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

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

All patients receiving a second treatment will be identified as patients receiving repeat treatments in any reports of results of this trial.

Information on all concomitant medications, administered blood products, as well as interventions occurring during the study must be recorded on the patient's eCRF.

**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 2 adverse events that will be recorded:
  a. Unexpected events that are possibly, probably, or definitely related to the research.
  b. Expected events that are possibly, probably or definitely related to the study interventions will be recorded only for the first year after the infusion.
  c. All Infections proven by culture, PCR, antigen detection or other laboratory methods will be recorded for the first year after infusion regardless of attribution.
- All grade 3, 4, and 5 adverse events will be recorded regardless of attribution.
- All serious events regardless of attribution

## 6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

**What data will be shared?**

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

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

_x__ Coded, linked data in BTRIS (automatic for activities in the Clinical Center)

_x__ 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:

_x__ An NIH-funded or approved public repository. Insert name or names: ClinicalTrials.gov.

_x__ BTRIS (automatic for activities in the Clinical Center)

_x__ Approved outside collaborators under appropriate individual agreements.

_x__ Publication and/or public presentations.

**When will the data be shared**

_x__ Before publication.

_x__ At the time of publication or shortly thereafter.

## 6.3 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.

## 6.4 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 178 and Recommendations for Initial Evaluation, Staging, and Response Assessment of Hodgkin and Non-Hodgkin Lymphoma: The Lugano Classification Journal of Clinical Oncology, 2014179)

6.4.1 Response criteria:

6.4.1.1 Complete Remission (CR):

- CR requires all of the following: Complete disappearance of all detectable clinical evidence of disease and disease-related symptoms if present before therapy. Regardless of FDG-avidity, if all extranodal masses and lymph nodes are less than or equal to 1.5 cm or less in longest diameter, the patient is considered to be in CR.
    - 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.
    - Variably FDG-avid lymphomas/FDG avidity unknown: in patients without a pretreatment PET scan, or if a pretreatment PET scan was negative, all lymph nodes and nodal masses must have regressed to normal size (≤ 1.5 cm in greatest diameter if > 1.5 cm before therapy). Previously biopsy-proven involved nodes that were 1.1 to 1.5 cm in their long axis and more than 1 cm in their short axis before treatment must have decreased to ≤ 1.0 cm in their short axis after treatment.
    - 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.
    - 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.

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

- ≥ 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.
- No increase in size of nodes, liver or spleen and no new sites of disease.
- If multiple splenic and hepatic nodules are present, they must regress by ≥ 50% in the SPD. There must be a ≥ 50% decrease in the greatest transverse diameter for single nodules.
- 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.
- 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.

6.4.1.3 Progressive Disease (PD):

- Defined by at least one of the following:
  - ≥ 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.
  - Appearance of a new lesion greater than 1.5 cm in any axis even if other lesions are decreasing in size
  - Greater than or equal to a 50% increase in size of splenic or hepatic nodules
  - At least a 50% increase in the longest diameter of any single previously identified node more than 1 cm in its short axis.
  - 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)

6.4.1.4 Stable Disease (SD):

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD. PET should be positive in typically FDG-avid lymphomas.

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

6.4.2 Response criteria for CLL:

(Hallek et al. International workshop on chronic lymphocytic leukemia (iwCLL) guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Blood 2018 )[180]

6.4.2.1 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 Hu1928-Hu20BB CAR-transduced T cell infusion:

- No lymph nodes ≥ 1.5 cm (physical exam, relevant CT scans)
- No hepatomegaly or splenomegaly (physical exam, relevant CT scans)
- Absence of constitutional symptoms
- Normal CBC as exhibited by platelets >100,000/ul and hemoglobin > 11.0 g/dl (untransfused), and neutrophils ≥ 1500/ul,
- Blood B lymphocyte count<4,000/ul
- Bone marrow aspirate and biopsy should be performed only after requirements #1-#5 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) fits CR criteria. If lymphoid nodules are detected on marrow examination, IHC or flow cytometry should be performed to determine whether these are composed of CLL cells versus T cells or other B cell types. If the nodules are not composed of CLL cells, a CR can be documented. If the marrow is hypocellular, a repeat determination should be performed in one month.

- Patients who fulfill all of the criteria for CR except for having bone marrow lymphoid nodules will be considered to be in a PR

6.4.2.2 Partial Remission:

The designation of partial response requires at least two of the following: a ≥ 50% decrease in peripheral B lymphocyte count from pre-treatment value, or a ≥ 50% reduction in lymphadenopathy, or a ≥ 50% reduction in splenomegaly/hepatomegaly for a period of at least 8 weeks based on physical exam and relevant CT scans. If the patient had only 1 of the abnormal parameters above prior to starting on study, then improvement of that parameter meets criteria for PR. 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:

- Platelets > 100,000/ul or 50% improvement from pre-treatment value
- Hemoglobin > 11.0 g/dl (untransfused) or 50% improvement from pre-treatment value

6.4.2.3 Progressive Disease:

The designation of progressive disease is characterized by any one of the following:

- A ≥ 50% increase in the greatest diameter of any lymph node that was enlarged pre-treatment.
- The appearance of new palpable lymph nodes
- A ≥ 50% increase in the absolute number of circulating B lymphocytes (value must exceed 5,000/ul)
- ≥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.
- Transformation to a more aggressive histology
- Patients not fulfilling the above criteria for progressive disease but demonstrating a decrease in hemoglobin value of > 2 gm/dl from baseline or a decrease of > 50% in platelet or granulocyte count will not be considered as evidence of progressive disease, because these changes may occur as both a consequence of many therapies or of underlying CLL/SLL; in such cases, a repeat bone marrow biopsy is recommended.

6.4.2.4 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.

## 6.5 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 5.0 can be downloaded from the CTEP web site

(http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). Cytokine release syndrome will be graded based on the Lee 2019 ASTCT system of reporting CRS (**Appendix F**)181 (A)

# 7 NIH Reporting Requirements / DATA 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 REPORTING

### 7.2.1 Expedited Reporting

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

### 7.2.2 NIH Intramural 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, deaths not reported to the OHSRP that occur within 30 days after receiving a research intervention regardless of attribution 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 Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

## 7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

### 7.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of CAR-T cells 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 CAR-T cells, 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.

### 7.4.3 Clinical Trial Information

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

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

### 7.4.4 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

The clinical research team will meet approximately weekly when patients are being actively treated on the trial to discuss each patient.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate 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.

### 7.5.1 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 NIH Intramural IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC 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.

Special addition to safety monitoring plan as requested by NIH IRBO: There will be a pause to enrollment only for Cohort 2 (Hodgkin Lymphoma) after 3 patients have been treated on this cohort. At this time, a report will be sent to the Safety Monitoring Committee to allow an assessment of the safety of this cohort before more patients are enrolled on Cohort 2. The SMC report will be forwarded to the IRB for the IRB to determine if enrollment can continue on this cohort.

# 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 8.1.3)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing

condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.

- A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient convenience) is not considered a serious adverse event.
- Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.

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

8.1.3 Life-threatening

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

8.1.4 Severity

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

8.1.5 Relationship to Study Product

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

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

## 8.2 ASSESSMENT OF SAFETY EVENTS

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

SAEs will be:

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

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

## 8.3 REPORTING OF SERIOUS ADVERSE EVENTS

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

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

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=157942842

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

## 8.4 REPORTING PREGNANCY

### 8.4.1 Maternal exposure

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

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

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

### 8.4.2 Paternal exposure

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

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

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

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

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

## 9 CLINICAL MONITORING

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

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

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

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

## 10 STATISTICAL CONSIDERATIONS

### 10.1 STATISTICAL HYPOTHESIS

#### 10.1.1 Primary objectives

The primary endpoint of this trial is to determine the safety and feasibility of administering CAR-expressing T cells to patients with relapsed or persistent B-cell malignancies.

#### 10.1.2 Secondary objectives

Secondary objectives of this trial are to measure persistence and peak blood levels of CAR-expressing T cells, to assess anti-malignancy activity of the CAR T cells, and to assess the relationship between expression of CD19 and CD20 and response.

### 10.2 SAMPLE SIZE DETERMINATION

Patients will be enrolled on 2 separate cohorts: **Cohort 1**: Patients who have B-cell malignancies including lymphoma and CLL and **Cohort 2** Patients who have Hodgkin lymphoma. 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 one cohort or the other due to the different

characteristics of B-cell malignancies compared with Hodgkin lymphoma. DLTs occurring on one cohort's dose escalation will not affect dose escalation of the other cohort. Maximum tolerated, and maximum administered doses will be determined independently for each cohort. Both cohorts can undergo repeat treatments only after a maximum tolerated or maximum administered dose is determined for the cohort in question.

For each cohort, a dose escalation scheme with up to 4 doses and up to 6 patients per dose level will be carried out (maximum 24 patients) In addition, up to 10 additional patients can be treated at the MTD/maximum administered dose for each cohort to establish additional safety and toxicity data at that level. Thus, with two cohorts, up to 2 x (24+10) =68 patients may be enrolled onto the trial. In addition, we will allow 20 additional patients to allow the trial to be completed as planned if some patients who enroll are not treated due to reasons such as rapid clinical deterioration requiring cancellation of protocol participation.

The degree of persistence of Hu1928-Hu20BB CAR-transduced T cells will be evaluated by a quantitative measure (flow cytometry and/or quantitative PCR) in all patients. Anti-malignancy effects will be measured by clinical response and categorized according to Section **6.4**. 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.

## 10.3 Populations For Analyses

All treated patients receiving at least one dose of CAR T cells will be included in the safety and efficacy analysis dataset.

## 10.4 Statistical Analyses

### 10.4.1 General Approach

This is a standard 3+3 dose escalation study. Toxicity data will be obtained and used to determine the MTD of the treatment.

### 10.4.2 Analysis of the Primary Endpoints

The toxicity obtained on each patient will be determined and used to report the number of patients at each dose level who experience a DLT, following standard phase I procedures.

### 10.4.3 Analysis of the Secondary Endpoint(s)

Secondary objectives of this trial are to measure persistence and peak blood levels of CAR-expressing T cells, to assess anti-malignancy activity of the CAR T cells, and to assess the relationship between expression of CD19 and CD20 and response. The persistence and peak blood levels of CAR-expressing T cells will be reported using descriptive statistics, at each dose level, as well as overall. The anti-malignancy activity of the CAR T cells will be assessed by response criteria as separately defined for each disease treated, with 95% confidence intervals provided about the rates of response. Comparison of peak blood CAR T-cell levels in patients obtaining an anti-malignancy response versus patients not obtaining an anti-malignancy response will be done by testing the peak levels between responders and non-responders using an exact Wilcoxon rank sum test, both at all dose levels individually and overall.

10.4.4 Safety Analyses

The study will determine the toxicity experienced on each patient enrolled on the trial and report this by dose level.

10.4.5 Baseline Descriptive Statistics

None will be provided in a formal manner; brief descriptions may be incorporated in a final report.

10.4.6 Planned Interim Analyses

Toxicity will be evaluated at each dose level as the patients accrue to the trial. Stopping criteria are described in section 3.6.

10.4.7 Sub-Group Analyses

None

10.4.8 Tabulation of individual Participant Data

None

10.4.9 Exploratory Analyses

The following evaluations will be performed using exploratory techniques. Assessing for associations between blood cytokine levels and response and toxicity will be done by comparing cytokine levels between responders and non-responders, as well as between those with a DLT or not, both at all dose levels individually and overall, using exact Wilcoxon rank sum tests.

A variety of clinical predictors of anti-lymphoma responses and toxicity may be evaluated, using non-parametric tests.

The phenotypes of infused CAR T cells and CAR T cells from the blood of patients will be evaluated and will be reported descriptively.

When any statistical tests are performed, no formal adjustment for multiple comparisons will be done since the evaluations are being done to generate hypotheses, but the results will be reported in the context of the number of tests performed. Since the evaluations are being done to generate hypotheses, and the results are expected to be based on very limited subjects, the results will be interpreted cautiously.

# 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

- 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 targeting CD20 in addition to CD19

### 12.3 PARTICIPATION OF CHILDREN

Children (younger than 18 years) 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 12.5 ), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

### 12.5 RISK/BENEFIT ASSESSMENT

12.5.1 Known Potential Benefits

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.

12.5.2 Known Potential Risks

The primary risk to patients participating in this research study is from the toxicity of chemotherapy and CAR-T cells.

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.[70,141] 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 Hu1928-Hu20BB CAR with proteins other than CD19 or CD20 in vivo. A 5$^{th}$ possible toxicity is hypogammaglobulinemia. Hypogammaglobulinemia has been a complication of many patients on clinical trials of anti-CD19 CAR-expressing T cells.[70,71] Hypogammaglobulinemia in these patients was routinely treated with infusions of intravenous immunoglobulins.[70]

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.[73-75]

12.5.2.1 Risk of Biopsy

All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent.

12.5.2.2 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, two (2) CT-Guided biopsies and seven (7)18FDG-PET/CT in the first year of the study.

The total additional radiation dose for research purposes will be approximately 19.1 rem in the first year of the study. Being exposed to too much radiation can cause harmful side effects such as an increase in the risk of cancer.

Most patients will obtain much less than the maximum levels of radiation exposure since PET scans will immediately cease as soon as CR is documented, and patients not obtaining CRs almost always go off-study in less than 6 months due to progressive lymphoma. In addition, CT imaging will be modified based on lymphoma locations. For example, many patients will not receive any

post-CAR T-cell infusion neck CT on the study since many patients do not have baseline neck involvement with lymphoma.

12.5.2.3 Research Blood Collection Risks

Risks of blood draws include pain and bruising in the area where the needle is placed, lightheadedness, and rarely, fainting. When large amounts of blood are collected, low red blood cell count (anemia) can develop.

12.5.2.4 Other Risks

Risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document or this protocol document. Frequent monitoring for adverse effects will help to minimize the risks associated with administration of the study agents.

12.5.2.5 Non-Physical Risks of Genetic Research

**Risk of receiving unwanted information**

Anxiety and stress may arise as a result of the anticipation that unwanted information regarding disease related RNA sequencing or disease tendencies, or misattributed paternity. Patients will be clearly informed that the data related to RNA sequencing and genetic analysis is coded, investigational and will not be shared with patients, family members or health care providers.

**Risk related to possibility that information may be released**

This includes the risk that data related to genotype, RNA sequencing or risk for disease tendency or trait can be released to members of the public, insurers, employers, or law enforcement agencies. Although there are no plans to release results to the patients, family members or health care providers, this risk will be included in the informed consent document

12.5.3 Assessment of Potential Risks and Benefits for Recipients

The experimental treatment has a chance to provide clinical benefit although it is quite possible that patients will obtain no clinical benefit. A goal of this study is to improve upon the number of patients who may benefit from adoptive cell therapy by using genetically modified T-cells, specifically CAR T cells. The risks of the study fall into 5 general categories. First, chemotherapy that could cause cytopenias is part of the protocol. As with any chemotherapy that causes neutropenia and thrombocytopenia, this chemotherapy could cause toxicities such as infections and bleeding. The second category of toxicity is cytokine-release type toxicities such as high fevers, hypotension, and fever. A third area of toxicity is neurological toxicity such as delirium, obtundation, myoclonus, seizures, headache, and transient focal neurological toxicities including aphasia and focal paresis. Cytokine-release-type toxicities and neurological toxicities have appeared in other clinical trials of CAR T cells during the first 2 weeks after CAR T cells were infused.[70,141] 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 Hu1928-Hu20BB CAR with proteins other than CD19 or CD20 in vivo. A 5$^{th}$ possible toxicity is hypogammaglobulinemia. Hypogammaglobulinemia has been a complication of many patients on clinical trials of anti-CD19 CAR-expressing T cells.[70,71] Hypogammaglobulinemia in these patients was routinely treated with infusions of intravenous immunoglobulins.[70]

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.[73-75]

The success of this clinical trial cannot be predicted at this time. A number of clinically appropriate strategies to minimize risks to patients have been built into the protocol through the means of inclusion/exclusion criteria, monitoring strategies, and management guidelines. 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.

Potential adverse reactions attributable to the administration of the study drugs utilized in this trial are discussed in the FDA packet insert. All care will be taken to minimize side effects, but they can be unpredictable in nature and severity.

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

## 13 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. REGULATORY AND OPERATIONAL CONSIDERATIONS

### 13.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, associate investigators, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

### 13.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

### 13.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NCI has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

## 14 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.

## 14.1 GAMMA-RETROVIRAL VECTOR CONTAINING THE ANTI-CD19/ANTI-CD20 CAR GENE

### 14.1.1 Source/Acquisition and Accountability

After cells are obtained by apheresis, further cell processing to generate CAR-expressing T cells will occur in the Department of Transfusion Medicine according to standard operating procedures (SOPs). Individual single IV bags accordingly dose requirements of the dose-escalation plan described in Section **3.1.4** will be prepared for each study participant by DTM personnel. Each single IV bags will be delivered from the Department of Transfusion Medicine to patient care unit where drug will be infused to the patient.

### 14.1.2 Cells manufacturing

The retroviral vector supernatant (MSGV1-Hu1928-Hu20BB) encoding 2 CARs directed against CD19 and CD20 was prepared and preserved following cGMP conditions in the University of Cincinnati Medical Center Vector Production Facility. The retroviral vector utilizes the MSGV1 retroviral vector backbone and consists of 7007 bps including the 5' LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor sites, the Hu1928-Hu20BB CAR construct encodes 2 separate proteins: (1.) The Hu19-CD828Z CAR containing a signal peptide from human CD8-alpha signal sequence, the human Hu19 scFv, CD8-alpha hinge and transmembrane domains, CD28 (cytoplasmic region), and TCR-zeta (cytoplasmic region), followed by an F2A ribosomal skip domain and (2.) A second CAR called Hu20-CD8BBZ that includes a signal peptide from human CD8-alpha signal sequence, the human Hu20 scFv, CD8-alpha hinge and transmembrane domains, CD28 (cytoplasmic region), and TCR-zeta (cytoplasmic region) after this second CAR comes the murine stem cell virus 3'LTR.

The supernatant will be stored at –80$^{o}$C or shipped on dry ice and stored in the Dept. of Transfusion Medicine, NIH or at NCI Frederick Biorepository, Frederick, MD. Both storage facilities are equipped with around-the-clock temperature monitoring. Upon request, supernatant will be delivered on dry ice to be used in *in vitro* transductions of T cells. There will be no re-use of the same unit of supernatant for different patients. Retroviral titer has been shown to be stable after immediate thawing and immediate use. Handling of the vector should follow the guidelines of Biosafety Level-2 (BSL-2). The specific guidelines for Biosafety Level-2 (BSL-2) can be viewed at http://bmbl.od.nih.gov/sect3bsl2.htm

### 14.1.3 Toxicities

Please refer to section **1.2.15**

### 14.1.4 Administration procedures

Please see section **3.3**.

## 14.2 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

### 14.2.1 Cyclophosphamide

#### 14.2.1.1 Source/Acquisition and Accountability

Cyclophosphamide will be provided by the NIH Clinical Center Pharmacy according to standard pharmacy procedures. Individual IV bags will be prepared for each study participant according to assigned dose by NIH Pharmacy personnel. IV bags will be delivered from NIH Pharmacy to patient unit where drug will be infused to the patient.

14.2.1.2 Administration procedures

The cyclophosphamide used in this regimen will be given as Intravenous infusion over 30 minutes.

14.2.2 FLUDARABINE

14.2.2.1 Acquisition and Accountability

Fludarabine monophosphate will be provided by the NIH Clinical Center Pharmacy according to standard pharmacy procedures. Individual IV bags will be prepared for each study participant according to assigned dose by NIH Pharmacy personnel. IV bags will be delivered from NIH Pharmacy to patient unit where drug will be infused to the patient.

14.2.2.2 Administration procedures

Fludarabine is administered as an IV infusion in 100 mL 0.9% sodium chloride, USP over 30 minutes.

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# 16 APPENDICES

## 16.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

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

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## 16.2 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
- Tumor Histology and date of confirmation
- CD19/CD20 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

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

- Dates anti-CD19 and anti CD20 CAR-transduced T cells given
- Dose level, actual dose, schedule and route given
- Height, weight, and body surface area at start of each course (a course is defined as chemotherapy followed by a CAR T-cell infusion)
- Response assessment for each restaging performed
- Concomitant medications will not be collected in C3D

### C. Laboratory and Diagnostic Test Data

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

### D. Adverse Events

Please see section **6.1.1** Adverse Event Reporting

### E. Tumor response and measurements

- Restaging studies performed at protocol specified time points and as clinically indicated.

- Any physical exam findings, will be collected as Adverse Events and labs results.

**F. Off study**

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

## 16.3 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.

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 possible to avoid killing or impairing the function of the CAR T cells.

**General supportive care guidelines**

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

- Any history of syncope or near-syncope within 1 month before CAR T-cell infusion or any time after CAR T-cell infusion.
- Any blood pressure reading of less than 100 mm Hg systolic blood pressure after anti-CD19 CAR T-cell infusion if 100 mm Hg is lower than the patients baseline systolic blood pressure.
- Heart rate greater than 100 beats per minute.

13. Any patient with syncope, near-syncope, or light-headedness will have orthostatic blood pressure and heart rate checked and receive intravenous fluids as appropriate. These patients will also receive an ECG.

14. A CBC will be obtained twice daily while the patient is inpatient. If the absolute neutrophil count becomes less than 500/microliter, Filgrastim will be initiated at a dose of 300 micrograms daily for patients under 70 kg in weight and a dose of 480 micrograms daily for patients over 70 kg in weight only in patients with absolute neutrophil counts less than 500/microliter. Filgrastim will be discontinued as soon as the absolute neutrophil count recovers to 2000/microliter.

15. Hypotension is a common toxicity requiring intensive care unit (ICU) admission. In general patients should be kept well-hydrated. Maintenance I.V. fluids (normal saline (NS) should be started on most patients with high fevers especially if oral intake is poor or the patient has tachycardia. I.V. fluids are not necessary for patients with good oral intake and mild fevers. For patients who are not having hypotension or tumor lysis syndrome, a generally even fluid balance should be strived for after allowing for insensible fluid losses in patients with high fevers. The baseline systolic blood pressure is defined for this protocol as the average of all systolic blood pressure readings obtained during the 24 hours prior to the CAR T-cell infusion. The first treatment for hypotension is administration of IV NS boluses.

    - **Patients with a systolic blood pressure that is less than 80% of their baseline blood pressure and less than 100 mm Hg should receive a 1 L NS bolus.**
    - **Patients with a systolic blood pressure less than 90 mm Hg should receive a 1 L NS bolus regardless of baseline blood pressure.**

    These I.V. fluid management suggestions may need to be modified based on the clinical characteristics of individual patients such as pulmonary status, cardiac function, edema and other factors.

16. Patients receiving more than 1 fluid bolus for hypotension should have a stat EKG and troponin, and a cardiac echocardiogram as soon as possible.

**ICU transfer**

Patients should be transferred to the ICU after consultation with the ICU physicians 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 70% the patient's baseline blood pressure and less than 100 mm Hg after administration of a 1L NS bolus.
- Anytime the systolic blood pressure is less than 90 mm Hg after a 1L NS bolus.

- Continuous tachycardia with a heart rate higher than 125 beats per minute on at least 2 occasions separated by 2 hours.
- Oxygen requirement of more than a 4L standard nasal cannula

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

**Immunosuppressive drug administration**

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

**Tocilizumab administration**

Tocilizumab should be administered under the following circumstances if the listed disorders are thought to be due to cytokine release from CAR T cells. Tocilizumab is administered at a dose of **8 mg/kg** infused IV over 1 hour (dose should not exceed 800 mg). If clinical improvement is not observed after the first dose, up to three additional doses of tocilizumab may be administered until clinical improvement is seen.

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

- Creatine kinase greater than 3x upper limit of normal
- AST or ALT of 400 U/L or higher
- Serum sodium of 127 mmol/L or lower

**Intermediate-dose methylprednisolone for toxicities not responsive to tocilizumab**

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

**High-dose methylprednisolone should be given <u>immediately</u> under these circumstances:**

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

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

**Neurological toxicity**

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

neurological toxicity, so it should not be given when patients have isolated neurological toxicity.

1. Inability of patient to follow simple commands such as “squeeze my fingers”.
2. Any generalized seizure
    1. Somnolence that is different than normal sleep such as constant sleep or difficult to arouse or any difficulty with airway protection or Grade 3 somnolence (stupor).
    2. Ataxia severe enough to preclude ambulation
    3. Disorientation to person or place that persists longer than 48 hours
    4. Neurologic toxicity lasting more than 2 hours that is severe enough to interfere with self-care activities of daily living (ADLs)
    5. Cerebral edema

## 16.4 APPENDIX D: 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 by cell processing. | |
| 2. Verify the physician orders:<br>- to administer the cells<br>- for the date of administration<br>- for premedication orders<br>- protocol number | a. Premeds are acetaminophen 650 mg PO and diphenhydramine 12.5 mg IV. |
| 3. Verify that the protocol consent is signed | |
| 4. Ensure that emergency and monitoring equipment are available in the patient's room:<br>- oxygen<br>- suction<br>- vital sign monitor with pulse oximeter and thermometer | |
| 5. Provide patient education covering infusion procedure, potential complications and associated symptoms to report. | |
| 7. Measure and record baseline vital signs, respiratory and circulatory assessments. | |

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

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

## 16.5 APPENDIX E: CARTOX (ADAPTED FROM NEELAPU ET AL, 2018):

- One point is assigned for each task. Normal function defined by overall score of 10.
- TASKS:
  - Orientation to year, month, city, hospital and President of country (maximum 5 points)
  - Name three objects (ex: point to clock, pen, button) (maximum of 3 points)
  - Write a standard sentence (1 point)
  - Count backwards from 100 in tens (1 point)

## 16.6 APPENDIX F: CYTOKINE RELEASE SYNDROME GRADING ASSESSMENT (ADAPTED FROM LEE ET AL, 2019). ASTCT CRS CONSENSUS GRADING

Table 2 ASTCT CRS Consensus Grading

| CRS Parameter | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|---|---|---|---|---|
| Fever*** | Temperature ≥38°C | Temperature ≥38°C | Temperature ≥38°C | Temperature ≥38°C |
| With | | | | |
| Hypotension | None | Not requiring vasopressors | Requiring a vasopressor with or without vasopressin | Requiring multiple vasopressors (excluding vasopressin) |
| And/or†† | | | | |
| Hypoxia | None | Requiring low-flow nasal cannula‡‡ or blow-by | Requiring high-flow nasal cannula‡‡, facemask, nonrebreather mask, or Venturi mask | Requiring positive pressure (eg, CPAP, BiPAP, intubation and mechanical ventilation) |

Organ toxicities associated with CRS may be graded according to CTCAE v5.0 but they do not influence CRS grading.

*Fever is defined as temperature ≥38°C not attributable to any other cause. In patients who have CRS then receive antipyretic or anticytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia. Hypotension is defined as a systolic blood pressure <90 mm Hg.
†CRS grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a patient with temperature of 39.5°C, hypotension requiring 1 vasopressor, and hypoxia requiring low-flow nasal cannula is classified as grade 3 CRS.
‡Low-flow nasal cannula is defined as oxygen delivered at ≤6L/minute. Low flow also includes blow-by oxygen delivery, sometimes used in pediatrics. High-flow nasal cannula is defined as oxygen delivered at >6L/minute.