

Phase 1 Trial of Autologous HER2-specific CAR T cells in Pediatric Patients with Refractory or Recurrent Ependymoma

ABSTRACT & SCHEMA

This is a multicenter, Phase 1 and Surgical study of the treatment HER2-specific CAR T cells for patients with refractory or recurrent ependymoma.

Ependymoma is the third most common central nervous system (CNS) tumor in children and is associated with poor long-term survival. Ten-year progression free survival is 30% and overall survival is 50% in these children.¹ Multiple recurrences and repeated aggressive surgical and radiation treatments can cause debilitating effects even in survivors.² About 90% of ependymomas in children are intracranial, with most arising from the posterior fossa.^{1,3} Standard of care for newly diagnosed ependymoma is maximal safe surgical resection followed by local adjuvant radiation therapy.⁴ Most patients have minimal long-term benefit from chemotherapy, and the risk of relapse is very high, with the primary tumor site being the most common site of recurrence. Gross total surgical resection correlates with slightly improved survival; nevertheless, many patients develop and eventually succumb to recurrent disease even years later.¹ At the time of recurrence, aggressive surgical re-resection, additional radiation therapy, and cytotoxic chemotherapy may be used for palliation and to prolong survival but there is no standard or known curative treatment in this group. Immunotherapeutic approaches that employ alternative mechanisms of tumor cell killing may potentially benefit these patients.

Phase 1

The primary objectives of the Phase I study are to determine the safety of intravenous injection of HER2-specific CAR T cells after lymphodepleting chemotherapy, and to evaluate the multicenter feasibility of administering up to three infusions of HER2-CAR T cells after lymphodepletion.

Patients will receive one infusion of HER2 specific CAR T cells after lymphodepleting chemotherapy. Following recovery from their first treatment (no earlier than 8 weeks and no later than 12 weeks), patients will resume treatment with HER2-specific CAR T cells for up to 2 infusions after lymphodepleting chemotherapy if they meet laboratory parameters defined in [Section 3.2.1.4](#).

The length of time on study for patients enrolled on the Phase 1 study is anticipated to be 9 months on treatment. Patients will then be followed until 15 years past last CAR T cell infusion.

Surgical Study

The objective of the Surgical study is to evaluate the post-treatment tumor tissue for presence of HER2-specific CAR T cells administered intravenously in children undergoing surgical resection. The Surgical study will be initiated following completion of the safety evaluation period of 6 patients treated in the Phase 1 study.

Once the Surgical study is open for enrollment, all patients who have clinical indication for surgery, except those needing urgent surgery, will be eligible for enrollment to the surgical

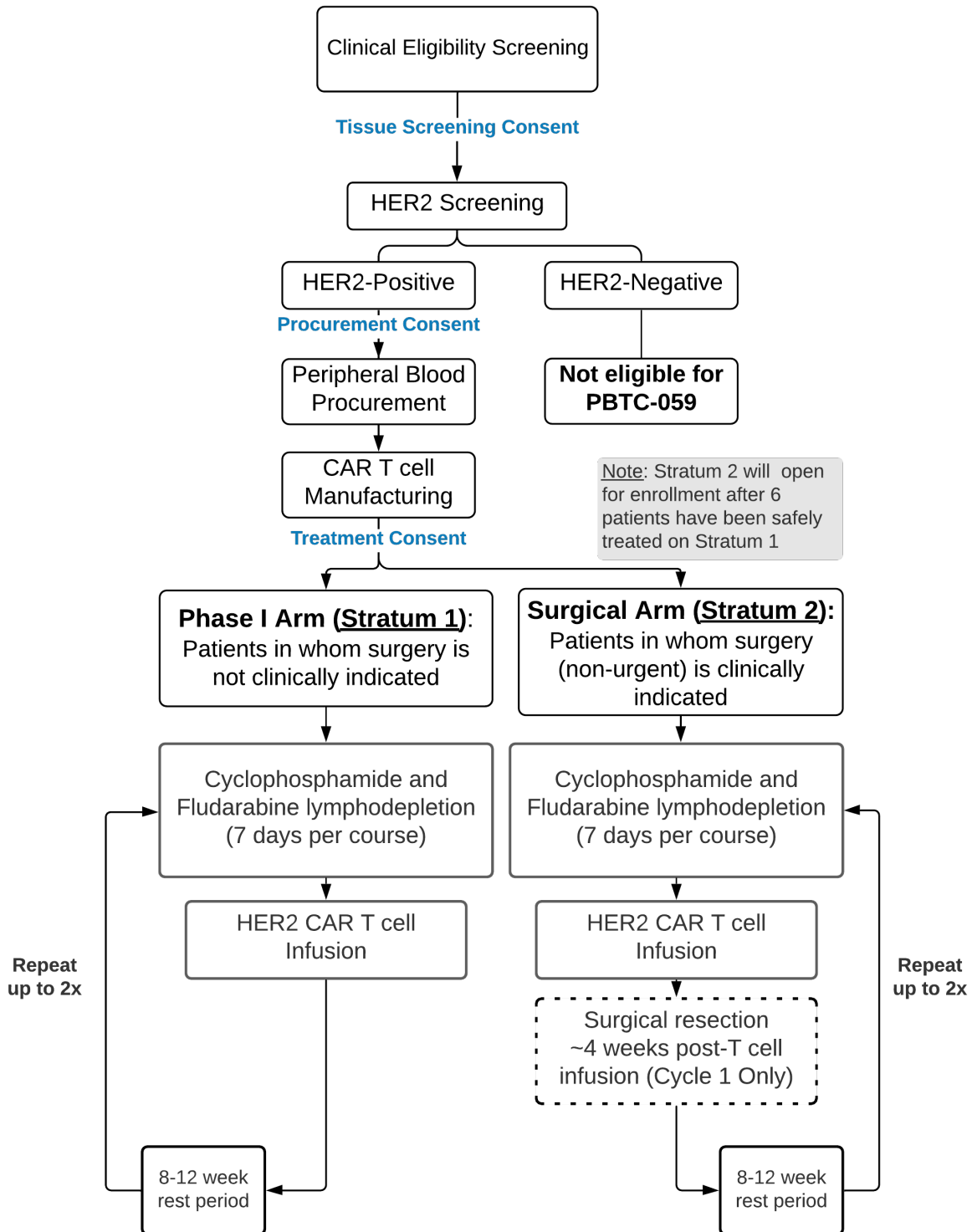
study. Patients will receive one infusion of HER2-specific CAR T cells after lymphodepleting chemotherapy 4-6 weeks before surgical resection of their tumor, at which time samples will be taken for analysis. Following recovery from surgery (no earlier than 8 weeks and no later than 15 weeks), patients will resume treatment with HER2-specific CAR T cells for up to 2 infusions if they meet laboratory parameters defined in [Section 3.2.1.4](#).

The first patient in the Surgical study will complete a 6-week safety evaluation period prior to enrollment of the subsequent patient. The length of time on study for patients enrolled on the Surgical study is anticipated to be 10 months on treatment. Patients will then be followed until 15 years post last CAR T cell infusion.

All patients on Phase 1 and Surgical study will receive HER2 CAR T cells at a patient-specific Dose Level 1 (8×10^7 CAR-positive T cells/m²) for infusion. The cell dose will be based on the patient weight and height obtained by the treating institution at the time of procurement. For patients whose BMI is greater than 95th percentile for given age and sex, the BSA will be calculated using the ideal body weight.

In the event that Dose Level 1 is found to have excessive toxicity, three additional doses of CAR T cells at Dose Level -1 (5×10^7 CAR-positive T cells/m²) will be made to be used in the event that dose de-escalation occurs before a patient is enrolled for treatment.

Dosing Schedule	
Dose Level	HER2-specific CAR T Cell Dose(s)
Level -1	5×10^7 CAR-positive T cells/m ²
Level 1*	8×10^7 CAR-positive T cells/m ²
*Starting Dose	



1.0 OBJECTIVES

1.1 Primary Objectives

- 1.1.1 To determine the safety of intravenous injection of autologous HER2-specific CAR T cells after lymphodepleting chemotherapy in patients with progressive or recurrent HER2-positive ependymoma.
- 1.1.2 To evaluate the multicenter feasibility of administering up to three infusions of HER2-CAR T cells after lymphodepletion in children with progressive or recurrent HER2-positive ependymoma.

1.2 Secondary Objectives

- 1.2.1 To study the expansion, distribution, and persistence of adoptively transferred CAR T cells.
- 1.2.2 To assess the antitumor effect of the infused HER2-specific CAR T cells.
- 1.2.3 To examine the ability of quantitative MR diffusion/weighted imaging/ADC mapping to provide early assessment of tumor behavior and specifically distinguish pseudoprogression/tumor inflammation from tumor progression.
- 1.2.4 To explore the use of serial MR permeability (DCE) and MR perfusion (DSC) to determine if elevated rCBV and ktrans can distinguish pseudoprogression/tumor inflammation from tumor progression in tumors treated on this protocol.

1.3 Exploratory Objectives

- 1.3.1 To evaluate the effect of CAR T cells on expression of tumor-associated antigens and immune ligands.
- 1.3.2 To characterize endogenous immune responses following CAR T cell infusion.
- 1.3.3 To evaluate both tumor and CAR T cell molecular profiles (i.e., DNA methylation and RNA-seq) to uncover determinants of function and response.

2.0 PATIENT SELECTION

All subjects must meet the following inclusion and exclusion criteria. No exceptions will be given. Imaging studies, physical examination, and laboratory evaluations done within the previous 4 weeks for clinical care will be used to establish eligibility for screening.

2.1 Eligibility Criteria for Screening

2.1.1 Tumor

Patient must have a diagnosis of ependymoma that is recurrent or progressive. All tumors must have histologic verification either at the time of diagnosis or recurrence.

2.1.2 Prior Therapy

Patient must have received standard of care therapy including maximal safe surgical resection followed by local adjuvant radiation therapy prior to enrollment.

2.1.3 Adequate Pre-trial Tumor Tissue

Patient must have adequate pre-trial tumor material available to determine HER2 status. Tumor tissue from the most recent resection or biopsy of recurrent disease is preferred. If unavailable, tumor tissue from prior recurrences or from the time of initial diagnosis is acceptable.

- One exception will be patients who have previously received HER2-directed therapy (including but not limited to trastuzumab); these patients will need evaluation of tumor HER2 status after stopping treatment due to the possibility of HER2 downregulation or loss.

Tumor biopsy will not be performed for the purpose of HER2 screening. Patients will not be eligible for screening on PBTC-059 if tumor tissue is not available or inadequate for HER2 testing. Tumor screening by IHC will be done centrally using the testing method validated at Texas Children's Hospital.^{13,14,29,77-79} Sample for screening must be shipped within 7 days of enrollment for screening (see [Section 5.2.1](#)).

2.1.4 Known HIV Positivity

Patients that are known to be HIV-positive are ineligible due to the unknown safety and efficacy of infusing these patients with CAR T cells genetically modified using retroviral vectors. Additionally, the immunosuppression used for treatment in this study will pose an unacceptable risk.

2.1.5 Age

Patient must be ≥ 1 but ≤ 21 years of age at the time of screening consent.

2.1.6 Screening Consent

The patient or parent/guardian can understand the consent and is willing to sign a written informed consent document according to institutional guidelines. Age- and developmentally appropriate assent should be obtained as required by institutional guidelines.

2.1.7 Potential Eligibility for Study Enrollment

Patients are screened for this trial should be reasonably anticipated to meet the criteria for treatment described in [Section 3.3](#) if their tumor is HER2-positive.

2.2 Eligibility Criteria for Procurement

All subjects must meet following inclusion and exclusion eligibility criteria at the time of peripheral blood procurement for manufacturing the HER2 CAR T cell product. No exceptions will be given. All clinical and laboratory evaluations to establish eligibility for procurement must be done within 14 days prior to enrollment. See [Section 6.1](#) for details of laboratory requirements and planning of procurement blood collection date.

2.2.1 Inclusion Criteria

2.2.1.1 Tumor

Patient must have been screened and determined to have a diagnosis of a HER2-positive recurrent or progressive ependymoma.

2.2.1.2 Performance Score

Karnofsky Performance Scale (KPS for > 16 years of age) or Lansky Performance Score (LPS for ≤ 16 years of age) (Appendix C) assessed within one week of procurement must be ≥ 60%. Patients who are unable to walk because of neurologic deficits, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score provided the neurological deficit is stable as described in [Section 3.3.1.7](#).

2.2.1.3 Prior Therapy

Patients must have received last dose of cytotoxic chemotherapy greater than 21 days preceding the date of enrollment for procurement.

2.2.1.4 Organ Function

Patient must have adequate organ and bone marrow function as defined below:

- Peripheral absolute neutrophil count (ANC) ≥ 1.0 x 10⁹ cells/L
- Platelet count ≥ 100 x 10⁹ cells/L (unsupported, defined as no platelet transfusion within 4 days)
- Hemoglobin ≥ 8 g/dL (may receive red blood cell transfusions)
- Total bilirubin ≤ 1.5 x institutional upper limit of normal (ULN) for age
- ALT(SGPT) and AST(SGOT) ≤ 3 x institutional upper limit of normal (ULN) for age
- Serum creatinine < 1.5 x institutional upper limit of normal for age and gender. Patients that do not meet the criteria but have a 24-hour Creatinine Clearance or GFR (radioisotope or iothalamate) ≥ 70 mL/min/1.73 m² are eligible.
- Pulmonary Function
 - Oxygen saturation as measured by pulse oximetry is ≥ 93% on room air

2.2.1.5 Concomitant Medications

Patients who are receiving systemic corticosteroids must be on a stable or decreasing dose for at least two weeks prior to procurement, and corticosteroid dose must be less than or equal to dexamethasone 0.75 mg/m²/day (or equivalent). Use of topical, ocular, intranasal, or inhaled corticosteroids are permitted.

2.2.1.6 *Procurement Consent*

The patient or parent/guardian can understand the consent and is willing to sign a written informed consent document according to institutional guidelines. Age- and developmentally appropriate assent should be obtained as required by institutional guidelines.

2.2.1.7 *Potential Eligibility for Study Enrollment*

Patients whose blood samples have been successfully procured for this trial should be reasonably anticipated to meet the criteria for treatment described in [Section 3.3](#) and to begin treatment within 180 days from the date of procurement. The treatment slot will not be held beyond the specified 180 days, and such patients may not be able to receive treatment on this study depending on slot availability.

2.2.2 Exclusion Criteria

2.2.2.1 *Known HIV Positivity*

Patients who are known to be HIV-positive are ineligible due to the unknown safety and efficacy of infusing these patients with CAR T cells genetically modified using retroviral vectors. Additionally, the immunosuppression used for treatment in this study will pose an unacceptable risk.

2.3 Eligibility Criteria for Treatment

All Phase I (Stratum 1) and Surgical (Stratum 2) subjects must meet following inclusion and exclusion eligibility criteria at the time of enrollment for treatment. No exceptions will be given. Imaging studies must be done within 14 days prior to enrollment. All other clinical and laboratory evaluations to establish eligibility for treatment must be done 7 days prior to enrollment.

2.3.1 Inclusion Criteria

2.3.1.1 *Diagnosis*

Patients with a histologically confirmed diagnosis of HER2 positive ependymoma that is recurrent or progressive. Histologic verification may be from time of diagnosis or time of recurrence. In cases where there is question of recurrence, histologic verification, or verification of progression on follow up imaging is required prior to enrolling for protocol treatment.

2.3.1.2 *Disease Status*

2.3.1.2.1 Phase I (Stratum 1)

Patients must have evaluable disease in the central nervous system to be eligible. Evaluable disease includes either measurable OR non-measurable disease, defined as follows:

- Measurable disease (enhancing or non-enhancing tumor):
 - a. at least 1 cm, or
 - b. at least two times (in both perpendicular diameters) the MRI slice thickness, plus the interslice gap.
- Non-measurable disease (tumor that is too small to be accurately measured):

- a. less than 1 cm in at least one perpendicular dimension, or
- b. less than two times the MRI slice thickness, plus the interslice gap, or
- c. no tumor visible on imaging but presence of malignant cells on cytologic examination of CSF.

Note: Leptomeningeal disease is considered non-measurable but evaluable.

2.3.1.2.2 Surgical Study (Stratum 2)

Patients with measurable disease ([Section 3.3.1.2.1](#)) in whom tumor resection is clinically indicated and feasible after the CAR T cell infusion.

2.3.1.3 Age

Patient must be ≥ 1 but ≤ 22 years of age at the time of enrollment for treatment.

2.3.1.4 HER2 CAR T cell product

The patient must have, at a minimum, one prescribed dose of the cryopreserved, autologous HER2 CAR T cell product available for infusion.

2.3.1.5 Prior Anti-neoplastic Therapy

- *Cytotoxic chemotherapy:* Patients must not have received cytotoxic chemotherapy for at least 28 days prior to study enrollment for treatment and must have recovered from the acute treatment related toxicities (defined as \leq grade 1 if not defined in eligibility criteria; excludes alopecia) prior to entering this study.
- *Biological, targeted, or investigational agents (anti-neoplastic):* Patients must have a period of at least 28 days from the last receipt of said drug and must have recovered from all acute toxic effects.
 - For agents that have known acute adverse events occurring beyond 28 days after administration, this period must be extended beyond the time during which adverse events are known to occur.
- *Monoclonal antibodies, checkpoint inhibitors, and other agents with known prolonged half-lives:* Patient must have recovered from any acute toxicity potentially related to the agent and received their last dose of the agent ≥ 28 days prior to study enrollment.
- *Adoptive cellular therapies:* Patient must have recovered from any acute toxicity potentially related to the cellular product and received their last dose of the cellular product at least 90 days prior to study enrollment. (Note: Patients who have previously received an adoptive cellular therapy may continue long-term follow up evaluations per the prior study's evaluation schedule as needed for assessment of long-term toxicities including genotoxicity.)
- *Radiation:* Patients must have had their last fraction of:
 - Craniospinal irradiation, whole brain radiation, or radiation to $>50\%$ of pelvis or spine ≥ 3 months prior to enrollment (90 days) prior to enrollment.
 - Focal palliative irradiation to the tumor ≥ 42 days prior to enrollment.

- Patients who receive tumor-directed radiation (non-palliative) should have confirmed disease progression on the imaging study done at least 6 weeks after the completion of the last fraction of radiation.
- *Surgery*: Patients must have not had surgery within 14 days of enrollment for treatment and must have adequate wound healing and recovered from other acute effects from surgery. One exception is the placement of central venous catheter which will be allowed at any time point until treatment initiation on the study.

2.3.1.6 *Growth Factors*

Patients must be off all colony-forming growth factor(s) for at least 7 days prior to enrollment (e.g., filgrastim, sargramostim, or erythropoietin). 14 days must have elapsed if the patient received a long-acting formulation.

2.3.1.7 *Corticosteroids*

Patients who are receiving systemic corticosteroids must be on a stable or decreasing dose for at least 14 days prior to enrollment for treatment, and corticosteroid dose must be less than or equal to dexamethasone 0.5 mg/m²/day (or equivalent) during the 14 days preceding enrollment. Use of topical, ocular, intranasal, or inhaled corticosteroids are permitted.

2.3.1.8 *Neurologic Status*

- In patients with neurological deficits, deficits should be stable for a minimum of 7 days prior to enrollment. A baseline detailed neurological exam should clearly document the neurological status of the patient at the time of enrollment for treatment on the study.
- Patients with seizure disorders may be enrolled if seizures are well controlled.

2.3.1.9 *Performance Status*

Karnofsky Performance Scale (KPS for > 16 years of age) or Lansky Performance Score (LPS for ≤ 16 years of age) (Appendix C) assessed within one week of enrollment must be ≥ 60%. Patients who are unable to walk because of neurologic deficits, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

2.3.1.10 *Organ Function*

Patients must have adequate organ and bone marrow function as defined in [Section 3.2.1.4](#).

2.3.1.11 *Pregnancy Prevention*

Patients of childbearing or child fathering potential must be willing to use a medically acceptable form of birth control, which includes abstinence, while being treated on this study.

2.3.1.12 *Informed Consent*

The patient or parent/guardian can understand the consent and is willing to sign a written informed consent document according to institutional guidelines. Age- and developmentally appropriate assent should be obtained as required by institutional guidelines.

- Patients who meet eligibility criteria per [Section 3.3.1.2.1](#) must be enrolled using Phase I

treatment consent (Stratum 1).

- Patients who meet eligibility criteria per [Section 3.3.1.2.2](#) must be enrolled using Surgical Study treatment consent (Stratum 2).

2.3.2 Exclusion Criteria

2.3.2.1 *Patients with Bulky Tumors on Imaging Studies*

Bulky tumors will be defined as those:

- > 6 cm in single maximum dimension, or
- tumor causing uncal herniation or mass effect leading to midline shift with or without symptoms or signs of impending herniation or
- obstruction to CSF flow.

2.3.2.2 *Infratentorial tumors*

Infratentorial tumors with symptoms or signs arising from brain stem involvement by the tumor. Patients with stable cranial nerve deficit(s) secondary to prior surgery will not be excluded.

2.3.2.3 *Surgical study (Stratum 2)*

Patients who have urgent need for surgical resection of tumor.

2.3.2.4 *Pregnancy or Breast-feeding*

Pregnant women or nursing mothers are excluded from this study.

- Female patients of childbearing potential must have a negative serum or urine pregnancy test within 7 days of start of enrollment for treatment. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

Pregnant or breast-feeding women are excluded from this study because there is an unknown but potential risk of adverse events to the fetus or the nursing infant with the use of T cells genetically modified to express HER2 CAR. Pre-clinical studies in mice demonstrate the target antigen HER2 is necessary for normal fetal development of cardiac trabeculae, cranial sensory ganglia, and motor neuron development.⁸⁰ Additionally, the lymphodepleting chemotherapy drugs fludarabine and cyclophosphamide are both Pregnancy Class D drugs.

2.3.3 Concurrent Illness

- Patients with active autoimmune disease, documented history of autoimmune disease/syndrome, or any other condition that requires ongoing systemic steroids or systemic immunosuppressive agents, except
 - Patients with vitiligo or resolved asthma/atopy
 - Patients with hypothyroidism stable on hormone replacement or Sjogren's syndrome
 - Patients requiring physiologic doses of corticosteroids (up to 0.5 mg/m²/day dexamethasone equivalent)
- History of or ongoing pneumonitis or significant interstitial lung disease
- Ongoing or active uncontrolled infection
- Patients with any clinically significant unrelated systemic illness (serious infections or

significant cardiac, pulmonary, hepatic or other organ dysfunction), that in the opinion of the investigator, would compromise the patient's ability to tolerate protocol therapy, put them at additional risk for toxicity or would interfere with the study procedures or results.

- Patients with any of the following cardiac diseases
 - New York Heart Association (NYHA) functional class III or IV
 - Clinically significant cardiac arrhythmia including, but not limited to, Torsade de pointes or requiring a pacemaker
 - Left ventricular ejection fraction below 50% as determined by echocardiography (ECHO)
- Known HIV positivity
HIV-positive patients are ineligible due to the unknown safety and efficacy of infusing these patients with CAR T cells genetically modified using retroviral vectors. Additionally, the immunosuppression used for treatment in this study will pose an unacceptable risk.

2.3.3.1 *Concomitant Medications*

- Patients who are receiving any other anti-cancer or investigational drug therapy are ineligible.
- Patients who have received the last vaccination of a live vaccine \leq 30 days prior to enrollment are ineligible.
 - Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines and must meet timeline for live vaccine.
- Herbal preparations/medications (except for vitamins) including, but not limited to: St. John's wort, Kava, ephedra (ma huang), ginkgo biloba, dehydroepiandrosterone (DHEA), yohimbe, saw palmetto, black cohosh, and ginseng. Patients should stop using all herbal medications and dietary supplements at least 7 days prior to enrollment.

2.3.3.2 *Inability to participate*

Patients who in the opinion of the investigator are unwilling or unable to return for required follow-up visits or obtain follow-up studies required to assess toxicity to therapy or to adhere to drug administration plan, other study procedures, and study restrictions.

2.3.3.3 *Allergy*

Patients with a history of allergic reactions attributed to compounds of similar chemical or biologic composition (murine protein-containing products, DMSO, or dextran 40).

2.4 **Inclusion of Women and Minorities**

NIH policy requires that women and members of minority groups and their subpopulations be included in all NIH-supported biomedical and behavioral research projects involving NIH-defined clinical research unless a clear and compelling rationale and justification establishes to the satisfaction of the funding Institute & Center (IC) Director that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research. Exclusion under other circumstances must be designated by the Director, NIH, upon the recommendation of an IC

Director based on a compelling rationale and justification. Cost is not an acceptable reason for exclusion except when the study would duplicate data from other sources. Women of childbearing potential should not be routinely excluded from participation in clinical research. Please see <http://grants.nih.gov/grants/funding/phs398/phs398.pdf>.

The PBTC remains committed to offering participation in our trials to subjects of all races and ethnic groups.

2.5 Treatment at the Primary Institution

All experimental protocol therapy should be dispensed and all on treatment imaging studies should be obtained at a PBTC institution. Laboratory studies, excluding pharmacokinetic and biologic assays, may be performed at a CLIA certified laboratory of the investigator's choice. Imaging utilized to determine eligibility may be performed at an outside institution if all required imaging sequences are included and the study is deemed of adequate quality by the treating team. All required physical examinations, laboratory parameters need to be performed at the primary PBTC institution during the safety observation period of the protocol.

2.6 Criteria to Start Treatment

- Subjects must start therapy (lymphodepletion) within 7 days of enrollment for treatment.
- Imaging studies for pre-treatment disease evaluation must be no older than 14 days prior to enrollment.
- Laboratory studies for organ function evaluation must be no older than 7 days prior to the start of lymphodepleting chemotherapy. If laboratory organ function studies are repeated for any reason after enrollment for treatment but before treatment start and do not meet the criteria listed below, they may again be repeated within the allowed window for treatment start. Initiation of treatment may then proceed if repeat laboratory values meet the criteria below.
 - Total bilirubin ≤ 1.5 x institutional upper limit of normal (ULN) for age
 - ALT(SGPT) and AST(SGOT) ≤ 3 x institutional upper limit of normal (ULN) for age
 - Serum creatinine < 1.5 x institutional upper limit of normal for age and gender. Patients that do not meet the criteria but have a 24-hour Creatinine Clearance or GFR (radioisotope or iothalamate) ≥ 70 mL/min/1.73 m² are eligible.
- All required pre-treatment evaluations must be completed as per the study calendar.
- See [Section 6.2.1.1](#) for additional criteria.
- If treatment does not proceed for the reasons outlined above or for any other reason, the subject will be removed from the assigned treatment slot but will be allowed to re-enroll for treatment provided the treatment eligibility criteria outlined in [Section 3.3](#) are again met and treatment slots remain.