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TITLE: Pilot study of tisagenlecleucel, CD19-targeted chimeric antigen receptor (CAR) T cells, in patients with primary central nervous system lymphoma.

Coordinating Center:	**Massachusetts General Hospital (MGH)**
Principal Investigator (PI):	**Matthew J. Frigault, MD** **MGH Cancer Center**
Other Investigators:	**Marcela Maus, MD, PhD** **Jorg Dietrich, MD, PhD** **Yi-Bin Chen, MD** **MGH Cancer Center**
IND Sponsor:	**Matthew J Frigault, MD** **MGH Cancer Center**

IND #: 19195

Protocol Type / Version # / Version Date: Version 4/August 8, 2023

Name of Sponsor/Company:
DF/HCC Investigator Sponsored – Matthew Frigault, MD

Name of Investigational Product:
Tisagenlecleucel (Kymriah)

Name of Active Ingredient:
Tisagenlecleucel (Kymriah)

Title of Study (Protocol Title):
Pilot study of tisagenlecleucel, CD19-targeted chimeric antigen receptor (CAR) T cells, in patients with primary central nervous system lymphoma.

Protocol Number 19-319

Study center(s): MGH Cancer Center

Principal Investigator: Matthew J. Frigault, MD
Investigator: Yi-Bin Chen, MD

Studied period (years): 15	**Phase of development:** Pilot
Estimated date first patient enrolled: quarter III, 2019 Estimated date last patient completed: quarter III, 2020	

Objectives:

Primary:

- To assess the safety of tisagenlecleucel in patients with primary CNS diffuse large B-cell lymphoma (PCNSL).

Secondary:

- To assess the activity of tisagenlecleucel in patients with primary CNS diffuse large B-cell lymphoma (PCNSL).

Exploratory:

- To assess long-term efficacy of tisagenlecleucel in patients with PCNSL.
- Evaluate the expansion, persistence, and phenotype, of tisagenlecleucel T cells following infusion.
- Evaluate cytokine profiles in the peripheral blood and CSF of subjects after infusion of tisagenlecleucel.
- To assess impacts of tisagenlecleucel on quality of life and neurocognitive function post infusion.
- To assess for correlative markers of tumor response/progression.

Route and Regimen:

Tisagenlecleucel will be administered intravenously as a one-time rapid infusion following lymphodepleting chemotherapy.

Methodology/Study Design: This is a pilot study. A total of 6 subjects with PCNSL will be enrolled to this trial.

Bridging Therapy

At time of diagnosis/disease progression patients will be consented and screened for study eligibility. Bridging therapy will be allowed at the discretion of the study PI as is clinically indicated during CAR-T manufacturing.

Lymphodepletion

Subjects will receive 3 days of lymphodepleting chemotherapy starting Day -5, before the infusion of tisagenlecleucel on Day 0. Lymphodepletion can be performed either as an outpatient or inpatient per the investigator's medical judgement.

Lymphodepletion treatment plan

Drug	Dose	Days
Cyclophosphamide	250 mg/m^2 IV infusion over 30 min	-5, -4, -3
Fludarabine	25 mg/m^2 IV infusion over 30 minutes administered immediately after the cyclophosphamide (fludarabine dose should be reduced based on renal function) [a]	-5, -4, -3

[a] Subjects with creatinine clearance 50 to 60 mL/min should have a 20% dose reduction of each daily fludarabine dose. Subjects with creatinine clearance of 30 to 49 mL/min should have a 40% dose reduction of each daily fludarabine dose.

Target Dose

A single dose level of 0.6 to 6.0 x10^8 CAR-positive T cells will be utilized based on the FDA approved product label.

Cytokine Release Syndrome (CRS), Neurotoxicity and Monitoring

The primary acute toxicity observed to date with CAR T cells have been CRS and neurotoxicity and this protocol will follow the recommendations and management for CRS using established consensus guidelines.

For this protocol, CRS is defined as a constellation of symptoms which may include (but are not limited to) fever, chills, hypotension, dyspnea, hypoxia, confusion, mental status changes, seizures, myalgias, nausea and vomiting, and laboratory abnormalities including elevated AST, ALT, bilirubin, CRP, D-dimers, PT/INR, ferritin, urea and/or creatinine. Monitoring for CRS should include a physical exam, vital signs, and lab testing per the clinical trial schedule of events (SOE), unless otherwise clinically indicated, in which case additional clinical assessments or interventions should be performed at the discretion of the investigator or treating physician.

Any subject with a fever ≥100.0°F within 28 days of tisagenlecleucel infusion should have a work up for CRS. The diagnostic work-up of a CRS includes an evaluation for an infectious etiology (e.g., blood cultures, urine culture, chest X-ray, as required). Clinical laboratory tests include measurements of serum ferritin, C-reactive protein (CRP), comprehensive chemistries, coagulation, and blood counts. In addition, research blood tests for CAR+ T cells and cytokine assessments should be performed.

Within the first 28 days following infusion, subjects experiencing a fever ≥ 100.0°F, rapidly rising CRP, altered mental status, unstable vital signs, abnormal laboratory findings, or any other concerning medical conditions should be admitted for monitoring and further workup, at the discretion of the investigator or treating physician.

Treatment of CRS will follow institutional guidelines and may be modified in the future as newer standards become available. If admitted, subjects should be afebrile for 24 hours with declining inflammatory markers and resolution of any signs or symptoms suggesting of CRS and neurologic toxicity prior to discharge.

Neurologic Toxicity

Neurologic toxicity has been reported in anti-CD19 and anti-BCMA CAR T cell clinical trials, including confusion, agitation, obtundation, aphasia, seizures and myoclonus. To date, the neurologic toxicity has been reversible in a majority of cases however isolated events of fatal neurologic toxicity have been seen in CARs containing a CD28 costimulatory domain [2-4]. Compared to CD28 containing CARs, tisagenlecleucel contains a 4-1BB costimulatory domain, which in comparison, has substantially lower neurotoxicity [5]. Additionally, no deaths from severe neurotoxicity have been reported with any 4-1BB containing CAR supporting its use in PCNSL. Initially there was been concern that the presence of CNS disease may predispose patients to increased rates, and more severe neurotoxicity, however emerging data now suggests that CRS and neurotoxicity may be a product of a larger systemic inflammatory response as opposed to isolated CNS disease [6,7].

In the event of neurologic toxicity, it is recommended that investigators thoroughly assess and manage subjects for possible etiologies according to institutional guidelines, which may be modified in the future as more published guidelines become available. Treatment intervention

with corticosteroids and/or cyclophosphamide has demonstrated success in other studies.

During the post-infusion period, subjects treated on this protocol will be monitored with routine neurocognitive assessments as per the SOE unless otherwise clinically indicated. In the event of suspected neurotoxicity, patients will undergo additional workup which may include a lumbar puncture, EEG, and/or CT/MRI imaging as clinically indicated and in coordination with neuro-oncology as per standard institutional practice. Neurotoxicity will be managed with dexamethasone, anakinra, and/or other agents as per the judgement of the clinical investigator. All patients will be started on prophylactic levetiracetam prior to CAR-T infusion.

Long-term Follow-up

Subjects will be transitioned to long-term follow-up (LTFU) following disease progression and/or completion of the 24 month active treatment phase to maintain compliance with the requirements for subjects receiving genetically modified cells containing lentiviral vectors under IND. Subjects will be followed for up to 15 years. Subjects will be asked to participate in a separate 15-year registry study, such as CIBMTR. For subjects who consent to the registry study, data for LTFU will be collected with the registry study.

In the event that patients are required to be enrolled to a separate LTFU protocol, as requested by FDA/Novartis, patients will be allowed to transition to this separate master protocol in compliance with regulatory authorities.

Safety evaluations, including documentation of AEs and blood assessments, will be evaluated in all subjects until they have undetectable vector copy number (VCN) (where the definition of undetectable VCN is <0.0003 vector copies per diploid genome) in peripheral blood cells for 2 consecutive measurements at least 1 month apart, at least 6 months after drug product infusion. For subjects with no disease progression and undetectable VCN, these evaluations will be performed for up to 5 years post-CAR T cells infusion, or until disease progression.

Efficacy evaluations, including disease specific response assessments, will only be evaluated in subjects without disease progression, or until the time of disease progression/relapse in LTFU phase. Among these subjects, efficacy will be evaluated for at least 5 years post-CAR T cells infusion if VCN is undetectable, and up to 15 years post-tisagenlecleucel infusion if VCN remains detectable.

Diagnosis and selection criteria: Primary CNS Lymphoma

Inclusion criteria for enrollment/apheresis:

1. ***Primary CNS Lymphoma in high risk elderly patients***
    1. New diagnosis of primary CNS lymphoma.
    2. Voluntarily sign informed consent form(s)
    3. ≥60 years of age at the time of signing informed consent
    4. Eastern Cooperative Oncology Group (ECOG) performance status 0 - 2
    5. Have failed or are unable to tolerate definitive first-line methotrexate-based therapy as defined by:
        i. Grade 3+ AKI and/or transaminitis preventing repeat treatment exposure and/or,
        ii. Failure to achieve a complete response (per IPCG) following two

cycles of first line therapy,

1. Definitive first-line therapies must include high dose methotrexate-based therapy but may also include temozolomide, high dose cytarabine, pemetrexed, lenalidomide, ibrutinib and rituximab.

iii. Whole-brain irradiation, lenalidomide monotherapy and ibrutinib monotherapy are considered first line therapy if patient was not eligible for methotrexate-based chemotherapy at time of initial treatment but now meets study eligibility criteria.

6. Adequate absolute lymphocyte count (ALC > 500 cells/ul) within one week of apheresis.
7. Adequate bone marrow function defined by absolute neutrophil count (ANC) >1000 cells/mm^3without growth factor support, and untransfused platelet count >50,000 mm^3 within 7 days.
8. Left ventricular ejection fraction >40%
9. Adequate hepatic function defined by aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) <2.5 × upper limit of normal (ULN) and direct bilirubin <1.5 × ULN
10. Adequate renal function defined by creatinine clearance >30 ml/min using the Cockcroft-Gault formula
11. International ratio (INR) or partial thromboplastin time (PTT) <1.5 × ULN, unless on a stable dose of anticoagulant for a thromboembolic event.
12. The effects of tisagenlecleucel T cells on the developing human fetus are unknown. For this reason, women of child-bearing potential and men with partners of childbearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to leukapheresis for at least 1-year post tisagenlecleucel infusion and until CAR T cells are no longer present by qPCR on two consecutive tests. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men with partners of childbearing potential treated or enrolled on this protocol must also agree to use adequate contraception prior to leukapheresis and until 4 months after tisagenlecleucel T cells administration. Additional follow-up requirements are described in 7.4.6.
13. Ability and willingness to adhere to the study visit schedule and all protocol requirements

2. ***Relapsed/Refractory Primary CNS Lymphoma***

1. Diagnosis of relapsed/refractory PCNSL having received at least one prior line of CNS directed therapy.
2. Voluntarily sign informed consent form(s)
3. ≥18 years of age at the time of signing informed consent
4. Eastern Cooperative Oncology Group (ECOG) performance status 0-2
5. Adequate absolute lymphocyte count (ALC > 500 cells/ul) within one week of apheresis.

6. Adequate bone marrow function defined by absolute neutrophil count (ANC) >1000 cells/mm^{3}without growth factor support, and untransfused platelet count >50,000 mm^3.
7. Left ventricular ejection fraction >40%
8. Adequate hepatic function defined by aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) <2.5 × upper limit of normal (ULN) and direct bilirubin <1.5 × ULN
9. Adequate renal function defined by creatinine clearance >30 ml/min using the Cockcroft-Gault formula
10. International ratio (INR) or partial thromboplastin time (PTT) <1.5 × ULN, unless on a stable dose of anticoagulant for a thromboembolic event.
11. The effects of tisagenlecleucel T cells on the developing human fetus are unknown. For this reason, women of child-bearing potential and men with partners of childbearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to leukapheresis for at least 1-year post tisagenlecleucel infusion and until CAR T cells are no longer present by qPCR on two consecutive tests. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men with partners of childbearing potential treated or enrolled on this protocol must also agree to use adequate contraception prior to leukapheresis and until 4 months after tisagenlecleucel T cells administration. Additional follow-up requirements are described in 7.4.6.
12. Ability and willingness to adhere to the study visit schedule and all protocol requirements

Inclusion Criteria for Lymphodepletion/Cell Infusion:

3. No Active, uncontrolled, systemic bacterial, viral, or fungal infection.
4. Adequate renal function defined by creatinine clearance >30 ml/min using the Cockcroft-Gault formula

Exclusion criteria:

1. Prior treatment with an any investigational cellular therapy.
2. Ongoing treatment with chronic immunosuppressants (e.g., cyclosporine). Systemic steroids are allowed up to a dose of dexamethasone 4mg daily or equivalent.
3. Ongoing systemic immunosuppression for acute and/or chronic GVH as a result of previous allogeneic bone marrow transplant.
4. Significant co-morbid condition or disease which in the judgment of the Principal Investigator would place the subject at undue risk or interfere with the study; examples include, but are not limited to, cirrhotic liver disease, sepsis, and/or recent significant traumatic injury.
5. Active, uncontrolled, systemic bacterial, viral, or fungal infection.
6. Active hepatitis B or hepatitis C infection.
7. HIV infection.

8. Subjects with a history of class III or IV congestive heart failure or non- ischemic cardiomyopathy.
9. Subjects with second malignancies if the second malignancy has required therapy in the last 3 years or is not in complete remission; exceptions to this criterion include successfully treated non-metastatic basal cell or squamous cell skin carcinoma, or prostate cancer that does not require therapy other than hormonal therapy.
10. Pregnant or lactating women
11. Live virus vaccines within 2 weeks prior to planned start of lymphodepleting chemotherapy.

Inclusion criteria for lymphodepletion/cell infusion:

1. No active, uncontrolled, systemic bacterial, viral, or fungal infection. If febrile, the patient must be afebrile for 24 hours or blood cultures negative for 48 hours on appropriate antibiotic therapy
2. Oxygen saturation >92% on room air while awake
3. Infusion may be delayed by up to 7 days after completion of LD chemo in the event that these issues resolve in that time frame.

Endpoints:

Primary:

Incidence and maximal grade of adverse events (AEs) related to tisagenlecleucel including cytokine release syndrome and neurotoxicity per ASBMT 2018 consensus criteria[8].

Secondary:

Overall response rate (ORR) at 1, 3, 6 and 12 months after tisagenlecleucel treatment per IPCG response criteria.

Exploratory endpoints:

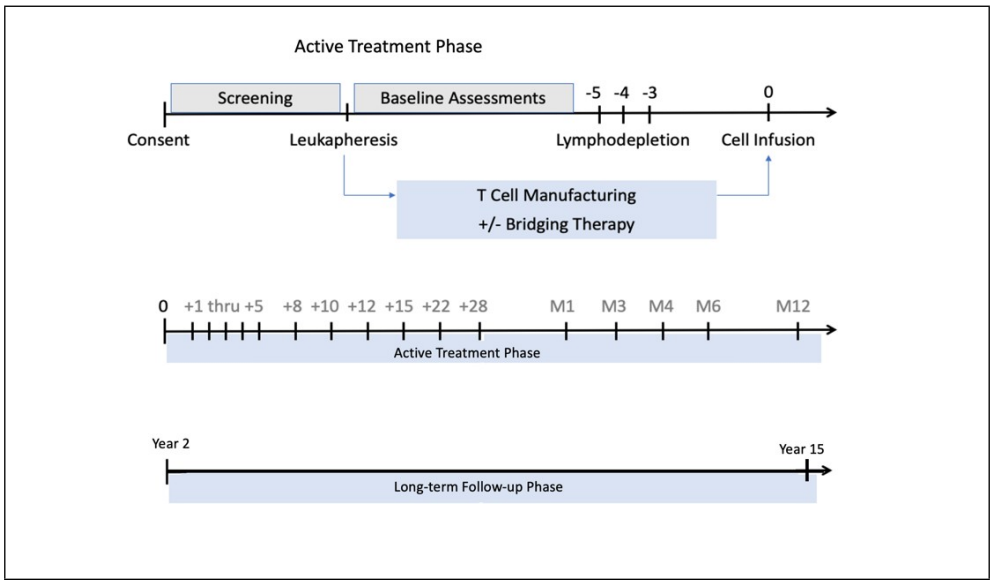
- Overall survival and progression-free survival
- Detection and quantification of tisagenlecleucel in the peripheral blood and CSF.
- Assessment of cytokine/chemokines profiling in peripheral blood and CSF, including IL-2, IL-6, IL-10, IFNγ, TNFα etc.
- Detection of tumor specific RNA/DNA in initial biopsies, peripheral blood and CSF.
- Longitudinal neurocognitive evaluation as per IPCG guidelines.
- When available, pre/post tumor samples for gene and immune-profiling.

Duration of Subject Participation:

Subjects will be followed for AEs, clinical status, and laboratory parameters for up to 24 months after the infusion of tisagenlecleucel, unless they terminate early due to disease progression, or withdraw for a different reason. All patients who receive tisagenlecleucel will be transitioned to the long-term follow-up (LTFU) phase of this protocol following disease progression and/or completion of the 24-

month active treatment phase to maintain compliance with the requirements for subjects receiving genetically modified cells containing lentiviral vectors under IND. Subjects will be followed for up to 15 years. Subjects will be asked to participate in a separate 15-year registry study, such as CIBMTR. For subjects who consent to the registry study, data for LTFU will be collected with the registry study.
Statistical methods: Analyses will be primarily descriptive due to the preliminary nature of this study.

SCHEMATIC OF CLINICAL STUDY



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1. OBJECTIVES

1.1 Study Design

This is a pilot study. A total of 12 subjects with primary CNS lymphoma be enrolled to this trial. This study will enroll two patient populations to include primary CNS lymphoma in high risk elderly patients, and relapsed/refractory primary CNS lymphoma following standard therapy. Primary CNS lymphoma is differentiated from secondary CNS lymphoma which occurs in the setting of systemic disease.

1.2 Primary Objectives and Endpoints

The primary objective of this study is to assess the safety of tisagenlecleucel in patients with primary CNS diffuse large B-cell lymphoma (PCNSL). The primary endpoint of this study is the incidence of adverse events (AEs) related to tisagenlecleucel including cytokine release syndrome and neurotoxicity.

1.3 Secondary Objectives and Endpoints

The secondary objective for this study is to assess the activity of tisagenlecleucel in patients with primary CNS diffuse large B-cell lymphoma (PCNSL). The main secondary endpoint for this study will be overall response rate (ORR) at 1, 3, 6 and 12 months after tisagenlecleucel treatment per IPCG response criteria.

1.4 Exploratory Objectives and Endpoints

The main exploratory objectives are to evaluate overall, and progression-free survival of patients treated with tisagenlecleucel with PCNSL. Additional objectives include detection and quantification of tisagenlecleucel and tumor specific mutations in biopsies, the peripheral blood and CSF, assessment of cytokine/chemokine profiling in peripheral blood and CSF, longitudinal neurocognitive evaluation as per IPCG guidelines, and when available, assess pre/post tumor samples for gene and immune-profiling.

2. BACKGROUND

2.1 Primary CNS Lymphoma (PCNSL)

Primary central nervous system lymphoma (PCNSL) is an extranodal non-Hodgkin lymphoma (NHL) confined to the brain, leptomeninges, eyes and/or spinal cord [9]. The prognosis of PCNSL is inferior to that of other NHL subtypes including other organ-specific subtypes of extranodal NHL. The 5- and 10-year survival proportions for PCNSL are 29.3% and 21.6%, respectively. An estimated 1,425 cases of PCNSL were diagnosed each year in the United States from 2007 to 2011 and the number of cases is expected to increase further with the aging of the U.S. population

[10]. Between 1970 and 2000, the incidence of PCNSL increased, largely due to the human immunodeficiency virus (HIV) pandemic. Since 2000, there has been a further increase in the incidence of PCNSL, especially in the elderly, with nearly half of all cases of PCNSL occurring in patients older than 60 [11]. Age itself is negatively prognostic as the overall median survival for patients over the age of 65 with newly diagnosed PCNSL is only 7 months with standard first line therapy only demonstrating a 2-year PFS of 37.3% [12,13]. Given these low long-term response rates, high-dose chemotherapy followed by autologous stem cell transplantation (HDC-ASCT) has been investigated as consolidation following CR1. HDC-ASCT is a promising approach, however treatment related toxicity is often significant and limiting in patients over the age of 60 demonstrating the need for newer, less toxic therapies in this patient population [14].

In the setting of relapsed or refractory PCNSL, optimal therapy has yet to be determined and has only been studied in relatively small studies using heterogeneous therapies. Despite high initial response rates with methotrexate-based induction therapy, most patients with PCNSL relapse. In general, prognosis for patients with relapsed or progressive PCNSL is poor with a median survival of approximately 4 months [15]. In a study of 256 PCNSL patients with relapsed or refractory PCNSL survival was worse in those patients who had refractory PCNSL and in those who relapsed within one year versus those who relapsed after one year [16]. Although relapses in PCNSL are predominantly within the CNS, relapses in extraneural organs are reported in up to 17% of patients. Late relapses also appear to occur more commonly in primary CNS DLBCL versus systemic DLBCL. In a long-term follow-up study 26% of all relapses were after 5 years and 1.7% were after 10 years [17]. Treatment for relapsed or refractory PCNSL has not been standardized and prognosis is poor for most of this patient population. Response rates to salvage chemotherapy in patients with relapsed or refractory PCNSL have not been systematically reported for cytotoxic or biological agents. Given the lack of a standard of care and the poor prognosis, older patients with poor functional status and patients with relapsed or refractory PCNSL represent an unmet medical need.

2.2 CD19 and tisagenlecleucel

CD19 is a 95-kDa glycoprotein present on B Cells from early development until differentiation into plasma cells. It is a member of the immunoglobulin superfamily and a component of a B-cell surface signal transduction complex that positively regulates signal transduction through the B-cell receptor. CD19 is an attractive therapeutic target because it is expressed by most B-cell malignancies, including B-cell non-Hodgkin lymphoma. Importantly, the CD19 antigen is not expressed on hematopoietic stem cells or any normal tissue apart from those of the B-cell lineage. CD19-specific chimeric antigen receptors (CARs) are single chain variable fragments (scFv) fused to a transmembrane domain and cytoplasmic signaling domains. Expression of the CD19-directed CAR in autologous T cells is achieved by ex vivo transduction using a recombinant retroviral or lentiviral vector. The CAR is expressed on the T cell surface and redirects the transfected T cells to CD19-expressing lymphoma cells, leading to CD19-specific tumor cell recognition, lysis, cytokine secretion and T cell proliferation. In clinical studies, CD19-targeted CARs have demonstrated encouraging activity in adult and pediatric subjects with refractory/relapsed B-cell acute lymphoblastic leukemia (ALL) and B-cell NHL. Based on these successful data

tisagenlecleucel was approved for the treatment of patients up to 25 years of age with relapsed/refractory B-cell precursor acute lymphoblastic leukemia (ALL) and adults with relapsed refractory diffuse large B-cell lymphoma (DLBCL). In the setting of DLBCL, it is provided in a single-dose unit containing 0.6 to 6.0 x 10^8 chimeric antigen receptor (CAR)-positive T cells and infused following a lymphodepleting chemotherapy regimen consisting of cyclophosphamide and fludarabine or bendamustine. Tisagenlecleucel was recently FDA approved for relapsed/refractory DLBCL based on Phase II data from the JULIET study (NCT02445248) demonstrating an overall response rate of (ORR) 50% with median duration of response having not been met [5]

2.3 Rationale for Study

2.3.1 Rationale for CAR-T in PCNSL

CAR T cells have shown significant activity in the treatment of certain relapsed/refractory CD19+ B-cell malignancies. Of the available US based clinical trials currently open involving CAR T cells for lymphoma/leukemia, none specifically address primary CNS lymphoma which has a 5- and 10- year survival of 29.3 and 21.6% respectively. Anecdotal experience in secondary CNS lymphoma suggest both activity and safety of CAR-T cells for DLBCL [18,19]. Despite case reports of CD19 directed CAR-T cell activity in the CNS, patients with PCNSL are excluded from the recently approved large cell lymphoma labels and ongoing clinical trial efforts. More recent data has suggested the viability of CAR-T cells for secondary CNS lymphoma. A 68-year-old female with DLBCL and cerebral involvement refractory to several chemotherapy regimens including allogeneic stem cell transplant was treated on NCT02631004 with CD19-directed CAR-T cells and achieved a CR at 1-month follow-up [18]. Similar activity was also seen in children and adults with refractory or relapsed ALL with CNS involvement suggesting that tisagenlecleucel may be a viable treatment option for patients with CNS based B-cell malignancies such as PCNSL[1,19]. Interestingly in both pediatric and adult patients with ALL, and adults with CLL, prior studies with tisagenlecleucel demonstrated higher levels of CAR transgene in the CSF than compared to peripheral blood at day 28 and months 3 and 6 when expressed per microgram of genomic DNA (Figure 1A). As CSF penetration will be critical for the treatment of PCNSL, it is important to also note that no central nervous system relapses have been observed in patients with pediatric B-ALL[1] implying these levels are biologically active and sufficient for disease clearance. Despite CNS penetration, quantification of CAR-T cells in the CSF did not correlate with neurologic toxicity suggesting the mere presence of T cells within the CNS space does not mediate high grade or fatal neurotoxicity (Figure 1B).

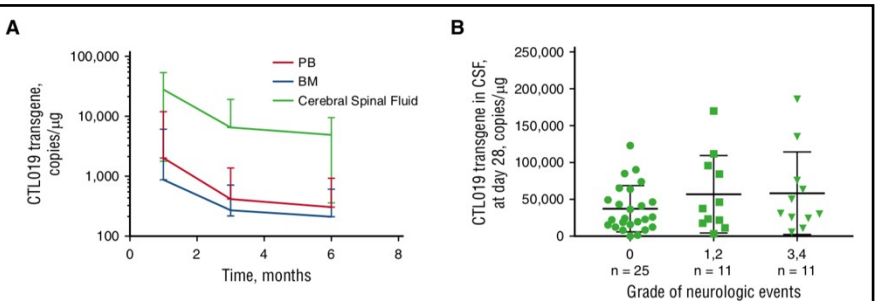

Figure 1 – Mueller et al[1].

2.3.2 **Rationale for Starting Dose**

The starting dose for this trial was determined based on the current FDA approved dose of 0.6 to 6.0 x 10^8. To date no relationship between tisagenlecleucel dose, maximal in vivo expansion, clinical responses has treatment related toxicities[20].

2.3.3 **Rationale for Lymphodepletion**

Clinical data from previous studies suggest that the expansion and activity of adoptively transferred T cells can be enhanced by using lymphodepleting agents.

Lymphodepletion acts via several mechanisms to augment T cell activity post transfer, including to increase the efficacy of the transferred T cells both by several potential mechanisms: 1) reducing tumor burden, 2) making 'space' for engraftment of the transferred T cells, 3) triggering increase in homeostatic cytokines production and 4) by reducing the number of immunosuppressive regulatory T cells. The removal of endogenous T cells and natural killer cells leads to the stimulation of IL-15 and IL-7, which increases T cell function and anti-tumor activity [21].

Fludarabine and cyclophosphamide are effective lymphodepleting agents and have been used as a lymphodepletive regimen in other CAR T cell therapy trials that have demonstrated persistence and activity of transferred cells [22]. The effectiveness of the combined use of both fludarabine and cyclophosphamide is evidenced in its effect on indoleamine 2,3-dioxygenase (IDO), and inhibitor of immune cells and CAR T activity: fludarabine and mafosfamide (a cyclophosphamide analog) downregulates IDO expression, and in combination with CD19+ CAR T cells, produced a better response than fludarabine or CAR+ T cells alone [23].

A dose of 250 mg/m^2 of cyclophosphamide and 25 mg/m^2 of fludarabine administered daily for 3 days has been selected as the regimen for this trial. This regimen has been tolerated by adult large cell lymphoma patients in both the JULIET study and commercially following FDA approval[24,25].

2.3.4 **Rationale for Long-Term Follow-up Phase**

The FDA recommends long-term follow-up for subjects treated with gene therapy drug products to monitor for delayed adverse events (AEs) due to the hypothetical safety concern that any ex vivo-gene modified autologous cell could include insertional mutagenesis. However, the potential for insertional mutagenesis and/or tumorigenicity is considered negligible for CAR T cells [26,27], based upon their terminal differentiation and the extensive clinical experience to date with long-term follow-up of patients receiving other CAR T cell products [28]. Ex vivo cultured primary human T cells were the first targets for human gene transfer experiments, using gamma-retroviral vectors performed at the National Cancer Institute (NCI) beginning in 1989 [29]. Thus, there exist 25 years of data concerning the biological implications of retroviral vector transduction of mature human T cells. To date, there is no report of a transformational event associated with a vector insertion in mature primary human T cells using either gamma-retroviral or lentiviral vectors. In addition to ongoing AE monitoring, patients will also be followed for durability of clinical response.

Therefore, after monitoring of subjects in the parent study has been completed (24 months after tisagenlecleucel T cells infusion, or <24 months after tisagenlecleucel T cells due to disease

progression) subjects will be transitioned to the long-term follow-up phase and followed for up to 15 years on protocol or in separate 15-year registry, such as CIBMTR.

2.4 Exploratory Studies Background

Correlative studies will be performed on peripheral blood, bone marrow/tumor biopsies and, if available, CSF samples to evaluate PK/PD markers related to safety and/or efficacy. The effectiveness of tisagenlecleucel T cells may depend on the expansion and persistence of CAR T cells in subjects. Therefore, the expansion and persistence will be monitored by flow cytometry and PCR analysis using peripheral blood samples as per standard SOPs. Samples will be collected per the protocol Schedule of Events (see **10.1 Schedule of Events**). Periodic monitoring of anti-inflammatory cytokines secretion will be performed to determine correlation between tisagenlecleucel effectiveness and/or toxicity.

Additional testing may be done depending on the clinical condition; for instance, patients with symptoms suggestive of cytokine release syndrome or with skin rash or other findings that may concern the investigators may have more frequent monitoring to enhance the safety of this trial.

If tumor tissues or bone marrow aspirates become available as part of routine clinical care, a sample will be collected for research analysis. Tissue samples (including CSF) will be analyzed for the presence of tisagenlecleucel T cells by PCR and/or flow cytometry as well as tumor specific RNA/DNA. Additionally, different T cell subsets may have demonstrated different effector function and persistence and, therefore, T cell immunophenotyping will be performed by flow cytometry. Peripheral blood samples may also be analyzed for cytokine/chemokine levels by Luminex technology. Tumor tissue will be analyzed for CD19 expression by immunohistochemistry and/or flow cytometry. In addition, baseline leukapheresis and tisagenlecleucel final product will be banked and may be analyzed by immunophenotyping, PCR and gene expression profiling.

Neuro-cognitive assessments will be performed per published schedules and in collaboration with neuro-oncology as per the SOE **(see 10.1 Schedule of Events).**

2.5

3. PARTICIPANT SELECTION

3.1 Inclusion Criteria

Primary CNS Lymphoma in high risk elderly patients

1. New diagnosis of primary CNS lymphoma.
2. Voluntarily sign informed consent form(s)
3. ≥60 years of age at the time of signing informed consent
4. Eastern Cooperative Oncology Group (ECOG) performance status 0 - 2
5. Have failed or are unable to tolerate definitive first-line methotrexate-based therapy as defined by:
    i. Grade 3+ AKI and/or transaminitis preventing repeat treatment exposure and/or,
    ii. Failure to achieve a complete response (per IPCG) following two cycles of first line therapy,
        1. Definitive first-line therapies must include high dose methotrexate-based therapy but may also include temozolomide, high dose cytarabine, pemetrexed, lenalidomide, ibrutinib and rituximab.
    iii. Whole-brain irradiation, lenalidomide monotherapy and ibrutinib monotherapy are considered first line therapy if patient was not eligible for methotrexate-based chemotherapy at time of initial treatment but now meets study eligibility criteria.
6. Adequate absolute lymphocyte count (ALC > 500 cells/ul) within one week of apheresis.
7. Adequate bone marrow function defined by absolute neutrophil count (ANC) >1000 cells/mm^3without growth factor support, and untransfused platelet count >50,000 mm^3 within 7 days.
8. Left ventricular ejection fraction >40%
9. Adequate hepatic function defined by aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) <2.5 × upper limit of normal (ULN) and direct bilirubin <1.5 × ULN
10. Adequate renal function defined by creatinine clearance >30 ml/min using the Cockcroft-Gault formula
11. International ratio (INR) or partial thromboplastin time (PTT) <1.5 × ULN, unless on a stable dose of anticoagulant for a thromboembolic event.
12. The effects of tisagenlecleucel T cells on the developing human fetus are unknown. For this reason, women of child-bearing potential and men with partners of childbearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to leukapheresis for at least 1-year post tisagenlecleucel infusion and until CAR T cells are no longer present by qPCR on two consecutive tests. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men with partners of childbearing potential treated or enrolled on this protocol must also agree to use

adequate contraception prior to leukapheresis and until 4 months after tisagenlecleucel T cells administration. Additional follow-up requirements are described in 7.4.6.

13. Ability and willingness to adhere to the study visit schedule and all protocol requirements

Relapsed/Refractory Primary CNS Lymphoma

1. Diagnosis of relapsed/refractory PCNSL having received at least one prior line of CNS directed therapy.
2. Voluntarily sign informed consent form(s)
3. ≥18 years of age at the time of signing informed consent
4. Eastern Cooperative Oncology Group (ECOG) performance status 0-2
5. Adequate absolute lymphocyte count (ALC > 500 cells/ul) within one week of apheresis.
6. Adequate bone marrow function defined by absolute neutrophil count (ANC) >1000 cells/mm^3without growth factor support, untransfused platelet count >50,000 mm^3, and untransfused hemoglobin >9 g/dL.
7. Left ventricular ejection fraction >40%
8. Adequate hepatic function defined by aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) <2.5 × upper limit of normal (ULN) and direct bilirubin <1.5 × ULN
9. Adequate renal function defined by creatinine clearance >30 ml/min using the Cockcroft-Gault formula
10. International ratio (INR) or partial thromboplastin time (PTT) <1.5 × ULN, unless on a stable dose of anticoagulant for a thromboembolic event.
11. The effects of tisagenlecleucel T cells on the developing human fetus are unknown. For this reason, women of child-bearing potential and men with partners of childbearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to leukapheresis for at least 1-year post tisagenlecleucel infusion and until CAR T cells are no longer present by qPCR on two consecutive tests. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men with partners of childbearing potential treated or enrolled on this protocol must also agree to use adequate contraception prior to leukapheresis and until 4 months after tisagenlecleucel T cells administration. Additional follow-up requirements are described in 7.4.6.
12. Ability and willingness to adhere to the study visit schedule and all protocol requirements

Inclusion Criteria for Lymphodepletion/Cell Infusion:

1. No Active, uncontrolled, systemic bacterial, viral, or fungal infection.
2. Adequate renal function defined by creatinine clearance >30 ml/min using the Cockcroft-Gault formula

3.2 Exclusion Criteria

1. Prior treatment with an any investigational cellular therapy.
2. Ongoing treatment with chronic immunosuppressants (e.g., cyclosporine). Systemic steroids are allowed up to a dose of dexamethasone 4mg daily or equivalent.
3. Ongoing systemic immunosuppression for acute and/or chronic GVH as a result of previous allogeneic bone marrow transplant.
4. Significant co-morbid condition or disease which in the judgment of the Principal Investigator would place the subject at undue risk or interfere with the study; examples include, but are not limited to, cirrhotic liver disease, sepsis, and/or recent significant traumatic injury.
5. Active, uncontrolled, systemic bacterial, viral, or fungal infection.
6. Active hepatitis B or hepatitis C infection.
7. HIV infection.
8. Subjects with a history of class III or IV congestive heart failure or non- ischemic cardiomyopathy.
9. Subjects with second malignancies if the second malignancy has required therapy in the last 3 years or is not in complete remission; exceptions to this criterion include successfully treated non-metastatic basal cell or squamous cell skin carcinoma, or prostate cancer that does not require therapy other than hormonal therapy.
10. Pregnant or lactating women
11. Live virus vaccines within 2 weeks prior to planned start of lymphodepleting chemotherapy.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

MGH and DF/HCC Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of any protocol-specific therapy or intervention. Any participant not registered to the protocol before protocol-specific therapy or intervention begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol-specific therapy and/or intervention. Issues that would cause treatment delays should be discussed with the Overall Principal

Investigator (PI). If the subject does not receive protocol therapy following registration, the subject must be taken off-study in the CTMS (OnCore) with an appropriate date and reason entered and another patient may be screened for enrollment.

4.1 Registration Process for DF/HCC Institutions

DF/HCC policy (REGIST-101) must be followed.

4.2 General Guidelines for Other Investigative Sites

N/A

4.3 Registration Process for Other Investigative Sites

N/A

5. TREATMENT PLAN

5.1 Study treatment

The overall treatment plan for this study (Protocol #), including screening, leukapheresis, cell infusion, and follow-up visits described in the **Schematic of the Clinical Study.**

Tisagenlecleucel is an autologous cellular immunotherapy product that is comprised of CD3+ T cells that have undergone ex vivo T cell activation, gene modification, expansion and formulation in infusible cryo media. The transgene to be expressed via lentiviral vector transduction is a CAR targeted against the CD19 antigen. The CAR contains a murine scFv that targets CD19 linked to a trans membrane region derived from the CD8 receptor, which is linked to an intracellular bipartite signaling chain of TCR-ζ (or CD3-ζ) and 4-1BB intracellular signaling do mains. The extracellular scFv with specificity for CD19 is derived from a mouse monoclonal antibody. T cells which are enriched from a patient leukapheresis unit are expanded ex vivo using commercially available magnetic beads that are coated with anti-C D3 and anti-CD28 monoclonal antibodies. The cells are transduced with the CD19 CAR lentiviral vector which ensures that only peripheral white blood cells enriched for lymphocytes are exposed to the vector. The residual non-integrated vector is washed away during the process. Tisagenlecleucel cells will be expanded ex vivo for up to 10 days. At the end of the culture, the tisagenlecleucel cells are depleted of magnetic beads, washed, concentrated, and cryopreserved. Results from a release testing procedure are required prior to release of the product for infusion.

5.2 Leukapheresis

Prior Anti-Cancer Treatment

Subjects should not receive any prior systemic therapy or anti-tumor directed therapy within 2 weeks prior to lymphodepletion. No lymphodepleting agents (e.g., cyclophosphamide, bendamustine) should be administered within 2 weeks of leukapheresis, and other therapies (e.g., immunomodulatory agents or antibodies) should be discussed with the medical monitor. BTKi and IMiDs are allowed prior, during, and following leukapheresis.

Avoidance of Corticosteroids

Therapeutic doses of steroids should ideally be stopped >72 hours prior to leukapheresis and/or tisagenlecleucel infusion, however may be continued if deemed medically necessary. If continued, they should not exceed 4mg dexamethasone daily or equivalent may be higher at the discretion of the Principle Investigator.

Use of Previously Frozen Leukapheresis Product

If patients have previously frozen leukapheresis product that meets incoming product specification for subsequent manufacturing, such product may be used following discussion with Novartis.

5.3 Dosing Regimen

A single dose level of 0.6 to 6.0 x10^8 CAR-positive T cells will be utilized based on the FDA approved product label.

5.4 Lymphodepleting chemotherapy

It is anticipated that many patients will have been receiving chemotherapy for relapsed or resistant disease. Prior to tisagenlecleucel cell infusion, an additional lymphodepleting chemotherapy cycle is planned.

When given, lymphodepleting chemotherapy should be started no more than 2 weeks before tisagenlecleucel infusion so that the tisagenlecleucel cells will be given within 14 days following completion of the lymphodepleting chemotherapy. Ideally patients will receive tisagenlecleucel 48-72 hours following completion of lymphodepleting chemotherapy. The purpose of this chemotherapy is to induce lymphopenia in order to facilitate engraftment and homeostatic expansion of tisagenlecleucel cells.

Lymphodepletion treatment plan

Drug	Dose	Days
Cyclophosphamide	250 mg/m^2 IV infusion over 30 min	-5, -4, -3

Fludarabine	25 mg/m^2 IV infusion over 30 minutes administered immediately after the cyclophosphamide (fludarabine dose should be reduced based on renal function) [a]	-5, -4, -3

[a] Subjects with creatinine clearance 50 to 60 mL/min should have a 20% dose reduction of each daily fludarabine dose. Subjects with creatinine clearance of 30 to 49 mL/min should have a 40% dose reduction of each daily fludarabine dose.

Chemotherapy should be administered per institutional guidelines. On Days -5, -4, and -3, subjects should receive pre-hydration with 1000 mL 0.9% sodium chloride IV over 1 to 3 hours.

Anti-emetics will be administered and include the use of ondansetron, oral or IV, or similar serotonin inhibitor, on days -5, -4 and -3 prior to chemotherapy but dexamethasone or other steroids are not to be administered for nausea. Subjects may receive education and prescriptions for anti-emetics such as ondansetron, lorazepam, or prochlorperazine for use when not in clinic.

After the administration of fludarabine on Days -5, -4 and -3, subjects should receive 1000 mL 0.9% sodium chloride IV over 1 to 2 hours. Diuretics may be used as needed based on clinical assessment.

On Days -2 and -1, there are no required clinical interventions except those for supportive care including continued anti-emetics for nausea. Subjects should be encouraged to increase fluid intake to minimize bladder toxicity or be supplemented with IV hydration at clinical discretion.

Anti-epileptic drugs (e.g. Keppra) should be given to all patients started on D-5 of lymphodepletion if not on already. In the event that a patient does not tolerate Keppra, an alternative agent will be determined in collaboration with neuro-oncology.

5.5 Tisagenlecleucel infusion

The tisagenlecleucel cell product will be prepared and released by the manufacturing facility to the study site approximately 3 to 4 weeks after manufacturing has commenced, provided all required safety and quality release criteria have been met. Upon receipt of the cryopreserved tisagenlecleucel cell product, an inventory must be performed, and a drug receipt log filled out and signed by the person accepting the shipment. The cryopreserved tisagenlecleucel cell product should be kept in the vapor phase of liquid nitrogen until tisagenlecleucel infusion. For details on the cryopreserved components, and the specific storage and handling requirements of tisagenlecleucel, see the commercial product handling manual.

All patients will be staggered by 28 days between infusions to allow for full safety evaluation following infusion.

All patients will be required to be on anti-epileptic drugs (eg. Keppra 500mg BID), prior to T cell infusion. In the event that a patient does not tolerate Keppra, an alternative agent will be determined in collaboration with neuro-oncology.

Prior to tisagenlecleucel infusion the following criteria must be met:

1. No active, uncontrolled, systemic bacterial, viral, or fungal infection. If febrile, the patient must be afebrile for 24 hours or blood cultures negative for 48 hours on appropriate antibiotic therapy
2. Oxygen saturation >92% on room air while awake

If patients are taking any of the following medications, their infusion must be delayed until the medications have been stopped according to the below:

1. **Steroids**: Therapeutic doses of steroids should ideally be stopped >72 hours prior to tisagenlecleucel infusion, however may be continued if deemed medically necessary. If continued, they should not exceed 4mg dexamethasone daily or equivalent.
2. **Antiproliferative Therapy:**
    - All antiproliferative therapies must have been stopped ≥ 2 weeks prior to tisagenlecleucel infusion (excluding BTKi or IMiDs).
    - Immunosuppressive therapies: Any drug used for immunosuppression must be stopped>4 weeks prior to tisagenlecleucel infusion (e.g. calcineurin inhibitors, methotrexate or other chemotherapy drugs, mycophenolate, steroids [see above], rapamycin, or immunosuppressive antibodies such as rituximab, anti-TNF, anti-IL6 or anti-IL6R).
    - Immune checkpoint blockade (eg. Pembrolizumab, ipilumimab, nivolumab, etc) must be held ≥ 4 weeks prior to tisagenlecleucel infusion.
3. **CNS disease prophylaxis:**
    - CNS prophylaxis treatment must be stopped >2 week prior to tisagenlecleucel infusion (e.g. intrathecal methotrexate).
4. **Myeloid growth factors**, particularly granulocyte macrophage- colony stimulating factor (GM-CSF), are not recommended during the first 3 weeks after tisagenlecleucel infusion or until CRS has resolved. Short acting granulocyte colony stimulating factor (G-CSF) should be stopped 72 hours prior to tisagenlecleucel infusion and long acting G-CSF should be stopped 10 days prior to tisagenlecleucel infusion.
5. **Radiotherapy**:
    - Non-CNS site of radiation must be completed > 2 weeks prior to tisagenlecleucel infusion
    - CNS directed radiation must be completed > 2 weeks prior to tisagenlecleucel infusion
6. **Anti T-cell Antibodies:** It is strongly recommended that administration of any T cell lytic or toxic antibody (e.g., alemtuzumab) within 8 weeks prior to tisagenlecleucel be discontinued since residual lytic levels may destroy the infused tisagenlecleucel cells and/or prevent their in vivo expansion.

Infusion may be delayed by up to 7 days after completion of LD chemo in the event that these issues resolve in that time frame.

5.6 General Concomitant Medication and Supportive Care Guidelines

Prior Medication

All medications taken within 30 days prior to the start of screening assessments are to be recorded in the Prior and Concomitant Medication CRF.

Concomitant Medications and Therapies

All concomitant medications and therapies (i.e., those that are taken by the subject after signing the informed consent form) will be recorded in the Concomitant Medication CRF.

Infection Surveillance and Antibiotic prophylaxis

- Subjects 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 if the subject has had one or more bacterial infections and is not neutropenic while enrolled on the protocol. 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.
- Subjects with a history of herpes simplex virus infection should receive herpes simplex virus prophylaxis at the discretion of the investigator.
- Subjects with a CD4 T cell count of <200 will be maintained on pneumocystis prophylaxis with trimethoprim-sulfamethoxazole 1 double-strength tablet every Monday-Wednesday-Friday. If subjects cannot tolerate trimethoprim sulfamethoxazole, an alternative pneumocystis prophylaxis will be used.
- Patients should be started on viral prophylaxis with initiation of lymphodepleting chemotherapy if not on already. Patients with prolonged neutropenia (>10 days) or who are started on steroids for management of Patients with neutropenia should be started on antimicrobial prophylaxis CRS/Neurotox should be started on antifungal prophylaxis.
    - Antimicrobial prophylaxis
        - Ciprofloxacin 500mg BID or levofloxacin 500mg BID
        - Acyclovir 400mg BID or famvir 500mg BID
        - Fluconazole 400mg daily.

Blood product support

Transfusion support of platelets and packed RBCs may be used at the discretion of the treating investigator. Leukocyte filters are encouraged for all platelet and packed RBC transfusions.

Anti-emetics, Anti-diarrheal and Growth Factors

Anti-emetics, anti-diarrheas and growth factor support may be administered at the discretion of the investigator and it is suggested utilization follow the American Society of Clinical Oncology guidelines. Steroids should NOT be used as an anti-emetic regimen. Myeloid growth factors, particularly G-CSF and GM-CSF, are not recommended during the first 3 weeks after tisagenlecleucel infusion or until CRS has resolved.

Guidelines for the management of Acute Toxicities occurring following the administration of tisagenlecleucel T cells

The management of CRS is discussed in Section 7 and Appendix B. These guidelines are published recommendations based on the observation and management of CRS in other active CAR T programs.

Anti-Cancer Therapy

After receiving tisagenlecleucel T cells, subjects should not receive any other therapy that is or may be active against the identified malignancy including other chemotherapies, biologics, radiation therapy or any other investigational agents other than tisagenlecleucel T cells. BTKi and IMIDs will be allowed as maintenance therapy before, during and after CAR-T infusion. Localized radiation for symptomatic relief may be allowed after discussion with the medical monitor. The use of bisphosphonates is acceptable at the discretion of the investigator.

5.7 Duration of Active Treatment Phase and Pausing Criteria

Subjects will be followed for AEs, clinical status, and laboratory parameters for up to 24 months after the infusion of tisagenlecleucel T cells, unless they terminate early due to disease progression, or withdraw for a different reason. All subjects who receive tisagenlecleucel T cells will be transitioned to the long-term follow-up (LTFU) phase following disease progression and/or completion of the 24-month active treatment period to maintain compliance with the requirements for subjects receiving genetically modified cells containing lentiviral vectors under IND.

Study enrollment will start at time of apheresis however only patients who are infused will be analyzed in subsequent data analysis.

Enrollment and treatment with drug product may be temporarily suspended for any of the following reasons pending review and recommendations from the DSMC or the Sponsor:

- Death that is not related to disease progression within 30 days of the tisagenlecleucel T cells infusion*
- Determination of unexpected, clinically significant, or unacceptable risk to subjects
- 2 or more instances of grade 4 CRS (ASTCT 2019), or CTCAE-defined grade 4 organ-specific toxicity affecting the kidney, liver, lungs, or heart, and for a single instance of grade 4 ICANS, cerebral edema, or other grade 4 CNS events such as hemorrhage.
- Any subject develops detectable replication competent lentivirus (RCL) during the study

- The study may be paused pending notification of the health authorities and the DSMC for investigation and possible protocol amendment if any subject experiences any of the following events within three weeks of the tisagenlecleucel infusion:
    - Life-threatening (grade 4) toxicity attributable to protocol therapy that is unmanageable, unexpected and unrelated to chemotherapy and attributable to protocol therapy. High fevers, hypotension, hypoxia, disseminated intravascular coagulation, encephalopathy (e.g. lethargy, confusion, aphasia, seizure), ICU admission, dialysis and mechanical ventilation are expected. The expected side effects can also result in grade 4 liver toxicity, nephrotoxicity and cardiac dysfunction.

*All events causing a study pause or stop will be reported to the FDA in an expedited manner.

*Any death that is not due to disease progression within 30 days after receiving tisagenlecleucel T cells will result in a hold of further enrollment and treatment with drug product until an investigation into the cause of death is performed. If it is determined that the death was not related to the drug product, then enrollment/treatment with drug product may restart. If the relationship between the drug product and the death is not clear, or it appears that the death may be related to study drug, enrollment and treatment with drug product will be held until after discussion with the sponsor.

5.8 Duration of Follow Up and Long-Term Follow-up Phase

All subjects who complete the 24 month active treatment period and/or progress, will be transitioned to the long-term follow-up (LTFU) phase of this protocol following disease progression and/or completion of the 24-month active treatment period to maintain compliance with the requirements for subjects receiving genetically modified cells containing lentiviral vectors under IND. Subjects will be followed for up to 15 years and will be asked to participate in a separate 15-year registry study, such as CIBMTR. For subjects who consent to the registry study, data for LTFU will be collected with the registry study.

Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.9 Criteria for Taking a Participant Off Study

Subjects have the right to withdraw from the study at any time for any reason, without prejudice to further medical follow up. Should a subject decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible. The Investigator and Sponsor also have the right to withdraw subjects from the study at any time due to protocol noncompliance, poor tolerance, or potential safety risks.

It is expected that the most common reason for withdrawal from the study will be disease progression. Subjects, however, may withdraw from this study at any time, for any reason. Other than progressive disease or death, other possible reasons for study withdrawal include:

- Toxicity
- Subject preference, including decision to undergo alternative treatment
- Physician preference
- Adequate cells are not collected during harvests, or failure of transduced cells to be dispositioned for clinical use
- Any medical condition which, in the opinion of the Investigators, would put the subject at risk for treatment or follow-up studies, or
- Closure of the study

If a subject is found to have disease progression based on defined response criteria, it is anticipated that they will be transitioned to this study's LTFU phase or followed on a registry/study that meets the requirements for subjects receiving genetically modified cells containing lentiviral vectors under IND. If a subject is found to have disease progression without meeting specific response criteria, the subject may be withdrawn at their own or the Investigator's discretion based on the subject's best interest, and then be transitioned to this study's LTFU phase or a registry/study that meets the requirements for subjects receiving genetically modified cells containing lentiviral vectors under IND.

Subjects withdrawn from active treatment at any time after receiving tisagenlecleucel T cells, regardless of the reason will be transitioned to the study's LTFU phase or appropriate registry/study and followed for up to 15 years .

The reason for transitioning to LTFU, and the date the participant was transitioned, must be documented in the case report form (CRF). In addition, the study team will ensure Off Treatment/Off Study information is updated in OnCore in accordance with DF/HCC policy RESIST-101.

6. DOSING DELAYS/DOSE MODIFICATIONS

In the case of medical or natural emergencies (i.e., holidays, snowstorms, acute infection) occurring after initiation of chemotherapy, the infusion of tisagenlecleucel may be delayed up to one week at the investigator's discretion. Subjects who do not initiate chemotherapy within 8 weeks of enrollment will be withdrawn; however, their tisagenlecleucel product will be stored for up to 1 year if they re-enroll at a later date.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

7.1 Expected Toxicities

7.1.1 Cytokine Release Syndrome (CRS) and Monitoring

The primary acute toxicity observed to date with CAR T cells has been CRS, and this protocol will follow the recommendations and management for CRS using established consensus guidelines. CRS and neurotoxicity grading will be performed utilizing the 2018 ASMBT consensus criteria.

For this protocol, CRS is defined as a constellation of symptoms which may include (but are not limited to) fever, chills, hypotension, dyspnea, hypoxia, confusion, mental status changes, seizures, myalgias, nausea and vomiting, and laboratory abnormalities including elevated AST, ALT, bilirubin, CRP, D-dimers, PT/INR, ferritin, urea and/or creatinine (see Table 1).

Monitoring for CRS should include a physical exam, vital signs, and lab testing per the clinical trial schedule of events (SOE), unless otherwise clinically indicated, in which case additional clinical assessments or interventions should be performed at the discretion of the investigator or treating physician.

Any subject with a fever ≥100.0°F within 28 days of tisagenlecleucel T cell infusion should have a work up for CRS. The diagnostic work-up of a CRS includes an evaluation for an infectious etiology (e.g., blood cultures, urine culture, chest X-ray, as required). Clinical laboratory tests include measurements of serum ferritin, C-reactive protein (CRP), comprehensive chemistries, coagulation, and blood counts. In addition, research blood tests for CAR+ T cells and cytokine assessments should be performed at least every other day in addition to the schedule of events.

Within the first 28 days following infusion, subjects experiencing a fever ≥ 100.0°F, rapidly rising

CRP, altered mental status, unstable vital signs, abnormal laboratory findings, or any other concerning medical conditions should be admitted for monitoring and further workup, at the discretion of the investigator or treating physician.

Treatment of CRS will follow protocol guidelines and may be modified in the future as newer standards become available.

If admitted, subjects should be afebrile for 24 hours with declining inflammatory markers and resolution of any signs or symptoms suggesting of CRS and neurologic toxicity prior to discharge.

Table 1: Clinical Signs and Symptoms Associated with CRS

Organ System	Symptoms
Constitutional	Fever +/- rigors, malaise fatigue, anorexia, myalgias, arthralgias, nausea, vomiting, headache
Skin	Rash
Gastrointestinal	Nausea, vomiting, diarrhea
Respiratory	Tachypnea, hypoxemia
Cardiovascular	Tachycardia, widened pulse pressure, hypotension, increased cardiac output (early), potentially diminished cardiac output (late)
Coagulation	Elevated D-Dimer, hypofibrinogenemia +/- bleeding
Renal	Azotemia
Hepatic	Transaminitis, hyperbilirubinemia
Neurologic	Headache, mental status changes, confusion, delirium, word finding difficulty or frank aphasia, hallucinations, tremor, dysmetria, altered gait, seizures

Guidelines for the management of toxicities related to tisagenlecleucel T cells infusion have been provided in Appendix B. These guidelines are based on available literature for the management of CRS and may need to be adjusted based on the individual clinical circumstances of each subject. The guidelines are not protocol required therapy as they may need to be adjusted but serve to provide a single consistent framework for the evaluation and management of CRS and tisagenlecleucel T cells related toxicity to mitigate risk to subject.

7.1.1.1 Temperature Self-Monitoring

Treated subjects must take their temperature every 6 to 8 hours from Day 0 through Day 10 post tisagenlecleucel T cells infusion and contact their treating investigator for any fever ≥100.0°F through Day 28 post tisagenlecleucel T cells infusion. Subjects should not take any nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen (Motrin, Advil), Naproxen Sodium (Aleve), aspirin or acetaminophen (Tylenol) because these can mask fevers. Fevers are a critically important sign that requires subjects to report to the treating institution as soon as possible. Fevers might possibly be the only warning of life-threatening toxicity that can quickly arise in subjects receiving CAR T cells. If subjects are in clinic for a visit when a self-monitoring temperature is to be done the temperature may be taken in the clinic by clinic staff. At the discretion of individual investigators, subjects may be hospitalized for AE monitoring if preferred.

Subjects are required to stay within a 30-mile radius of the treating institution; subjects should be evaluated and/or admitted to the treating institution due to the institution's familiarity with the treatment protocol and appropriate management of CRS.

7.1.2 Neurologic Toxicity

Neurologic toxicity has been reported in anti-CD19 and anti-BCMA CAR T cell clinical trials, including confusion, agitation, obtundation, aphasia, seizures and myoclonus. To date, the neurologic toxicity has been reversible in a majority of cases however isolated events of fatal neurologic toxicity have been seen in CARs containing a CD28 costimulatory domain [2-4,30].

Because these syndromes are only now being characterized in the setting of CAR T cell therapy, in the event of neurologic toxicity it is recommended that investigators thoroughly assess and manage subjects for possible etiologies according to institutional guidelines (See Appendix B), which may be modified in the future as more published guidelines become available. Treatment intervention with corticosteroids and/or cyclophosphamide has demonstrated success in other studies [31]. Grading and management of neurotoxicity will be performed as per 2018 Lee criteria[8].

During the post-infusion period, subjects treated on this protocol will be monitored with routine neurocognitive assessments as per the SOE unless otherwise clinically indicated. In the event of suspected neurotoxicity, patients will undergo additional workup which may include a lumbar puncture, EEG, and/or CT/MRI imaging as clinically indicated and in coordination with neuro-oncology as per standard institutional practice. Neurotoxicity will be managed with dexamethasone, anakinra, and/or other agents as per the judgement of the clinical investigator.

7.1.3 B cell aplasia and hypo/agammaglobulinemia

Transient or permanent B-cell aplasia with associated hypogammaglobulinemia or agammaglobulinemia is an expected-on target effect of tisagenlecleucel therapy in patients with sustained tumor response. This occurs since non-malignant B cells express CD19 and is expected to resolve if and when the tisagenlecleucel cells are cleared.

Monitor immunoglobulin levels after treatment with tisagenlecleucel. Hypo/agammaglobulinemia is typically managed with immunoglobulin replacement therapy dependent upon age specific, disease-specific and local institutional guidelines. Use infection precautions including antibiotic prophylaxis as appropriate and per local standard of care. In general B cell aplasia and hypogammaglobulinemia, of various causes, can be associated with increased rates of infection. Such infections are typically sinopulmonary but other sites and types of infections have also been reported.

Other potential complications of B cell aplasia include progressive multifocal leukoencephalopathy (PML) and reactivation of hepatitis B virus. Neither PML nor reactivation of hepatitis B virus have been observed to date with tisagenlecleucel. However, in other therapies associated with B cell aplasia these complications have been observed.

The safety of immunization with live viral vaccines during or following tisagenlecleucel treatment has not been studied. Vaccination with live virus vaccines is not recommended for at least 2 weeks prior to the start of lymphodepleting chemotherapy, during tisagenlecleucel treatment, and until immune recovery following treatment with tisagenlecleucel.

Pregnant women who have received tisagenlecleucel may have hypo/agammaglobulinemia. Immunoglobulin levels should be assessed in newborns of mothers treated with tisagenlecleucel.

7.1.4 Hypersensitivity Reactions

Hypersensitivity reactions may occur with an infusion of tisagenlecleucel. Serious hypersensitivity reactions, including anaphylaxis, may be due to the dimethyl sulfoxide (DMSO) or dextran 40 in tisagenlecleucel. Hypersensitivity prophylaxis should follow institutional standards, however steroids should not be given.

7.1.5 Serious Infections

Serious infections, including life-threatening or fatal infections, occurred in patients after tisagenlecleucel infusion. In the JULIET study, infections (all Grades) after tisagenlecleucel infusion occurred in 59% of the patients, including 35% with Grade 3-4 infections and 2 patients (3%) with fatal infections. In the JULIET study, infections (all Grades) after tisagenlecleucel infusion occurred in 34% of patients including 20% with grade 3-4 infections and 1 patient (1%) with a fatal infection. Prior to tisagenlecleucel infusion, infection prophylaxis should follow local guidelines, and after treatment the patient should be monitored for signs and symptoms of infection and treated appropriately.

Febrile neutropenia (Grade 3 or 4) was also observed in 37% of patients in JULIET and 17% of patients in JULIET. Febrile neutropenia may be concurrent with CRS. In the event of febrile neutropenia, the patient should be evaluated for infection and managed with broad-spectrum antimicrobials, fluids and other supportive care as medically indicated.

7.1.6 Cytopenias Note Resolved by Day 28

Patients may exhibit cytopenias for several weeks following lymphodepleting chemotherapy and tisagenlecleucel infusion. In JULIET, Grade 3 and 4 cytopenias not resolved by day 28 following tisagenlecleucel treatment included neutropenia (40%), and thrombocytopenia (27%) among responding patients. At 56 days following tisagenlecleucel, 17% and 12% of responding patients had Grade 3 and 4 neutropenia or thrombocytopenia, respectively.

In JULIET, Grade 3 and 4 cytopenias not resolved by day 28 following tisagenlecleucel treatment included neutropenia (24%), and thrombocytopenia (40%) among responding patients. At data cutoff 5% and 16% of responding patients had Grade 3 and 4 neutropenia or thrombocytopenia, respectively.

Prolonged neutropenia has been associated with increased risk of infection. Myeloid growth factors, particularly GM-CSF, are not recommended during the first 3 weeks after tisagenlecleucel infusion or until CRS has resolved.

7.1.7 Progressive Multifocal Leukoencephalopathy (PML)

PML is rare but well described with antibody therapies causing B cell aplasia[32]. It is a demyelinating disease of the central nervous system, resulting from infection of oligodendrocytes and astrocytes, mostly with JC virus. PML classically has a subacute clinical presentation with focal neurologic deficits, such as weakness, speech difficulties, unsteady gait and hemiparesis. Ophthalmic symptoms are relatively common, occurring as homonymous hemianopia which progresses to cortical blindness. Seizure and headache are uncommon. Dementia, manifesting as deficits in cognition, personality changes, and memory impairment are also common, but rarely occurs in the absence of the focal neurologic deficits of PML. Lesions identified by radiographic assessment are generally confined to the white matter with occipitoparietal lobe lesions without mass effect being most common.

In general, patients with known B cell aplasia are at increased risk for PML. Patients should be monitored at regular intervals for any new or worsening neurological symptoms or signs that may be suggestive of PML. The clinician should evaluate the patient to determine if the symptoms are indicative of neurological dysfunction, and if so, whether these symptoms are possibly suggestive of PML. Consultation with a neurologist should be considered as clinically indicated.

7.1.8 Uncontrolled T Cell Proliferation

Tisagenlecleucel transduced cells could theoretically proliferate without t h e control of normal homeostatic mechanisms. In pre-clinical studies and clinical experience to date tisagenlecleucel transduced cells have only proliferated in response to physiologic signals or upon exposure to CD19 antigen[33-35]. In the context of tisagenlecleucel therapy, it is expected that the T cells will proliferate in response to signals from the CD19 expressing malignant tumor and normal B cells. This could be beneficial or harmful depending on the extent of proliferation.

If uncontrolled T cell proliferation occurs (e.g., expansion of T cells in the absence of CD19 antigen), patients may be treated with corticosteroids such as methylprednisolone (2 mg/kg/d i.v.) or chemotherapy, such as high dose cyclophosphamide. Investigators should further discuss this with Novartis. Toxicity associated with allogeneic or autologous T cell infusions has been managed with a course of pharmacologic immunosuppression. T cell-associated toxicity has been reported to respond to systemic corticosteroids[36]. This theoretical toxicity is distinct from the toxicity associated with a CRS that develops during T cell proliferation upon exposure to CD19 expressing cells. CRS associated with T cell expansion is managed with anti-cytokine therapy, not immunosuppressants.

7.2 Adverse Events

Monitoring of AEs will be conducted from informed consent through the duration of the study. AEs for all subjects, will be recorded in the CRFs from the time informed consent is signed through discontinuation or completion of study.

For tisagenlecleucel T cells infused subjects who discontinue from the study for reasons other than withdrawal of consent and who do not enroll in the long-term follow up study, all ongoing AEs will be monitored until they are completely resolved or determined to be a stable or chronic condition, and no new AEs will be recorded unless they are related to study procedure or drug product, as determined by the Investigator.

For subjects who discontinue from the study after leukapheresis but before CAR T cells infusion, ongoing AEs will be followed up for 30 days post-discontinuation, and no new AEs will be recorded unless they are related to study procedure, as determined by the Investigator.

An AE is any untoward medical occurrence associated with the use of a drug in subjects, whether or not considered drug related. An AE may include a change in physical signs, symptoms, and/or clinically significant laboratory change occurring in any phase of a clinical study. This definition includes intercurrent illnesses or injuries, and exacerbation of pre-existing conditions. A pre-existing condition is a clinical condition (including a condition being treated) that is diagnosed before the subject signs the informed consent form and is documented as part of the subject's medical history.

Serious Adverse Events

An SAE is any AE occurring at any dose and regardless of causality that:

- Results in death.
- Is *immediately* life-threatening; i.e. the subject was at immediate risk of death at the time of the event. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.
- Requires in in-patient hospitalization or prolongation of existing in-patient hospitalization. Hospitalization admissions occurring during the study period that are for procedures *planned prior to study entry* do not meet these criteria, unless there is a complication resulting from procedure that prolongs hospitalization.
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a subject's ability to conduct normal life functions.
- Is a congenital anomaly/birth defect.
- Is an important medical event. An important medical event is an event that may not result in death, be life-threatening, or require hospitalization, but may be considered serious when, based upon appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed above

Medical Events of Special Interest (ESIs)

Grade 3 or greater CRS events and Grade 3 or greater events of neurologic toxicity are medical ESIs, and therefore immediately reportable to the Sponsor, even if the events do not meet SAE criteria.

All ESIs will be reported to the Sponsor within 24 hours of the Investigator's first knowledge of the event, even if the experience does not appear to be related to tisagenlecleucel T cells infusion. All ESIs should be reported to the Sponsor as specified in the SOM.

7.3 Adverse Event Characteristics

Adverse Event Assessment

For all AEs, the Investigator must determine both the severity of the AE and the relationship of the AE to tisagenlecleucel T cells treatment.

- **CTCAE term (AE description) and grade**: The descriptions and grading scales found in the revise 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

- **For expedited reporting purposes only:**
  - AEs for agents utilized in this study should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
  - Other AEs for the protocol that do not require reporting are outlined in the next section (Expedited Adverse Event Reporting) and under the sub-heading of Protocol-Specific Expedited Adverse Event Exclusions.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.4 Procedures for AE and SAE Collection and Reporting

7.4.1 Each subject must be carefully monitored for the development of any AEs. This information should be obtained in the form of non-leading questions (e.g., "How are you feeling?") and from signs and symptoms detected during each examination, laboratory assessments, observations of study personnel, and spontaneous reports from subjects. AEs for all subjects will be recorded in the AE CRF. Any clinically significant laboratory abnormality or other clinically significant findings is considered an AE and must be recorded on the appropriate pages of the CRF. Refer to the CRF Completion Guidelines for additional instructions on documenting AEs.

7.4.2 In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the Overall PI.

7.4.3 Investigators **must** report to the Overall PI any adverse event (AE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7.4.4 **Instructions for rapid notification of serious adverse events and Reporting responsibility**

Each serious adverse event (but not pregnancies) must be reported by the investigator to Novartis within 24 hours of learning of its occurrence, even if it is not felt to be treatment-related. Follow-up information about a previously reported serious adverse event must also be reported to Novartis within 24 hours of receiving it. If the serious adverse event has not been previously documented (new occurrence) and it is thought to be related to study drug (or therapy), the Medical Safety Expert of the Drug Safety & Epidemiology (DS&E) Department may contact the investigator to obtain further information. If warranted, an investigator alert may be issued, to inform all investigators involved in any study with the same drug (or therapy) that this serious adverse event has been reported.

7.4.5 Reporting Procedures

The investigator must complete the FDA MedWatch 3500a form and Novartis SAE coversheet in English, assess the relationship to study treatment and send the initial completed MedWatch form and Novartis SAE coversheet by fax 1.877.778.9739 or by email:clinicalsafetyop.phuseh@novartis.com, within 24 hours to the local Novartis Drug Safety & Epidemiology (DS&E) Department. The investigator must then ensure that the form and coversheet are accurately and fully completed with follow-up information and fax those to Novartis DS&E Department within 2 to 3 calendar days for deaths or life-threatening events and 5 calendar days for other serious adverse events. The original and the duplicate copies of the FDA MedWatch form, Novartis SAE coversheet, and the fax confirmation sheet must be kept with the case report forms at the study site.

Follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not, and whether the patient continued or discontinued study participation. The MedWatch form, Novartis SAE coversheet, and fax confirmation sheet must be retained. Pregnancy follow-up should describe the outcome of the pregnancy, including any voluntary or spontaneous termination, details of the birth,

and the presence or absence of any congenital abnormalities or birth defects. Additionally, specific events will trigger direct reporting to Novartis as below:

Interventional Clinical Trial WITH a Novartis IMP	
Collection by Sponsor	**Transfer to Novartis**
• All SAEs • All reports of drug exposure during pregnancy and pregnancy outcome. Pregnancy reports and outcomes should be collected for the female partners of any males who took tisagenlecleucel in this study, provided consent has been obtained from the female partner • All non-serious AEs • All reports of misuse and abuse of tisagenlecleucel, other medication errors and uses outside of what is foreseen in the protocol (irrespective if a clinical event has occurred)	**Within 24 hours of awareness:** • All neurotoxicity AEs with severity ≥Grade 4 • Death (including fatal SAEs or fatal disease progression) irrespective of causality, if it occurs within 30 days post tisagenlecleucel infusion. After 30 days, Death (including fatal SAEs or fatal disease progression) should be reported as SAE only, if there is at least a possible causal relationship to tisagenlecleucel **Within 15 days of awareness:** • All collected SAEs in subjects exposed to tisagenlecleucel • All collected pregnancy reports and outcomes in subjects exposed to tisagenlecleucel. Pregnancy reports and outcomes should be collected for the female partners of any males who took tisagenlecleucel in this study, provided consent has been obtained from the female partner • All collected reports of abuse and misuse of tisagenlecleucel, other medication errors and uses outside of what is foreseen in the protocol (irrespective if a clinical event has occurred) **In periodic 1-monthly batches** • All non-serious AEs

7.4.6 Pregnancies

Any pregnancy that occurs during study participation should be reported. To ensure patient safety each pregnancy must also be reported to Novartis within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects, congenital abnormalities or maternal and newborn complications.

Women of child bearing potential (WOCBP) and sexually active males must be informed that receiving the study treatment may pose unknown risks to the fetus if pregnancy were to occur during the study, and agree that in order to participate in the study they must adhere to the contraception requirements outlined in the exclusion criteria. If there is any question that the subject will not reliably comply, they should not be entered or continue in the study. WOCBP and sexually active males who receive tisagenlecleucel must adhere to contraception requirements for at least 12 months following tisagenlecleucel infusion and until CAR T cells are no longer present by qPCR on two consecutive tests.

Women of child-bearing potential must have a negative urine pregnancy test performed within 24 hours before leukapheresis, lymphodepletion and prior to tisagenlecleucel infusion. Additional pregnancy testing might be performed if requested by local requirements.

7.4.5 **DF/HCC Adverse Event Reporting Guidelines**

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

7.5 Reporting to the Food and Drug Administration (FDA)

The Sponsor of this study will be responsible for all communications with the FDA. The Sponsor will report to the FDA, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.6 Reporting to the NIH Office of Biotechnology Activities (OBA)

The Study sponsor will be responsible for all communications with the OBA. The Sponsor will report to the OBA, any serious adverse event that meets the OBA's criteria for expedited reporting following the reporting requirements and timelines set by the OBA.

If the dosing site is a recipient of funding from the National Institutes of Health (NIH) for recombinant research, the dosing site will ensure that an Institutional Biosafety Committee (IBC) is in place that is composed of at least 5 appropriately-qualified members. The IBC will ensure that the site conforms to the requirements set forth in the Section IV-B-2 of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, promulgated by the NIH/Office of Biotechnology Activities (NIH/OBA). The Investigator will be responsible for petitioning the IBC and obtaining approval prior to enrolling any subject in the study. The Investigator will also be required to obtain and follow all biohazard safety guidelines promulgated by the IBC, and to report all findings as required to the IBC and to NIH/OBA.

This protocol and any accompanying material provided to the subject (such as subject information sheets, Informed Consent Form, or descriptions of the study used to obtain informed consent) will be submitted by the Sponsor and/or the site to the legally constituted and chartered Institutional Biosafety Committee (IBC). The clinical site will be approved by the IBC in accordance with local procedures and country specific regulatory requirements. Documentation of IBC approval must be in place prior to product shipment to the site. At the discretion of the specific IBC and within federal requirements, IBC oversight of individual sites is suggested to be terminated provided (1) all subjects at that site have completed dosing by at least 90 days, and (2) all investigational materials have been fully accounted for and either returned to the Sponsor, destroyed on site, or shipped to a duly licensed destruction facility.

7.7 Reporting to the Institutional Biosafety Committee (IBC)

Participating investigators will register and report on research protocols involving biohazards (i.e., recombinant DNA or infectious agents) according to the reporting requirements set by their respective IBC.

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB/IEC and other appropriate institutional regulatory bodies will review all appropriate study documentation in order to safeguard the rights, safety, and well-being of the subjects. The study will only be conducted at sites where IRB/IEC and other appropriate institutional regulatory body approvals have been obtained. The protocols, informed consents, written information given to the subjects, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB/IEC and other appropriate institutional regulatory bodies by the Investigator.

7.8 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports, sentinel events or unanticipated problems that require reporting per institutional policy.

7.9 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agent administered in this study can be found in Section 7.1.

8.1 Study drug preparation and dispensation

Upon release from the manufacturing facility, the cryopreserved tisagenlecleucel cell product is shipped to the investigator. Upon receipt of the cryopreserved tisagenlecleucel cell product, and inventory must be performed, and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable tisagenlecleucel cell product in a given shipment will be documented in the study files. The investigator must notify the sponsor of any damaged or unusable tisagenlecleucel cell product that was supplied to the investigator's site.

After logging the tisagenlecleucel cells, they will be stored safely and properly (Section 8.1.2). Please note the time between product thawing and completion of the infusion should not exceed 30 minutes to maintain maximum product viability. Therefore, to ensure this timeframe, the product should be thawed in close proximity to the patient's bedside. Additionally, after cell thawing the tisagenlecleucel cell product should NOT be washed prior to infusion, all contents will be infused. If the tisagenlecleucel cell product appears to have a damaged or leaking bag, or otherwise appears to be compromised, it should not be infused, and should be returned to the

manufacturing facility. This issue should be documented properly, and the manufacturing facility should be notified on handling of the product.

In rare instances a patient's incoming apheresis or outgoaing product may not meet commercial release testing criteria. These cases will be discussed with Novartis on a case by case basis prior to proceeding forward with patient re-collection or infusion on commercially non-conforming product.

8.1.1 Study drug packaging and labeling

As tisagenlecleucel is an FDA approved product, packaging and labeling will follow commercial formatting and documentation.

8.1.2 Drug supply and storage

Tisagenlecleucel cell product must be received, handled and stored safely and properly by designated personnel at the site. Upon receipt, the tisagenlecleucel cell product should be stored according to the instructions specified on the product labels. Personnel receiving, handling and storing tisagenlecleucel cell product must complete REMS or RMP training accordingly.

8.1.3 Study drug disposal and destruction

Tisagenlecleucel cell product may require disposal for a variety of reasons, including but not limited to: 1) Mislabeled product; 2) Condition of patient prohibits infusion, and/or 3) Patient refuses infusion. Any unused product and all used infusion supplies, including the infusion bag and tubing, must be disposed of according to local institutional standard operating procedures.

In the event that tisagenlecleucel study cell product manufactured by Novartis was not shipped to the site for infusion, it will be managed per manufacturing facility process. The tisagenlecleucel product will either be utilized for research purposes or it will be destroyed.

8.1.4 Administration

The treating physician should evaluate the patient just prior to infusion to ensure that tisagenlecleucel infusion is clinically appropriate. Prior to tisagenlecleucel preparation, the patient identity must be matched with the patient identifiers on the tisagenlecleucel infusion bag. Tisagenlecleucel is for autologous use only. Employ universal precautions to avoid potential transmission of infectious diseases when handling the product.

The infusion bag must be inspected for any breaks or cracks prior to thawing. If the bag is compromised, the contents should not be infused and Novartis should be called at 1-844-4KYMRIAH.

The timing of tisagenlecleucel thaw and infusion should be coordinated. If more than one bag is being infused for the treatment dose, wait to thaw/infuse the bag until it is determined that the previous bag is safely administered. The infusion time should be confirmed in advance, and the start time for thaw adjusted so that tisagenlecleucel is available for infusion when the recipient is ready.

The infusion bag must be placed inside a second, sterile bag in case of a leak and to protect ports from contamination. Tisagenlecleucel should be thawed at 37°C using either a water bath or dry thaw method until there is no visible ice in the infusion bag. The bag will be removed from thawing device immediately; and the product bag should not be stored at 37°C. Once thawed and at room temperature (20°C to 25°C), it should be infused within 30 minutes.

Tisagenlecleucel should not be washed, spun down, and/or re-suspended in new media prior to infusion. The contents of the thawed infusion bag should be inspected for any visible cell clumps. If visible cell clumps remain, the contents of the bag can be gently mixed. Small clumps of cellular material should disperse with gentle manual mixing. Tisagenlecleucel should NOT be infused if clumps are not dispersed, the infusion bag is damaged or leaking, or otherwise appears to be compromised. Call Novartis at 1-844-4KYMRIAH.

Tisagenlecleucel infusion should be performed using precautions for immunosuppressed patients. Protective isolation should follow institutional standards and policies. Emergency medical equipment should be available during the infusion in case the patient has a significant reaction to the infusion such as anaphylaxis or severe hypotension.

The site must confirm that two doses of tocilizumab are on site and available for administration prior to tisagenlecleucel infusion.

The tisagenlecleucel dose will be administered via intravenous (IV) infusion through a latex free i.v. tubing WITHOUT a leukocyte filter (approximately 10 – 20 mL per minute adjusted as appropriate for smaller children and smaller volumes). The volume in the infusion bag ranges from 10-50mL. All contents of the infusion bag should be infused. If more than one bag is being infused for the treatment dose, wait to thaw/infuse the bag until it is determined that the previous bag is safely administered. It is recommended that the infusion should be completed within 30 minutes of thawing the cryopreserved product. The tubing should be primed with saline and the setup should also contain a Y-arm with an attached supplemental saline bag to be used after the initial infusion is completed. This will allow any remaining product left behind within the bag and tubing to be recovered and infused while maintaining a closed tubing system. Cells from all bag(s) should be infused to complete a single dose.

Vital signs (temperature, respiration rate, pulse, pulse oximetry, and blood pressure) will be taken prior to, during and immediately after the infusion and then approximately every 15 minutes for one hour and repeated at 2 hours. If vital signs are unsatisfactory and unstable, continue to monitor the patient until vital sign stabilization.

Tisagenlecleucel contains human cells genetically modified with a lentivirus, all used infusion supplies, including the infusion bag and tubing, must be handled and disposed of according to local institutional biosafety standard operating procedures.

Following tisagenlecleucel infusion: Should emergency treatment be required in the event of life-threatening hypersensitivity or other acute infusion-related reaction, supportive therapy such as oxygen, bronchodilators, epinephrine, antihistamines, and corticosteroids should be given according to local institutional guidelines. Patients should be evaluated and carefully monitored until complete resolution of signs and symptoms. Patient or patient's caregiver should monitor the patient's temperature four times daily for the first 14 days post tisagenlecleucel infusion. The patient or patient's caregiver should be instructed to call the treating physician promptly with any signs and symptoms of CRS or neurologic toxicities for possible hospitalization.

Patients and their caregivers should plan to stay within 30 miles of the treatment site for at least 4 weeks after tisagenlecleucel infusion, unless otherwise indicated by the treating physician.

While patients are admitted, and at each study visit, ICE/ICANS assessments will be performed.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies

N/A

9.2 Laboratory Correlative Studies

The persistence, biodistribution and immunologic effects of tisagenlecleucel T cells will be evaluated using peripheral blood samples. For molecular studies (Q-PCR), immune phenotyping and functional assays, peripheral blood will be collected in Lavender top (K2EDTA) tubes. For cytokine analyses peripheral blood samples will be collected in red top (no additive) tubes. CSF will be stored and clear vials and processed as soon as possible.

Samples will be collected per the protocol Schedule of Events (see **Section 10.1 Schedule of Events**).

If tumor tissues, bone marrow aspirates, and/or CSF become available as part of routine clinical care, a sample will be collected for research analysis. Tissue samples will be analyzed for the presence of tisagenlecleucel T cells by PCR and/or flow cytometry. Peripheral blood samples may also be analyzed for cytokine levels by Luminex technology. Tumor tissue will be analyzed for CD19 expression by immunohistochemistry and/or flow cytometry.

All research samples will be delivered, processed, and frozen as per SOP to the Maus/Immune monitoring Laboratory at the MGH Cancer Center for storage and bulk analyses. Documentation

of sample receipt, processing, and storage and primary data from the research analyses will be collected and stored in the processing lab personnel. All research analyses will be performed based on assay-specific SOP using qualified and, if possible, validated assays.

10. STUDY CALENDAR

10.1 Schedule of Events

Evaluations and procedures identified in the Schedule of Events may be performed at unscheduled visits, as clinically indicated, at the Investigator's discretion in consultation with the Sponsor.

Study Procedures
The study consists of 1) a screening phase, 2) followed by an intervention/treatment phase consisting of infusion of tisagenlecleucel T cells and 3) follow up. Schedule of evaluations and infusion is included in **Section 10.1.**

Subject Screening and Registration

Subjects willing to participate in the study will provide written informed consent according to Good Clinical Practice (GCP). Written informed consent must be obtained before the conduct of any Screening tests.

Upon signing the informed consent and a manufacturing slot is confirmed, the subject will be registered and assigned a unique subject number. Once a subject number has been assigned, it cannot be reused, and the number stays with the subject even if the subject is subsequently determined to be ineligible for the study.

Leukapheresis must occur ≤28 days after the start of assessments in Screening. Thus, if re-screening is necessary:

Informed Consent and Demographics need not be repeated

Only new Medical History and Disease History since previous screening will be collected

All other assessments in Table 2 for enrollment must be repeated.

The subject will maintain their originally assigned unique subject number.

Re-screening of assessments during Baseline may be performed at the discretion of the Investigator and Sponsor.

Pre-leukapheresis evaluation

The following tests must be performed within four weeks (except where noted) prior to leukapheresis:

- Medical history including prior treatment, current medications, and drug allergies.

- Physical exam including vital signs, pulse oximetry, height, weight, and ECOG performance status
- Laboratory studies including CBC with differential, coagulation factors (INR/aPTT, d-dimer and fibrinogen), comprehensive metabolic panel (includes sodium, potassium, bicarbonate, BUN, creatinine, glucose, total bilirubin, alkaline phosphatase, AST, ALT, albumin, calcium, magnesium, phosphorus, LDH, uric acid) and inflammatory markers (CRP and ferritin)
- Serum pregnancy test (females of childbearing potential)
- Baseline IDM Panel – (HIV/HCV/HBV serology, and EBV/CMV serology)
- 12 lead electrocardiogram (EKG)
- Baseline echocardiogram or MUGA scan (to be completed within 4 weeks of leukapheresis).

Additional information regarding subject eligibility for this trial is listed in **Section 3**.

Screen Failures

Subjects are screen failures if:

they cannot finish assessments for Screening or are ineligible based on those assessments,

they meet eligibility criteria during Screening but do not undergo leukapheresis

Data collected on Screen Failure subjects will only include:

Demography

Eligibility

Note: Subjects who discontinue from the study after leukapheresis but prior to infusion with tisagenlecleucel T cells will be considered withdrawals. All study data collected through the point of withdrawal will be captured for subjects who discontinue early.

Long-Term Follow-up Phase

Patients will be transitioned to the long-term follow-up (LTFU) phase of this protocol following disease progression and/or completion of the 24-month active treatment phase to maintain compliance with the requirements for subjects receiving genetically modified cells containing lentiviral vectors under IND. Subjects will be followed for up to 15 years and will be asked to participate in a separate 15-year registry study, such as CIBMTR. For subjects who consent to the registry study, data for LTFU will be collected with the registry study.

Table 2: Schedule of Study Procedures and Assessments – Active Treatment Phase

Assessment	~ Wk (-) 6 to 4[15]	~ Wk (-) 4[15]	~Week (-) 2-1[21]	~ Day (-) 5-3[17]	~ Day (-) 1*	D 0*	D+1*	D+2[$] (+1d)	D+4[$] (+ 2d)	D+7 (+/- 2d)	D+10 (+/-2d)	D+14 (+/-3d)	D+21 (+/- 3d)	D+28 (+/-3d)	Monthly to 6 mo. (+/-2 wk)	Quarterly to Year 2[25] (+/- 1 mo)
	Screening	Apheresis	Re-Staging	LD Chemo-therapy	Pre –Infusion	Infusion	Post Infusion	Follow-up	Follow-up	Follow-up	Follow-up	Follow-up	Follow-up	Follow up	Response Endpoint/LP at 1, 3, 6 and 12 months and Follow - up	
Clinical Assessments																
Inform Consent	X															
Vital signs	X	X		X	X	X[1]	X	X	X	X	X	X	X	X	X	X
Recent Med. History, Physical Examination	X			X	X	X[11]	X	X	X	X	X	X	X	X	X	X
ECOG	X			X		X										
Concomitant Medications	X			X	X	X[11]	X	X	X	X	X	X	X	X	X	X
Venous Access Assessment	X															
Clinical Disease Staging/ Disease monitoring (PET/CT/MRI)/Tumor response assessments[4]	X		X											X	X	X
Lumbar Puncture[3] (cytology/flow)	X		X							X				X	X	X
ECHO/MUGA	X[13]															
EKG	X															
Patient monitoring[16]						X	X	X	X	X	X	X	X	X	X	X
AEs/SAEs						X[11]	X	X	X	X	X	X	X	X	X	X
ICE/ICANS assessment						X	X	X	X	X	X	X	X	X	X	X
Neurocognitive Assessments[18]	X				X[18]									X	X[18]	
Clinical Labs Testing																
Blood for Serum pregnancy test[2] (1 ml SST)	X															
Urine pregnancy test[2]		X		X	X											

Assessment	~ Wk (-) 6 to 4[15]	~ Wk (-) 4[15]	~Week (-) 2-1[21]	~ Day (-) 5-3[17]	~ Day (-) 1*	D 0*	D+1*	D+2$ (+1d)	D+4$ (+ 2d)	D+7 (+/- 2d)	D+10 (+/-2d)	D+14 (+/-3d)	D+21 (+/- 3d)	D+28 (+/-3d)	Monthly to 6 mo. (+/-2 wk)	Quarterly to Year 2[5] (+/- 1 mo)
	Screening	Apheresis	Re-Staging	LD Chemo-therapy	Pre –Infusion	Infusion	Post Infusion	Follow-up	Follow-up	Follow-up	Follow-up	Follow-up	Follow-up	Follow up	Response Endpoint/LP at 1, 3, 6 and 12 months and Follow - up	
Blood CBC, differential (5 ml EDTA)	X			X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Chemistry/Metabolic Panel[12] (3 ml SST)	X			X	X	X	X	X	X	X	X	X	X	X	X	X
Blood for CD3/CD4/CD8/CD19 FLOW (4ml lav)	X				X	X					X			X	X[6]	X[6]
Serology: IDM Panel (5ml)	X															
Coagulation Factors (PT, PTT, INR, fibrinogen, D-dimer)	X				X	X	X	X	X	X	X	X	X	X	X	X
Inflammatory markers (CRP, Ferritin)	X				X	X	X	X	X	X	X	X	X	X	X	X
Intervention																
Leukapheresis		X[8]														
Lymphodepletion				X												
Tisagenlecleucel infusion						X										
Translational lab testing peripheral blood mononuclear cells 25 cc (Lavender)[10, 14]																
DNA/VCN (qPCR)[9]					X	X	X	X	X	X	X	X	X	X	X	X
RCL testing[9]					X									X	X[20]	X[20]
PBMC (functional assays, Tisagenlecleucel T cells immunophenotyping, etc)				X[22]	X	X[14]	X	X	X	X	X	X	X	X	X	X
Translational lab testing peripheral blood serum 5 cc (Red)[7, 10]																
Multiplex cytokines				X[22]	X	X[7]	X	X	X	X	X	X	X	X	X	X
Translational lab testing CSF 4 cc black top[10]																

Assessment	~ Wk (-) 6 to 4[15]	~ Wk (-) 4[15]	~Week (-) 2-1[21]	~ Day (-) 5-3[17]	~ Day (-) 1*	D 0*	D+1*	D+2$ (+1d)	D+4$ (+ 2d)	D+7 (+/- 2d)	D+10 (+/-2d)	D+14 (+/-3d)	D+21 (+/- 3d)	D+28 (+/-3d)	Monthly to 6 mo. (+/-2 wk)	Quarterly to Year 2[5] (+/- 1 mo)
	Screening	Apheresis	Re-Staging	LD Chemo-therapy	Pre –Infusion	Infusion	Post Infusion	Follow-up	Follow-up	Follow-up	Follow-up	Follow-up	Follow-up	Follow up	Response Endpoint/LP at 1, 3, 6 and 12 months and Follow - up	
Multiplex cytokines			X							X				X	X	X
Immune cell subset immunophenotyping[3, 19]			X							X				X	X	X

* The Clinical Investigator will review all re-and post-infusion lab results to determine that it is appropriate to proceed with the infusion. Any abnormal results and that is a change from the previous value will be reviewed by the PI prior to the infusion. Any changes in lab results from the prior value will be reviewed by investigators.
$ Translational lab testing that falls on weekends, and out of study window, will not be collected or considered a deviation.

1. Vital signs should be taken prior to CAR T cells infusion, once during the infusion, once at the end of infusion, and then approximately every 15 minutes for one hour and repeated at 2 hours.
2. Pregnancy testing – for females of childbearing potential only (not required if documentation of menopause/hysterectomy ie. surgical, chemo induced, physiologic, or etc.)
3. May deferred if not clinically safe.
4. Baseline imaging for tumor response assessments will be done within 4 weeks of infusion. Imaging for tumor response assessments post Tisagenlecleucel T cell infusion will be done after months 1, 3, 6 and 12. Tumor response assessment criteria is described in Section 11. MRI studies should be performed with perfusion imaging if possible.
5. After completion of the main study protocol (up to 2 years), subjects will be enrolled into a designated long term follow up protocol for up to 15 years post Tisagenlecleucel T cell infusion. Subjects will be monitored for delayed adverse events associated with CAR T lentiviral gene therapy.
6. Blood for CD3/CD4/CD8/CD19 will be taken at 3, 6, 12, 18 at 24 months.
7. Blood for research testing (5 cc red top) will be taken prior infusion and between 20-120 minutes post-infusion.
8. 12-15 liter apheresis product will be delivered to Novartis.
9. These samples will be banked for later analysis.
10. Samples should be delivered to the testing labs as soon as possible. If required to keep samples after hours, please keep red top tubes upright, lavender top tubes should be kept at room temperature on rotating platforms. CSF should be kept at room temp and processed as soon as possible. In the event of unexpected events, research samples collection may be done as necessary. This should be done at the PI's discretion.
11. Medical history, physical exams and concomitant medications information need to be collected prior to T cells infusion on Day 0. Any adverse events will be collected from the starting the day of infusion of the Tisagenlecleucel T cell.
12. Laboratory studies using comprehensive metabolic panel (includes sodium, potassium, bicarbonate, BUN, creatinine, glucose, total bilirubin, direct bilirubin, alkaline phosphatase, AST, ALT, albumin, calcium, magnesium, phosphorus, LDH, uric acid, CPK). (See Section 10.2 for details)
13. Baseline echocardiogram or MUGA scan (to be completed within 6 weeks of planned CAR T cell infusion).
14. PBMC samples are only required before CAR-T infusion, multiplex cytokines should be drawn before CAR-T infusion and 20-120 min after infusion.
15. The intent of these windows is to ensure subjects are infused within 6 weeks of enrollment. If the subject completes these visits within a shorter timeframe than 6 weeks, this will not be considered a protocol violation/deviation.
16. Patient monitoring will occur from Day 0 through Day 7 post tisagenlecleucel infusion excluding weekends and holidays, and should include a physical exam and vital signs every 8 hours

while admitted unless otherwise clinically indicated. After discharge subjects must take temperature through D +28, and contact their treating investigator for any fever ≥100.0° F which will require hospitalization until the subject has been afebrile for 18 hours; Subjects must remain within 30 miles of site.

17. Only vital signs are required on D-4, and D-3 of lymphodepletion. Medical history, physical exam, ECOG, and concomitant medications are required D-5 only.
18. Neurocognitive assessments will follow IPCG guidelines and will be obtained D0, day 28 and month 6.
19. FLOW phenotyping and RNA profiling will be performed when samples allow.
20. RCL testing to be performed at baseline, 1 month, 3, 6, 9, 12 months, bi-annually thereafter up until year 2 and transition to LTFU phase.
21. Re-staging assessments may extend beyond the defined 1-2 week window, including up to the LDC window as clinically indicated.
22. PBMC and multiplex cytokines only need to be drawn on D-5 prior to initiation of lymphodepleting chemotherapy.

10.2 Study Assessments

Tumor cells CD19 Expression and Tumor Burden

Demographics and Medical History

Demographic data includes gender, age, race, and ethnicity.

A complete medical history should include all relevant prior and current medical history, and should also include anti-cancer therapies, including start and end dates of prior therapies, best response, date of progression or relapse, and reason for progression.

Physical Examination and Vital Signs

A physical examination includes general appearance; head eyes, ears, nose, and throat; cardiovascular; dermatologic, abdominal; genitourinary; lymph nodes; hepatic; musculoskeletal; respiratory; and neurological, and weight. Height is also to be measured at Screening.

Vital signs include systolic/diastolic blood pressure, pulse, respiration rate, and temperature. On the day of infusion, vital signs should be taken prior to Tisagenlecleucel T cells infusion, once during the infusion, once at the end of infusion, and then hourly for 4 hours.

Echocardiogram will also be performed.

Performance Status

Eastern Cooperative Oncology Group (ECOG) performance status assessment is to be assessed according to the Schedule of Events.

Response assessments will be made according to IPCG Consensus guidelines.

Laboratory Tests

Clinical Laboratory Tests

Clinical laboratory tests (Table 3) are to be performed by the local laboratory and reviewed by the Investigator or qualified designee (e.g., physician's assistant, nurse practitioner).

Table 3: Clinical Laboratory Tests

Hematology	Serum Chemistry	Coagulation	Enzymes & Liver Studies
CBC with differential ferritin fibrinogen	sodium potassium, chloride bicarbonate creatinine glucose blood urea nitrogen calcium uric acid phosphate magnesium C-reactive protein Creatinine phosphokinase	prothrombin time (PT)/ partial thromboplastin time (PTT), international normalized ratio (INR), fibrinogen, d-dimer	AST ALT alkaline phosphatase total and direct bilirubin albumin LDH

Abbrev.: CBC, complete blood count; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase

CBC includes hematocrit, hemoglobin, red blood cell (RBC) count, white blood cell (WBC) count with differential, and platelet count.

Blood will also be collected for analysis of T cell subsets, including CD4+ and CD8+ cells.

Additional clinical laboratory tests may be performed at the Investigator's discretion.

Additional Eligibility-Determining Laboratory Tests

During Screening, blood samples will be collected for additional eligibility-determining laboratory tests, as follows:

Serology

Screening serology will be evaluated using standard methods. The serology panel should include the following:

- HIV-1 and HIV-2
- hepatitis B virus core antibody (HBcAb)
- hepatitis B virus surface antibody (HBsAb)
- hepatitis B virus surface antigen (HBsAg)
- hepatitis C virus (HCV) antibody
- HCV RNA
- Cytomegalovirus (CMV) antibody
- Epstein-Barr Virus (EBV) antibody

Blood may also be drawn for additional serology testing if subject has risk factors or clinical evidence of infection with other communicable disease agents or disease.

Serology should be performed within 28 days prior to leukapheresis. Additional serology may be performed if required according to country-specific and institutional guidelines.

Serum β-human chorionic gonadotropin pregnancy test

Required for women of child-bearing potential.

Blood, Bone Marrow, Tissue and CSF Analysis for CAR and CD19

CAR+ T cells and CD19+ cells will be quantified in peripheral blood, bone marrow, tumor biopsies and CSF when possible.

Soluble factors/Multiplex Cytokines

Serum will be analyzed for GM-CSF, IL-1β, IL-2, IL-6, IL-8, IFN-γ, IL-10, IL-12p70, MCP-1, and TNFα, in addition to other cytokines as identified. Quantification of markers of endothelial activation, including d-dimer, PTT, INR, ANG1, ANG2, gal-3, VWF multimers, may also be examined as exploratory studies.

Blood Collection for Research

Blood will be collected and stored for potential analyses of B cell subtypes, T cell subtypes, and/or markers of T cell exhaustion.

CAR T Cell Phenotyping

Blood will be collected and analyzed for CAR T cell phenotyping, which includes the analysis of CAR T cell subsets and markers of memory, activation, and trafficking.

Gene Expression

Whole blood, bone marrow aspirate or tissue biopsies will be collected and may be analyzed for gene expression per standard SOPs and according to the NIH standards to avoid uncover single nucleotide polymorphisms (SNPs) that are unique to individuals.

Tissue Collection

Blood, Bone Marrow, CSF and Tumor for Future Research

Aside from protocol-specified bone marrow aspirate and biopsies, additional bone marrow and tissue samples may be collected outside the Schedule of Events and is encouraged in the event of unexpected clinical findings such as toxicity or delayed response. These samples, as well as leftover samples from protocol-specified procedures, may be used for biomarker analyses of proteins, DNA, RNA, and other molecules to study PCNSL, and/or gene therapy. Such samples may be stored until the samples are exhausted or until the repository is discontinued. The Sponsor will be the custodian of the samples in the repository and any unused samples will be destroyed at the Sponsor's discretion.

Collection and storage of the samples described above will be subject to discretionary approval from each center's Institutional Review Board (IRB) / Independent Ethics Committee (IEC) and the subject's specific written consent. Samples will be labeled with a unique identification number that includes no subject identifying information.

Note that leukapheresis product collected as part of the manufacture of the drug product may be used to study the manufacturing process. In particular extra leukapheresis product may be used to understand how the process may be improved or made more robust. These potential studies are not optional.

11. MEASUREMENT OF EFFECT

Although response is not the primary endpoint of this trial, participants with measurable disease will be assessed by standard criteria for their disease as would any large cell lymphoma patient treated with CAR-T. For the purposes of this study, participants should be assessed for response at day 28, 3 months, 6 months, 12 months and 24 months after tisagenlecleucel T cell infusion as is standard for commercial tisagenlecleucel. Staging imaging will be performed via MRI imaging of the brain with perfusion imaging. Additionally, lumbar punctures will be performed at these same time points as this is required for complete response assessment per IPCG consensus guidelines (Appendix C). MRI imaging will be performed with perfusion imaging.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the Office of Data Quality (ODQ) in accordance with DF/HCC policies.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of medical oncologists, research nurses, pharmacists and biostatisticians with direct experience in cancer clinical research. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year with the frequency determined by the outcome of previous reviews. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days of intervention and summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Collaborative Agreements Language

"N/A"

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

General Design Issues
This is a pilot study to evaluate the safety and tolerability, and persistence and engraftment of tisagenlecleucel T cells in primary CNS lymphoma. Failure to complete the study due to the stopping rules (described in Section 5.7) being invoked will be the main basis for determining safety of this study. This study is primarily intended to provide data to be used to plan a preliminary study to assess safety and feasibility.

Upon enrollment, patients will undergo baseline tumor assessment, and research blood samples will be collected. At dosing, patients will receive redirected autologous T cells against CD19 (Tisagenlecleucel T cells).

Patients will be monitored according to the Schedule of Events (Section 10.1). Observation and monitoring of patients will continue through week 24 post dosing, and every three months until two years.

This study aims to evaluate 12 patients from either treatment group. All subjects who are infused with Tisagenlecleucel T cells will be evaluated for safety.

Primary Endpoints
The primary endpoint of this study is rate and grade of adverse events including (but not limited to) cytokine release syndrome and neurotoxicity per 2018 ASBMT consensus criteria[8]. The primary objective is to assess the safety of tisagenlecleucel in patients with PCNSL. Safety will be evaluated based on monitoring of the occurrence of study related adverse events that are "possibly", "likely", or "definitely" related to study treatment any time from the first day of study treatment until Month 12 while the subject is enrolled on this study.

Secondary Endpoints

The secondary endpoint of this study is the proportion of patients with PCNSL with objective disease response to tisagenlecleucel per IPCG response criteria.

This will be assessed by evaluation of disease specific response at months 1, 3, 6, and 12.

Patients who are lost to follow up without a known date of progression or death due to any cause will be censored at the date of their last available tumor assessment.

Exploratory Endpoints

The exploratory objectives are to assess long-term efficacy of tisagenlecleucel in patients with PCNSL, to evaluate the expansion, persistence, and phenotype, of tisagenlecleucel T cells following infusion, evaluate cytokine profiles in the peripheral blood and CSF of subjects after infusion of tisagenlecleucel, to assess impacts of tisagenlecleucel on quality of life and neurocognitive function post infusion, to assess for correlative markers of tumor response/progression. Endpoints to include: a) to evaluate the overall and progression free survival of patients post tisagenlecleucel, 2) detection and quantification of tisagenlecleucel and tumor specific RNA/DNA in biopsies, the peripheral blood and CSF 3) Assessment of cytokine/chemokine profiles in peripheral blood and CSF, including IL-2, IL-6, IL-10, IFNγ, TNFα etc. 4) longitudinal neurocognitive evaluation as per IPCG guideline per published schedule, 5) when available, analyze pre/post tumor samples for gene and immune-profile.

13.2 Sample Size, Accrual Rate and Study Duration

This is a pilot study to assess tisagenlecleucel in PCNSL. A larger follow-up study may be designed pending results from this initial cohort.

Subjects will be followed for AEs, clinical status, and laboratory parameters for up to 24 months after the infusion of Tisagenlecleucel T cells, unless they terminate early due to disease progression, or withdraw for a different reason. All subjects who receive Tisagenlecleucel T cells will be will be transitioned to the long-term follow-up (LTFU) phase of this protocol following disease progression and/or completion of the 24-month active treatment period to maintain compliance with the requirements for subjects receiving genetically modified cells containing lentiviral vectors under IND. Subjects will be followed for up to 15 years and will be asked to participate in a separate 15-year registry study, such as CIBMTR. For subjects who consent to the registry study, data for LTFU will be collected with the registry study. We anticipate accruing the study population within 12 months.

13.3 Statistical Analysis

The statistical analysis will be primarily descriptive in keeping with the exploratory nature of the study. Incidence of CRS and/or NT will be tabulated, by grade and treatment attribution. Time to these AEs will also be analyzed. Summary statistics will be calculated (median, mean, range for continuous variables; frequency and percentage for discrete variables). Time to event outcomes

will be estimated using Kaplan Meier method or cumulative incidence curves. Only patients who are infused will be analyzed in subsequent data analysis.

13.4 Stratification Factors

N/A

13.5 Interim Monitoring Plan

No interim analysis is planned due to the small sample size.

13.6 Analysis of Primary Endpoints

The frequency, grade, and duration of all adverse events that are at least "possibly" related to the study drug will be listed and summarized.

13.7 Analysis of Secondary and Exploratory Endpoints

The number and proportion of patients with objective disease response at month 1, 3, 6 and 12 will be reported. Overall survival and progression-free survival will be plotted and summarized using the Kaplan-Meier method. Additional exploratory analyses will be primarily descriptive, with results presented graphically and/or as summary statistics.

13.8 Reporting and Exclusions

13.8.1 **Evaluation of Toxicity**

All participants will be evaluable for any drug related toxicity from the time of their first treatment. Adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). All subjects who initiate any study procedures will be included in the assessment of safety. Subject incidence rates of AEs, including SAEs, fatal and treatment related AEs will be reported through the conduct of the study. Changes in clinical laboratory test results, vital signs and physical examination findings will be summarized with descriptive statistics as appropriate. Laboratory parameters will be summarized for changes across study by using descriptive statistics including shifts relative to CTCAE criteria for laboratory abnormalities. Laboratory measures will also be compared with their corresponding normal ranges and the incidence of abnormally high and abnormally low laboratory values will be calculated for each relevant protocol-specified laboratory test. Laboratory values that are of Grade 3 severity or greater will be tabulated by dose and listed on an individual subject basis.

All treated subjects will be followed for overall survival for up approximately 15 years after T cell infusion.

13.8.2 **Evaluation of the Primary Efficacy Endpoint**

The primary endpoint for this trial will be related to safety, including the incidence of and maximal grade of CRS and neurotoxicity.

ADMINISTRATIVE REQUIREMENTS

13.9 Good Clinical Practice

The study will be conducted in accordance with the International Conference on Harmonization (ICH) Guideline for GCP and the appropriate regulatory requirement(s). The Investigator will be thoroughly familiar with the appropriate use of Tisagenlecleucel as described in the protocol. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files will be established at the beginning of the study, maintained for the duration of the study and retained per the appropriate regulations.

13.10 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB/IEC and other appropriate institutional regulatory bodies will review all appropriate study documentation to safeguard the rights, safety, and well-being of the subjects. The study will only be conducted at sites where IRB/IEC and other appropriate institutional regulatory body approvals have been obtained. The protocols, informed consents, advertisements (if applicable), written information given to the subjects, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB/IEC and other appropriate institutional regulatory bodies by the Investigator.

13.11 Biosafety Considerations

If the dosing site is a recipient of funding from the National Institutes of Health (NIH) for recombinant research, the dosing site will ensure that an Institutional Biosafety Committee (IBC) is in place that is composed of at least 5 appropriately-qualified members. The IBC will ensure that the site conforms to the requirements set forth in the Section IV-B-2 of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, promulgated by the NIH/Office of Biotechnology Activities (NIH/OBA). The Investigator will be responsible for petitioning the IBC and obtaining approval prior to enrolling any subject in the study. The Investigator will also be required to obtain and follow all biohazard safety guidelines promulgated by the IBC, and to report all findings as required to the IBC and to NIH/OBA.

This protocol and any accompanying material provided to the subject (such as subject information sheets, Informed Consent Form, or descriptions of the study used to obtain informed consent) will be submitted by the Sponsor and/or the site to the legally constituted and chartered Institutional Biosafety Committee (IBC). Additional materials may be submitted to the IBC according to the specific Committee and federal (United States' National Institutes of Health or foreign equivalent) requirements. Documentation of IBC approval must be in place prior to product shipment to the

site. At the discretion of the specific IBC and within federal requirements, IBC oversight of individual sites is suggested to be terminated provided (1) all subjects at that site have completed dosing by at least 90 days, and (2) all investigational materials have been fully accounted for and either returned to the Sponsor, destroyed on site, or shipped to a duly licensed destruction facility.

13.12 Subject Information and Informed Consent

After the study has been fully explained, written informed consent will be obtained from the subject or his/her legal representative prior to study participation. The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

13.13 Subject Confidentiality

In order to maintain subject privacy, all CRFs, accountability records, study reports, and communications will identify the subject by initials and the assigned subject number. The Investigator will grant monitor(s) and auditor(s) from the Sponsor or its designee and regulatory authority(ies) access to the subject's original medical records for verification of data gathered on the CRFs and to audit the data collection process. The subject's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

13.14 Protocol Compliance

The Investigator will conduct the study in compliance with the protocol provided by the Sponsor, and given approval/favorable opinion by the IRB/IEC and other appropriate institutional regulatory bodies. Modifications to the protocol should not be made without agreement of both the Investigator and the Sponsor. Changes to the protocol will require written IRB/IEC and other appropriate institutional regulatory body approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to subjects. The IRB/IEC may provide, if applicable regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval /favorable opinion of the IRB/IEC and other appropriate institutional regulatory bodies. The Sponsor will submit all protocol modifications to the regulatory authority(ies) in accordance with the governing regulations.

When immediate deviation from the protocol is required to eliminate an immediate hazard(s) to subjects, the Investigator will contact the Sponsor, if circumstances permit, to discuss the planned course of action. Any departures from the protocol must be fully documented in the CRF and source documentation.

13.15 Direct Access to Source Data

Monitoring and auditing procedures developed by the Sponsor will be followed, in order to comply with GCP guidelines.

The study will be monitored by the Sponsor or its designee. Monitoring will be done by personal visits from a representative of the Sponsor (site monitor) and will include on-site review of the CRFs for completeness and clarity, cross-checking with source documents, and clarification of

administrative matters will be performed. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.

The site monitor will ensure that the investigation is conducted according to protocol design and regulatory requirements by frequent communications (letter, e-mail, telephone, and fax).

Regulatory authorities, the IRB/IEC and other appropriate institutional regulatory bodies, and/or the Sponsor may request access to all source documents, CRFs, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities.

13.16 Case Report Form Completion

CRFs will be completed for each study subject. It is the Principal Investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the subject's CRF. Source documentation supporting the CRF data should indicate the subject's participation in the study and should document the dates and details of study procedures, AEs, and subject status.

The Principal Investigator or designated representative should complete the CRF screens as soon as possible after information is collected, preferably on the same day that a study subject is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

The Principal Investigator must sign and date the Investigator's Statement at the end of the CRF to endorse the recorded data.

For data to be collected on screen fails and early withdrawal, see Section 10.1.

13.17 Record Retention

The Investigator will maintain all study records according to ICH-GCP and applicable regulatory requirement(s). Records will be retained for at least 2 years following marketing application approval or 2 years after formal discontinuation of the clinical development of the investigational product or per applicable regulatory requirement(s). If the Investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. The Sponsor must be notified in writing if a custodial change occurs.

The Sponsor has full rights over any invention, discovery, or innovation, patentable or not, that may occur when performing the study.

13.18 Liability and Insurance

The Sponsor has subscribed to an insurance policy covering, in its terms and provisions, its legal liability for injuries caused to participating persons and arising out of this research performed strictly in accordance with the scientific protocol as well as with applicable law and professional standards.

14. PUBLICATION PLAN AND PRESENTATION OF THE STUDY FINDINGS

It is anticipated that the results of this study will be presented at scientific meetings and/or published in a peer reviewed scientific or medical journal.

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.

APPENDIX B: CYTOKINE RELEASE SYNDROME (CRS) AND NEUROTOXICITY MANAGEMENT

Monitoring and Management of late reactions following immune effector cell infusion

Monitoring: Patients will be assessed at a minimum of every 8 hours for signs and symptoms of CRS and graded per modified Lee criteria described in table 6. The following labs will be performed at least daily: CBC w/diff, CMP, Phos, LDH, PT/INR, PTT, fibrinogen, d-dimer, CRP, ferritin, and with increased frequency as clinically indicated. Grading of ICANS will be assessed per Lee 2019 described in tables 9 & 10.

Management: In the event that toxicity is suspected, patients will be placed on continuous telemetry including continuous O_2 monitoring. Vital checks will be assessed at an increased frequency as clinically indicated and supportive measures including fluid management, respiratory support, and vasopressors/inotropic support will be provided. Patients with rapid clinical decline, extensive comorbidities, or older age, will be evaluated by ICU triage. Patients with fevers/febrile neutropenia will be treated as septic/febrile neutropenia with broad spectrum antibiotics. Specific infectious prophylaxis will also be started at time of lymphodepletion per clinical discretion until resolution of symptoms. Patients with neurologic toxicity will be managed in collaboration with neuro-oncology.

Pharmacologic therapy may include:

1. Anti-IL6 therapy can be administered as clinically indicated per table 7 & 8.
2. Corticosteroids can be administered as clinically indicated per table 7 & 10.
3. Low and high dose vasopressor support as clinically indicated.
4. Antiepileptic drugs to include keppra at first sign of CRS/neurotoxicity, starting dose 500mg BID, additional recommendations per neuro-oncology immune effector toxicity management.

Table 4. CRS Grading System – ASBMT 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 either:				
Hypotension	None	Not requiring vasopressors	Requiring one 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, non-rebreather mask, or Venturi mask	Requiring positive pressure (eg: CPAP, BiPAP, intubation and mechanical ventilation)

CPAP: Continuous positive airway pressure; BiPAP: Bilevel positive airway pressure

† Fever is defined as temperature ≥38°C not attributable to any other cause. In patients who have CRS then receive antipyretics or anti-cytokine 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.

‡ 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 one vasopressor and hypoxia requiring low-flow nasal cannula is classified as having Grade 3 CRS.

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

^ Low-flow nasal cannula is defined as oxygen delivered at ≤ 6 liters/minute. Low flow also includes blow-by oxygen delivery, sometimes used in pediatrics. High-flow nasal cannula is defined as oxygen delivered at > 6 liters/minute.

Table 5. Management of CRS by Grade

CRS Grade	Anti-IL-6 Therapy	Corticosteroids	Additional Supportive Care
Grade 1	For prolonged CRS (>3 days) in patients with significant symptoms and/or comorbidities, consider tocilizumab as per Grade 2	N/A	• Empiric broad-spectrum antibiotics, consider granulocyte colony-stimulating factor (G-CSF) if neutropenic and concern for infectious etiology • Maintenance IV fluids for hydration • Symptomatic management of organ toxicities
Grade 2	Tocilizumab 8 mg/kg IV over 1 hour (not to exceed 800 mg/dose). Repeat in 8 hours if no improvement; no more than 3 doses in 24 hours, with a maximum of 4 doses total	For persistent refractory hypotension after 1–2 doses of anti-IL-6 therapy: Dexamethasone 10 mg IV every 6 hours (or equivalent)	• IV fluid bolus as needed • For persistent refractory hypotension after two fluid boluses and anti-IL-6 therapy: Start vasopressors, consider transfer to intensive care unit (ICU), consider echocardiogram, and initiate other methods of hemodynamic monitoring • Manage per Grade 3 if no improvement within 24 hours after starting anti-IL-6 therapy • Symptomatic management of organ toxicities
Grade 3	Anti-IL-6 therapy as per Grade 2 if maximum dose not reached within 24-hour period	Dexamethasone 10 mg IV every 6 hours (or equivalent). If refractory, manage as grade 4	• Transfer to ICU, obtain echocardiogram, and perform hemodynamic monitoring • Supplemental oxygen including high-flow oxygen delivery and noninvasive positive pressure ventilation • IV fluid bolus and vasopressors as needed. • Symptomatic management of organ toxicities
Grade 4	Anti-IL-6 therapy as per Grade 2 if maximum dose not reached within 24-hour period	Dexamethasone 10 mg IV every 6 hours (or equivalent). If refractory, consider methylprednisolone 1000 mg/day IV	• ICU care and hemodynamic monitoring • Mechanical ventilation as needed • IV fluid bolus and vasopressors as needed • Symptomatic management of organ toxicities

Table 6. Anti-IL6 Therapy and Dosing Recommendations

DRUG	RECOMMENDED DOSE FOR CRS AND/OR NEUROTOXICITY	MAXIMUM DOSE	MECHANISM OF ACTION	COMMENTS
TOCILIZUMAB	8 mg/kg IV for up to three doses in a 24-hour period (Maximum 4 doses total)	Maximum 800 mg per dose	IL-6 receptor antagonist	First line agent Doses can be given 8 hours apart

Table 7. Grading of Neurotoxicity (Lee et al. 2018[8])

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE Score^	7-9	3-6	0-2	0 (patient is unarousable and unable to perform ICE)
Depressed level of consciousness❖	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse. Stupor or coma
Seizure	N/A	N/A	Any clinical seizure focal or generalized that resolves rapidly; or Non-convulsive seizures on EEG that resolve with intervention	Life-threatening prolonged seizure (>5 min); or Repetitive clinical or electrical seizures without return to baseline in between.
Motor findings§	N/A	N/A	N/A	Deep focal motor weakness such as hemiparesis or paraparesis
Raised ICP / Cerebral edema	N/A	N/A	Focal/local edema on neuroimaging#	Diffuse cerebral edema on neuroimaging; Decerebrate or decorticate posturing; or Cranial nerve VI palsy; or

				Papilledema; or Cushing's triad

‡ ICANS grade is determined by the most severe event (ICE score, level of consciousness, seizure, motor findings, raised ICP/cerebral edema) not attributable to any other cause. For example, a patient with an ICE score of 3 who has a generalized seizure is classified as having Grade 3 ICANS.
^A patient with an ICE score of 0 may be classified as having Grade 3 ICANS if the patient is awake with global aphasia. But a patient with an ICE score of 0 may be classified as having Grade 4 ICANS if the patient is unarousable.
❖ Depressed level of consciousness should be attributable to no other cause (e.g. no sedating medication)
§ Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0 but they do not influence ICANS grading.
# Intracranial hemorrhage with or without associated edema is not considered a neurotoxicity feature and is excluded from ICANS grading. It may be graded according to CTCAE v5.0.
ICE: Immune effector Cell-associated Encephalopathy; ICP: Intracranial pressure; EEG: electroencephalogram.

Table 8. Encephalopathy assessment tools for grading of Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS).

Immune effector Cell-associated Encephalopathy (ICE)
• **Orientation:** Orientation to year, month, city, hospital: 4 points • **Naming:** Name 3 objects (e.g., point to clock, pen, button): 3 points • **Following commands:** (e.g., Show me 2 fingers or Close your eyes and stick out your tongue): 1 point • **Writing:** Ability to write a standard sentence (e.g., Our national bird is the bald eagle): 1 point • **Attention:** Count backwards from 100 by ten: 1 point

Score 10: No impairment
Score 7-9: Grade 1 ICANS
Score 3-6: Grade 2 ICANS
Score 0-2: Grade 3 ICANS
Score 0 due to patient unarousable and unable to perform ICE assessment: Grade 4 ICANS

Table 9. Management of ICANS

Treatment by Grade	
Grade 1	• Supportive care
Grade 2	• Supportive care • Dexamethasone 10 mg IV x 1. Can repeat every 6 hours or methylprednisolone 1 mg/kg IV every 12 h if symptoms worsen.
Grade 3	• ICU care is recommended. • Dexamethasone, 10 mg IV every 6 h • Consider repeat neuroimaging (CT or MRI) every 2–3 days if patient has persistent grade ≥3 neurotoxicity.
Grade 4	• ICU care, consider mechanical ventilation for airway protection. • High-dose corticosteroids, methylprednisolone 1000 mg/day IV x3 days

	• Consider repeat neuroimaging (CT or MRI) every 2–3 days if patient has persistent grade ≥3 neurotoxicity. • Treat convulsive status epilepticus per institutional guidelines.

APPENDIX C: IPCG RESPONSE CLASSIFICATION

Response Criteria for Primary Central Nervous System Lymphoma

Response	Brain Imaging	Corticosteroid Dose	Eye Examination	CSF Cytology
CR	No contrast enhancement	None	Normal	Negative
CRu	No contrast enhancement	Any	Normal	Negative
	Minimal abnormality	Any	Minor RPE abnormality	Negative
PR	50% decrease in enhancing tumor	Irrelevant	Minor RPE abnormality or normal	Negative
	No contrast enhancement	Irrelevant	Decrease in vitreous cells or retinal infiltrate	Persistent or suspicious
PD	25% increase in lesion	Irrelevant	Recurrent or new ocular disease	Recurrent or positive
	Any new site of disease: CNS or systemic			

Abbreviations: CR, complete response; CRu, unconfirmed complete response; RPE, retinal pigment epithelium; PR, partial response; PD, progressive disease.

REFERENCES:

1. Mueller, K.T.*, et al.* Cellular kinetics of CTL019 in relapsed/refractory B-cell acute lymphoblastic leukemia and chronic lymphocytic leukemia. *Blood* **130**, 2317-2325 (2017).
2. Maude, S.L., Barrett, D., Teachey, D.T. & Grupp, S.A. Managing cytokine release syndrome associated with novel T cell-engaging therapies. *Cancer J* **20**, 119-122 (2014).
3. Davila, M.L.*, et al.* Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med* **6**, 224ra225 (2014).
4. Bonifant, C.L., Jackson, H.J., Brentjens, R.J. & Curran, K.J. Toxicity and management in CAR T-cell therapy. *Mol Ther Oncolytics* **3**, 16011 (2016).
5. Schuster, S.J.*, et al.* Chimeric Antigen Receptor T Cells in Refractory B-Cell Lymphomas. *N Engl J Med* (2017).
6. Giavridis, T.*, et al.* CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. *Nat Med* **24**, 731-738 (2018).
7. Norelli, M.*, et al.* Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. *Nat Med* **24**, 739-748 (2018).
8. Lee, D.W.*, et al.* ASBMT Consensus Grading for Cytokine Release Syndrome and Neurological Toxicity Associated with Immune Effector Cells. *Biol Blood Marrow Transplant* (2018).
9. Batchelor TT, D.L.e. Lymphoma and Leukemia of the Nervous System, 2nd ed. New York: Springer; 2013. (2013).
10. Ostrom, Q.T.*, et al.* CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2007-2011. *Neuro Oncol* **16 Suppl 4**, iv1-63 (2014).
11. Dolecek, T.A., Propp, J.M., Stroup, N.E. & Kruchko, C. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005-2009. *Neuro Oncol* **14 Suppl 5**, v1-49 (2012).
12. Panageas, K.S., Elkin, E.B., Ben-Porat, L., Deangelis, L.M. & Abrey, L.E. Patterns of treatment in older adults with primary central nervous system lymphoma. *Cancer* **110**, 1338-1344 (2007).
13. Fritsch, K.*, et al.* High-dose methotrexate-based immuno-chemotherapy for elderly primary CNS lymphoma patients (PRIMAIN study). *Leukemia* **31**, 846-852 (2017).
14. DeFilipp, Z.*, et al.* High-dose chemotherapy with thiotepa, busulfan, and cyclophosphamide and autologous stem cell transplantation for patients with primary central nervous system lymphoma in first complete remission. *Cancer* **123**, 3073-3079 (2017).
15. Jahnke, K.*, et al.* Relapse of primary central nervous system lymphoma: clinical features, outcome and prognostic factors. *J Neurooncol* **80**, 159-165 (2006).
16. Langner-Lemercier, S.*, et al.* Primary CNS lymphoma at first relapse/progression: characteristics, management, and outcome of 256 patients from the French LOC network. *Neuro Oncol* **18**, 1297-1303 (2016).
17. Wang, N.F., C. Davis, M. Nayak, L. Batchelor, T. Defining Relapse Patterns in Primary CNS Diffuse Large B-Cell Lymphoma: A Multicenter Study (P6.282). *Neurology* **86**(2016).
18. Abramson, J.S.*, et al.* Anti-CD19 CAR T Cells in CNS Diffuse Large-B-Cell Lymphoma. *N Engl J Med* **377**, 783-784 (2017).

19. Susan R. Rheingold, L.N.C., Shannon L Maude, Richard Aplenc, Christine Barker, David M. Barrett, Colleen Callahan, Katherine Cebry, Irina Kulikovskaya, Simon F. Lacey, Bruce L. Levine, Jan J. Melenhorst, Pamela A Shaw, Anna Sparrow, David T Teachey, Mark Duckworth, Carl H. June, Stephan A. Grupp. Efficient Trafficking of Chimeric Antigen Receptor (CAR)-Modified T Cells to CSF and Induction of Durable CNS Remissions in Children with CNS/Combined Relapsed/Refractory ALL. *Blood* **126**, 3769 (2015).
20. Schuster, S.J.*, et al.* Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma. *N Engl J Med* (2018).
21. Kochenderfer, J.N.*, et al.* Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood* **116**, 4099-4102 (2010).
22. Kochenderfer, J.N.*, et al.* B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. *Blood* **119**, 2709-2720 (2012).
23. Ninomiya, S.*, et al.* Tumor indoleamine 2,3-dioxygenase (IDO) inhibits CD19-CAR T cells and is downregulated by lymphodepleting drugs. *Blood* (2015).
24. Kochenderfer, J.N. Anti CD19 CAR T Cells Administered after LowDose Chemotherapy Can Induce Remissions of Chemotherapy Refractory Diffuse Large B Cell Lymphoma. in *American Society of Hematology* (Abstract 550, 2014).
25. O'Brien, S.M.*, et al.* Results of the fludarabine and cyclophosphamide combination regimen in chronic lymphocytic leukemia. *J Clin Oncol* **19**, 1414-1420 (2001).
26. Hackett, P.B., Largaespada, D.A., Switzer, K.C. & Cooper, L.J. Evaluating risks of insertional mutagenesis by DNA transposons in gene therapy. *Transl. Res* **161**, 265-283 (2013).
27. Tey, S.K. Adoptive T-cell therapy: adverse events and safety switches. *Clin. Transl. Immunology* **3**, e17 (2014).
28. Jena, B., Dotti, G. & Cooper, L.J. Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor. *Blood* **116**, 1035-1044 (2010).
29. Rosenberg, S.A.*, et al.* Gene transfer into humans--immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. *N. Engl. J. Med* **323**, 570-578 (1990).
30. Kochenderfer, J.N.*, et al.* Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. *J Clin Oncol* **33**, 540-549 (2015).
31. Cohen, A.D.*, et al.* B-Cell Maturation Antigen (BCMA)-Specific Chimeric Antigen Receptor T Cells (CART-BCMA) for Multiple Myeloma (MM): Initial Safety and Efficacy from a Phase I Study. in *ASH* (San Diego, CA, 2016).
32. Weissert, R. Progressive multifocal leukoencephalopathy. *J Neuroimmunol* **231**, 73-77 (2011).
33. Milone, M.C.*, et al.* Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. *Mol Ther* **17**, 1453-1464 (2009).
34. Grupp, S.A.*, et al.* Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *New England Journal of Medicine* **368**, 1509-1518 (2013).
35. Porter, D.L.*, et al.* Chimeric antigen receptor T cells persist and induce sustained

remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med* **7**, 303ra139 (2015).

36. Lamers, C.H.*, et al.* Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. *J Clin Oncol* **24**, e20-22 (2006).