ANCHOR

ALLOGENEIC NATURAL KILLER T-CELLS EXPRESSING CD19 SPECIFIC CHIMERIC ANTIGEN RECEPTOR AND INTERLEUKIN-15 IN RELAPSED OR REFRACTORY B-CELL MALIGNANCIES

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CHECKLIST FOR PATIENT ELIGIBILITY FOR TREATMENT

PATIENT ID_____**PATIENT NAME** _____

YES	NO	VALUE/DATE	
Any "**NO**" answers will make a patient **ineligible** for study participation.			
			Diagnosis of CD19-positive B-cell lymphoma or leukemia (ALL or CLL).
			The disease is: • Relapsed or refractory after two or more lines of therapy, including a CD20 antibody, if an indolent lymphoma. • Relapsed or refractory after two or more lines of therapy, including ibrutinib and venetoclax, if CLL. • Relapsed or refractory after two or more lines of therapy, including a CD20 antibody and an anthracycline, and the patient is ineligible for autologous stem cell transplantation, if an aggressive or highly aggressive lymphoma. • Relapsed or refractory after two or more lines of therapy, if ALL. (Non-ALL = cohort A; ALL = cohort B)
			Measurable disease by current criteria
			Age ≥ 3 and ≤75 years
			Bilirubin < 2 times (3 times if Gilbert syndrome) upper limit of normal
			AST and ALT < 5 times upper limit of normal
			Estimated GFR ≥ 50 mL/min
			Pulse oximetry of ≥ 90% on room air
			Karnofsky or Lansky score of ≥ 70
			Recovered from the acute toxic effects of all prior chemotherapy based on the enrolling physician's assessment (if some effects of chemotherapy are expected to last long term—for example, residual neuropathy, anemia or alopecia—patient is eligible if meeting other eligibility criteria)
			Life expectancy of greater than 12 weeks.
			Sexually active patients must be willing to utilize one of the more effective birth control methods during the study and for 6 months after study is concluded. Male partner should use a condom.
			Informed consent explained to, understood by and signed by patient/guardian. Patient/guardian given copy of informed consent.

Signature of MD _____ **Date** _____

YES	**NO**	
Any "**YES**" answers will make a patient **ineligible** for study participation.		
		Currently receiving any investigational agents or having received any cellular therapies within the previous six weeks.
		History of grade 2 to 4 graft-versus-host disease (GVHD)
		History of hypersensitivity reactions to murine protein-containing products
		Pregnant or lactating
		Active infection with HIV or HTLV
		Active infection with HBV or HCV
		Uncontrolled active bacterial, fungal or other viral infection

Signature of MD _____ **Date** _____

1. Objectives

1.1. Primary Objective

To evaluate in patients with refractory/relapsed B-cell non-Hodgkin lymphomas (NHL) or leukemia (ALL or CLL) the safety of escalating doses of CD19.CAR-aNKTs. These are allogeneic invariant Natural Killer T cells (aNKT) genetically modified to express an artificial antigen receptor (chimeric antigen receptor or CAR) that targets the CD19 molecule (CD19.CAR) and incorporates a costimulatory domain (CD28), cytokine IL-15, and shRNA to reduce expression of HLA molecules.

1.2. Secondary Objective

1.2.1. To measure the anti-tumor effects of CD19.CAR-aNKTs in patients with NHL or leukemia.

1.3. Exploratory Objective

1.3.1. To measure the survival and function of CD19.CAR-aNKTs *in vivo*.

2. Background and Rationale

2.1. CD19-Positive B-Cell Malignancies

2.1.1. Acute Lymphoblastic Leukemia (ALL)

ALL has an overall incidence of 1.5 per 100,000 in the US. It has a bimodal distribution with peaks at 5 and 50 years of age.[1] It is the commonest childhood cancer and accounts for 80% of childhood leukemias. The majority of children (>80%) are cured with intensive chemotherapy, but high numbers of children still die because of the frequency of the disease.[2] In addition, adverse cytogenetics (Philadelphia chromosome, hypodiploidy), primary induction failure and early relapse predict a dismal outcome in the pediatric population. In these situations hematopoietic stem cell transplant (HSCT) often provides the only hope of long term disease free survival.[3, 4]

ALL accounts for 20% of adult leukemias[1] and even with intensive therapy, outcomes are inferior to pediatric counterparts. Long term survival is approximately 30-40% and prognosis worsens with increasing age.[5-8] Poor cytogenetics, increased white cell count and B-cell phenotype are more common in adults and correlate with a high relapse and low cure rates[7]. Intensive therapy does not appear to affect the survival of individuals with predictors of adverse outcome. Other studies have shown allogeneic HSCT performed in first remission benefits adults with standard risk disease in addition to those with high risk[9-11].

2.1.2. Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL)

Chronic lymphocytic leukemia (chronic lymphoid leukemia, CLL) is a monoclonal disorder characterized by a progressive accumulation of functionally incompetent lymphocytes. It is the most common form of leukemia found in adults in Western countries. More than 17,000 new cases of chronic lymphocytic leukemia are reported every year.[2] The most common abnormality is deletion of 13q, which occurs in more than 50% of patients. The presence of trisomy 12, which is observed in 15% of patients, is associated with atypical morphology and progressive disease. Deletion in the short arm of chromosome 17 has been

associated with rapid progression, short remission, and decreased overall survival in CLL. Deletions of bands 11q22-q23, observed in 19% of patients, are associated with extensive lymph node involvement, aggressive disease, and shorter survival.

Allogeneic stem cell transplantation is the only known curative therapy for CLL. The optimal timing and selection of most appropriate candidates for transplantation has been investigated.[12-15] It is known that delay of transplantation until development of refractory disease results in worse outcomes. The effectiveness of nonmyeloablative transplantation and donor lymphocyte infusions (DLI) has shown that there is a graft versus leukemia immune effect in CLL.[14, 16-18]

2.1.3. B-Cell NonHodgkin Lymphoma (NHL)

Approximately 65,000 patients are diagnosed with NHL each year in the US alone.[2] NHL is a heterogenous group of lymphoproliferative malignancies with differing patterns of behavior and responses to treatment.[19-21] NHL usually originates in the lymphoid tissues and can spread to other organs. However, unlike Hodgkin disease, NHL is much less predictable and has a far greater predilection to disseminate to extranodal sites. The prognosis depends on the histologic type, stage, and treatment. Most (i.e., 80-90%) NHLs are of B-cell origin ($CD19^{+}$).[22] NHL can be divided into 2 general prognostic groups: the indolent lymphomas and the aggressive lymphomas. Indolent lymphomas have a relatively good prognosis, with median survival time as long as 10 years, but they are not usually curable in advanced stages.[21] Most of the indolent types are nodular (or follicular) in morphology. The aggressive type of NHL has a shorter natural history, but a significant number of these patients can be cured with combination chemotherapy regimens.[20] In general, with modern treatment of patients with NHL, the overall survival rate at 5 years is approximately 50-60%. Thirty percent of patients with aggressive NHL can be cured. Most relapses occur in the first 2 years after therapy. The risk of late relapse is higher in patients with a divergent histology of both indolent and aggressive disease. A subset of aggressive lymphomas, Burkitt lymphoma and lymphoblastic lymphoma, were designated as high grade by the International Working Formulation (IWF) to reflect the rapidly progressive behavior of these subtypes. While indolent NHL is responsive to radiation therapy and chemotherapy, a continuous rate of relapse is usually observed in advanced stages. However, patients can often be re-treated with considerable success as long as the disease histology remains low grade. Patients who present with or convert to aggressive forms of NHL may achieve complete remission (CR) with combination chemotherapy regimens with autologous or allogeneic stem cell transplant.[23, 24]

Allogeneic stem cell transplantation for B-cell NHL can be associated with a relatively high mortality rate when complications of graft versus host disease arise.[24-26] However, it appears to have a lower risk of subsequent relapse, possibly because of graft versus lymphoma effect. An apparent plateau in failure-free survival rates suggests that allogeneic stem cell transplantation may offer cure to some patients with follicular lymphoma and may be appropriate for selected younger patients.[24, 27-29] With the advent of nonmyeloablative allogeneic stem cell transplants, this modality may be available to a larger group of patients because it does not carry the risks of acute early toxicity and mortality, unlike myeloablative stem cell transplants.

Despite the possibility of cure with first line chemotherapy or allogeneic stem cell transplantation, many patients experience disease relapses or are unable to achieve the remission required to proceed to transplant. Thus, novel therapies are needed.

2.2. CD19 as a Target Antigen

CD19 is a 95-kDa B lineage-specific transmembrane glycoprotein, which functions as a central response regulator in B-lymphocytes. With the important exception of hematopoietic stem cells, CD19 is expressed on B-cells during all stages of their differentiation, is down-regulated on plasma cells,[30] and is maintained on cells that have undergone neoplastic transformation. It is expressed on >95% of cells in patients with B-cell lymphoma or leukemia, and recent studies have also shown that this expression is maintained despite loss of CD20 expression following treatment with anti-CD20 antibodies.[31] CD19 has therefore become a primary target for cellular therapies directed to B-cell malignancies.

2.3. Chimeric Antigen Receptors

Chimeric receptors are most commonly generated by joining the heavy and light chain variable regions of a monoclonal antibody with a linker to form a single-chain Fv (scFv) molecule.[32] This scFv is then attached to the transmembrane and cytoplasmic portion of the T Cell Receptor's (TCR) ζ chain via a flexible hinge region. Engagement of the extracellular scFv of the chimeric receptor results in tyrosine phosphorylation of immune-receptor activation motifs present in the cytoplasmic domain, initiating T cell signaling to the nucleus. Human T lymphocytes genetically engineered to express these recombinant receptor genes exhibit specific lysis via the perforin/granzyme pathways, as well as cytokine secretion upon exposure to tumor cells expressing the target antigen.[33]

The advantages of CARs over the native antibodies or ligands from which they derive are a consequence of their physical association with effector T cells.[34] Thus, CAR-modified T cells can have an active biodistribution, with migration through multiple tissue planes along chemokine gradients, and can recruit the multiple cytotoxic effector mechanisms available to a T cell, rather than the more restricted cytotoxic machinery associated with, for example, the Fc component of an antibody. CARs also offer advantages over transfer of native αβ-TCRs of T lymphocytes. Firstly, target cell recognition by αβ-TCRs is MHC restricted, precluding the design of a "universal" receptor for the treatment of patients with different HLA polymorphisms; by contrast, CARs, like monoclonal antibodies, are essentially universal as their cytotoxic activity is MHC-unrestricted.[34] Moreover, many tumors down-regulate expression of MHC molecules and/or have dysfunctional antigen processing machinery, so that the target antigenic epitopes for αβ-TCR are simply not present: since CAR-modified T cells bind directly to native proteins expressed on the surface of target cells without the need for antigen processing or MHC-restricted presentation, they are unaffected by this immune evasion strategy. Finally, CARs can recognize non-protein antigens, unlike conventional αβTCRs.[34]

2.4. Extending the Survival of Cells Transduced with CARs

2.4.1. The Role of Costimulation

Despite consistent and robust expression of CAR molecules in T cells, early clinical studies of the approach were disappointing. A major problem of CAR-modified T cells is their lack of expansion and persistence *in vivo.*[35] A number of factors likely contribute to this discrepancy but a major contribution is the inability of CAR-engagement alone to recapitulate the complex costimulatory events that follow the physiologic engagement of the native αβ-TCR. Full activation and proliferation of T

cells requires not only TCR engagement (signal 1) but also co-stimulation provided by antigen presenting cells (APCs, signal 2) and cytokines (signal 3).[36] CAR-redirected T cells lack any such costimulation when they engage tumor cells, since these target cells are deficient in costimulatory molecule expression (e.g. CD80 and CD86) and do not release helper cytokines. On the other hand, CAR-modified T cells cannot receive activation through stimulation provided by professional antigen presenting cells (APCs) in secondary lymphoid organs since the native receptors on CAR-modified T cells are not specifically directed towards antigens on the hosts' APCs. Therefore, T cells grafted with first-generation artificial T-cell receptors have a limited capacity to become fully activated when the transgenic receptor engages the specific antigen.[37] The modification of these artificial receptors by incorporation of co-stimulatory molecules such as CD28 within the construct overcomes this limitation *in vitro*.[34] Furthermore, our group was able to demonstrate in a clinical trial that second-generation CD19-specific CAR-T cells are safe and that CD28 does indeed provide a co-stimulatory signal *in vivo* in patients with B-cell lymphoma compared to first generation CARs.[37] Comparable results were obtained by Kochenderfer et al.[38] and Brentjens et al.,[39] who also used a CD19.CAR with a CD28 endodomain in their studies.

2.4.2. The Role of a Lymphodepleted Environment

It is now well established that expansion of lymphocytes *in vivo* is subject to homeostatic mechanisms that control their total numbers.[40] Therefore, it has become apparent that a certain degree of lymphopenia will facilitate expansion of lymphocytes. While lymphopenia will always be present after autologous stem cell transplantation (given the nature of the conditioning regimens, which are myeloablative and lymphodepleting) and in some patients who may be receiving chemotherapy, subjects who have been off chemotherapy for prolonged periods of time will most likely not be lymphopenic. In this case, induction of lymphopenia, and the concomitant depletion of multiple other inhibitory effector cell populations, before lymphocyte infusion is highly desirable, as evidenced by the much higher rate of complete responses achieved in studies in which administration of cyclophosphamide and/or fludarabine preceded infusion of CAR-T cells.[41-43]

2.4.3. Safety and Efficacy of Targeting the CD19 Antigen with CAR-bearing T cells in Patients with B-Cell Malignancies

Autologous CD19-targeted CAR-T cells produce high rate of complete response (CR) in relapsed/refractory diffuse large B-cell lymphoma (DLBCL) and ALL, even in heavily pretreated patients.[39, 41-56] The largest clinical trials performed so far are summarized on Tables 1 and 2. Two specific acute toxicities have emerged after administration of CD19.CAR-T cells, cytokine release syndrome (CRS) and neurotoxicity.

CRS is a systemic inflammatory response (typically accompanied by a measurable increase in serum cytokines such as IL-6, TNFα and IL-1) that can range from a mild syndrome requiring minimally invasive supportive care to symptoms resembling hemophagocytic lymphohistiocytosis (HLH) and requiring intensive care, vasopressor support, and various degrees of respiratory support (severe CRS).[57, 58] CRS has been a frequent occurrence in all successful trials of autologous CD19 CAR T-cells, regardless of variations in construct, vector, manufacturing methods, or cytoreductive regimens. For unknown reasons, CRS occurs less frequently in trials targeting antigens other than CD19.[59] While neurotoxicity occurs in a smaller subset

of patients, it remains an important toxicity worthy of further study. The identification of IL-6 as the predominant cytokine responsible for the symptoms of sCRS has allowed successful treatment with tocilizumab (an anti-IL6 receptor blocker) without eradication of transduced T cells, while corticosteroids and other anti-inflammatory medications have been used as well.[59] More recently, several treatment algorithms have been created that guide the systematic management of sCRS related to CAR T cell infusion, which has reduced the morbidity of this phenomenon.[57]

Despite significant adverse events, the outstanding results observed in multicenter studies led to the recent approval by the FDA of two autologous CD19.CAR-transduced T-cell products for treatment of these diseases: tisagenlecleucel (using a second generation CAR that includes 4-1BB as a costimulatory domain) for ALL and DLBCL; and axicabtagene ciloleucel (using a second generation CAR that includes CD28 as a costimulatory domain).

Table 1: CD19.CAR-T Cells in Relapsed or Refractory ALL

Reference	Center	Population	Number of patients	Efficacy, CR
Maude, NEJM 2018[55]	Multicenter (University of Pennsylvania)	Pediatric and young adults	75	81%
Park, NEJM 2018[56]	Memorial Sloan-Kettering Cancer Center	Adults	53	83%
Lee, Lancet 2015[51]	National Cancer Insitute	Pediatric and young adults	21	67%*
Turtle, JCI 2016[60]	Fred Hutchinson Cancer Center	Adults	29	93%

Table 2: CD19.CAR-T Cells in Relapsed or Refractory DLBCL

Reference	Center	Population	Number of patients	Efficacy, CR (PR)
Kochenderfer, JCO 2015[61]	Multicenter (University of Pennsylvania)	Adults	30	53% (27%)
Schuster, NEJM 2017[54]	Memorial Sloan-Kettering Cancer Center	Adults	28	57% (7%)
Turtle, STM 2016[52]	National Cancer Institute	Adults	32	54% (28%)
Neelapu, NEJM 2017[53]	Multicenter (MD Anderson Cancer Center)	Adults	101	54% (28%)

2.5. Limitations of CD19.CAR-Transduced Autologous T-Cell Products

The use of autologous CAR-T cells is limited by the requirement for patient leukapheresis and the delay of therapy until the autologous product is manufactured, tested and released. In addition, patient heterogeneity results in variability of the manufactured autologous products. Moreover, autologous CAR T cell therapy is less effective in lymphoma patients, who have decreased frequencies of the more highly proliferative naïve T cell population, reducing their capacity for expansion and the rate of success in their manufacture and infusion.[62] In contrast, an allogeneic CD19 targeted therapy can be produced in advance in large quantities, with more consistent properties from dose to dose. These properties make an allogeneic product nearly immediately available without subjecting patients to leukapheresis procedures and treatment delay. However, the use

of allogeneic T cell products is limited by graft-versus-host disease (GVHD) and immune rejection (or host-versus-graft activity, HVG). Alternative strategies are thus needed to develop CAR-redirected immunotherapy that is consistently effective in lymphoma patients and does not pose a risk of GVHD or rapid rejection by the recipient.

2.6. Rationale for Using a CD19.CAR-Transduced NKT Cell Product

NKT cells are a subset of innate lymphocytes that can be (i) readily expanded to clinical scale; (ii) safely infused into immunosuppressed individuals without causing GvHD; and (iii) possess natural antitumor properties beyond their CAR-encoded specificity, decreasing the risk of therapy failure due to CAR epitope escape variants. NKTs express an invariant TCR α-chain and their cytotoxicity is restricted by CD1d,[63-65] which is expressed by both normal B lineage cells and B cell malignancies.[66-68] CD1d is detected in about 30% of tumors from NHL patients,[69] as well as on tumor-associated macrophages (TAMs) that are associated with poor outcome in DLBCL patients and killed directly by NKTs.[27,31] Furthermore, NKTs have been proven safe and well-tolerated in numerous clinical studies while also inducing objective clinical responses.[70,71] Since NKTs are often functionally defective in cancer patients,[72-74] we propose that allogeneic (or third-party) NKTs expressing a CD19 CAR could be used after cytoreductive host conditioning to treat patients with relapsed/refractory $CD19^+$ aggressive or indolent B-cell lymphomas and other $CD19^+$ malignancies. Using "off-the-shelf" donor-derived NKTs would eliminate the manufacturing delay characteristic of autologous cellular therapies, in addition to minimizing heterogeneity and maximizing yield of the final product. We will employ a CD19 CAR that is broadly comparable to other clinically validated constructs (Figure 1).

2.7. Rationale for Reducing HLA Class I and II Expression in CD19.CAR-NKTs and Including IL-15 in the NKT Cell Product

All normal nucleated cells express HLA class I, and therefore adoptively transferred therapeutic cells from HLA mismatched donors will eventually be eliminated by the host immune system. T and NKT cells can also transiently express HLA class II when activated, and HLA class II mismatch triggers donor cell elimination (HVG) by host CD4 T cells. To limit the severity and toxicity of immunosuppressive host conditioning prior to infusing these allogeneic cells, we propose to knockdown expression of HLA class I and II molecules on the transduced NKT cells by using targeted shRNAs encoded within the CAR construct to allow transduced NKTs an extended therapeutic window prior to rejection by the host immune system.

We will target B2M in donor-derived NKTs to disrupt cell surface expression of HLA class I molecules and prevent their recognition by host CD8 T cells.[75] We will use shRNA-mediated knockdown to achieve a level of B2M/HLA class I expression that is sufficient to prevent activation of host CD8 T cells without triggering host NK cell cytotoxicity.[76] To attenuate NKT cell rejection by host CD4 T cells, we will additionally target CD74 (also known as Class II invariant chain, or Ii), which facilitates the export of HLA class II from the endoplasmic reticulum and is required for proper antigen loading and surface expression of HLA class II-antigen complexes. We reasoned that combined targeting of B2M and Ii in NKTs would further prolong tolerance of cancer patients to therapeutic CD19 CAR NKTs from HLA-unmatched healthy donors and maximize the antitumor activity of these cells without the risk for GvHD.

To improve CD19 CAR NKT cell expansion, persistence, and antitumor activity, the human cytokine IL-15 will also be co-expressed from the CAR construct. IL-15 is critical for the development and peripheral maintenance of CD8 T, NK, and NKT lymphocytes,[77]

and increases the antitumor efficacy of CAR T and NK cells in pre-clinical models.[78, 79] The impact of co-expressing IL-15 with a CAR is under evaluation in two ongoing clinical trials, including the GINAKIT2 study at our center (NCT03294954).

3. Structure of the CD19-specific Vector

The CD19-specific CAR construct (CD19.CAR-28.15.Sh.B2m-Ii) contains the FMC63 scFv, a hinge and transmembrane domain from human CD8α, a costimulatory domain from human CD28 (signal 2), and a signaling domain from human CD3ζ (signal 1). The construct also encodes secreted human IL-15 (signal 3), and is further designed to reduce expression of class I and II HLA molecules in transduced NKT cells by knocking down expression of β2-microglobulin (B2M) and Ii (CD74) using shRNAs (Figure 1).

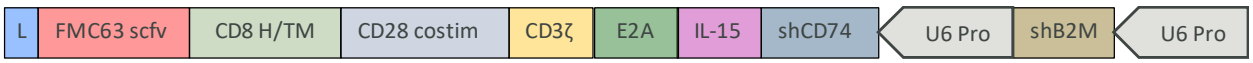

Figure 1. Schematic of CD19 CAR construct with shRNA sequences for expression in NKT cell product. scFv= single chain variable fragment, H= hinge, TM= transmembrane, sh= shRNA, U6 Pro= U6 promoter.

4. Generation of Transduced Allogeneic NKT Cells

All manufacturing procedures will have been performed in our GMP facility as dictated by Standard Operating Protocols (SOP). Brief summaries are given here.

4.1. Retroviral Production. The 293Vec-Galv cell line will be thawed from the Master Cell Bank, expanded and transfected with the CD19 CAR retroviral construct using the PEIpro-HQ reagent. The conditioned medium containing retroviral particles will be collected 48 h and 72 h later, centrifuged and purified by filtering via a 0.45 µm filter. The final product will be aliquoted, snap-frozen and subjected to the required testing procedures. All batches of retroviral supernatant will be tested again in particular to exclude Replication Competent Retrovirus (RCR) and issued with a certificate of analysis as directed by our SOPs and Vector Production master-file with the FDA. The remaining aliquots will be stored at -80°C.

4.2. Source Material. A healthy, consenting qualified donor will have undergone leukocyte apheresis as per our SOPs F03.01.XX and F03.02.XX. The apheresis product will have been used to generate the cellular components listed below for treatment and for infectious disease testing as per the same SOPs.

4.3. Manufacturing process. Briefly, NKT cells will have been positively selected from the leukapheresis product, using NKT-specific magnetic beads and a CliniMACS instrument (Miltenyi) followed by stimulation with the NKT-cell specific ligand α-galactosylceramide, retroviral transduction with CD19.CAR-28.15.ShB2M-Ii retroviral vector, and expansion in culture supplemented with IL-2 and IL-21. We will use the A4-4 artificial antigen-presenting cell (aAPC) line for restimulation of NKTs, and follow specific requirements for allogeneic donors.

4.4. Testing and Freezing. When sufficient numbers of CD19.CAR-aNKTs for infusion are achieved, cells will be collected and tested for cytotoxicity against CD19-positive tumor cells and CD19-negative cell lines to assess receptor function. The cellular product will be checked for identity, phenotype and microbiological culture, and cryopreserved prior to administration according to our SOPs. RCR testing will be performed as per FDA requirements. All results will be reviewed by QA prior to issuing a certificate of analysis.

4.5. Handling of Cells Prior to Administration. Products that meet study specific release criteria, as detailed on the CofA, will be infused as per Section 6.4. Immediately prior to infusion, CD19.CAR-aNKTs will be removed from liquid nitrogen storage and then

transported to the bedside for infusion.

5. Eligibility Criteria

5.1. Inclusion Criteria

Patients must meet the following eligibility criteria to be included for treatment:

5.1.1. Diagnosis of CD19-positive B-cell lymphoma or leukemia (ALL or CLL).

5.1.2. The disease is:

5.1.2.1. Cohort A (non-ALL patients):

5.1.2.1.1. Relapsed or refractory after two or more lines of therapy, including a CD20 antibody, if an indolent lymphoma.

5.1.2.1.2. Relapsed or refractory after two or more lines of therapy, including ibrutinib and venetoclax, if CLL.

5.1.2.1.3. Relapsed or refractory after two or more lines of therapy, including a CD20 antibody and an anthracycline, and the patient is ineligible for autologous stem cell transplantation, if an aggressive or highly aggressive lymphoma:

5.1.2.1.3.1. Ineligibility for autologous stem cell transplantation includes non-responsive disease after salvage therapy and failure to mobilize stem cells for transplant.

5.1.2.2. Cohort B (ALL patients):

5.1.2.2.1. Relapsed or refractory after two or more lines of therapy, if ALL.

5.1.3. Measurable disease by current criteria (Lugano criteria for lymphomas, IWG criteria for CLL, and detectable disease for ALL).

5.1.4. Age ≥ 3 and ≤75 years.

5.1.5. Bilirubin less than 2 times (3 times if the patient has Gilbert syndrome) the upper limit of normal.

5.1.6. AST and ALT less than 5 times the upper limit of normal.

5.1.7. Estimated GFR ≥ 50 mL/min.

5.1.8. Pulse oximetry of ≥ 90% on room air

5.1.9. Karnofsky or Lansky score of ≥ 70

5.1.10. Recovered from the acute toxic effects of all prior chemotherapy based on the enrolling physician's assessment (if some effects of chemotherapy are expected to last long term—for example, residual neuropathy, anemia or alopecia—patient is eligible if meeting other eligibility criteria).

5.1.11. Life expectancy of greater than 12 weeks.

5.1.12. Sexually active patients must be willing to utilize one of the more effective birth control methods during the study and for 6 months after the study is concluded. The male partner should use a condom.

5.1.12.1. Acceptable methods include long-acting reversible contraception (such as intra uterine device, IUD), hormonal contraception (such the pill or depot progestogen injections), barrier methods (such as condoms), or permanent

contraception (such as vasectomy or tubal ligation).

5.1.13. Patients must sign an informed consent indicating that they are aware this is a research study and have been told of its possible benefits and toxic side effects. Patients or their guardians will be given a copy of the consent form.

5.2. Exclusion Criteria

5.2.1. Currently receiving any investigational agents or received any cellular therapies within the previous 6 weeks.

5.2.2. History of grade 2 to 4 graft-versus-host disease (GVHD).

5.2.3. History of hypersensitivity reactions to murine protein-containing products.

5.2.4. Pregnant or lactating.

5.2.5. Active infection with HIV or HTLV.

5.2.6. Active infection with HBV or HCV

5.2.7. Uncontrolled active bacterial, fungal or other viral infection

6. Treatment Plan

6.1. Cytoreductive Chemotherapy

In accordance with the rationale described in Section 2.4.2, patients will receive 3 daily doses of cyclophosphamide (500 mg/m^2/day) together with fludarabine (30 mg/m^2) to induce lymphopenia, finishing at least 24 hours and at most 2 weeks before CD19.CAR-aNKT cell infusion. Infusions should be given following hospital/pharmacy recommendations. However, at a minimum, the cyclophosphamide should be infused over 1 hour and the fludarabine should be infused over 30 minutes. Mesna, IV hydration and anti-emetics should also be provided following local institutional guidelines.

6.2. CD19.CAR-aNKT Dosing

Four dose levels will be evaluated, using the strategy described in Section 10. We will study 2 cohorts of patients, based on potential distinct safety profiles and possible maximum tolerated dose of the conditions based on the nature of the underlying disease and disease burden: cohort A (non-ALL) and cohort B (ALL). Dose levels are defined based on number of transduced NKT cells. The cell dose escalation plan is based on data anticipated from the GINAKIT2 study of autologous CAR NKTs in neuroblastoma, where the starting dose is 3 × 10^6 transduced NKT cells/m^2. Body surface area (BSA) will be capped at 2.4 m^2. We will ensure that subjects will receive less than the maximum number of allogeneic T cells/kg allowed for any dose level (see Table).

Dose level	Number of CD19.CAR-aNKTs	Maximum allogeneic T cells
1	1×10^7/m^2	1×10^4/kg
2	3×10^7/m^2	3×10^4/kg
3	1×10^8/m^2	1×10^5/kg
4	3×10^8/m^2	3×10^5/kg

CD19.CAR-aNKT cell infusion should be delayed if the patient is experiencing unexplained fever, for example, fever not related to the underlying malignancy or to a known, controlled infection. There will be a gap of 4 weeks between the first and second

patient on each dose level. There will be a gap of 4 weeks between the second and third patient on dose level one and a gap of 2 weeks between the second and the third patient on additional dose levels. In addition, there will be a gap of 4 weeks between the last patient on any dose level and the first patient on a higher dose level. Subjects < 18 yrs of age will not be enrolled as the first patient on any dose level.

6.3. Pre-medication

Patients may be pre-medicated with diphenhydramine up to 1 mg/kg IV (max 50 mg) and acetaminophen up to 20 mg/kg PO (max 650 mg). Steroids should be avoided given their detrimental effect on the survival of the infused aNKT cells. Anti-emetics in appropriate dosage for each patient may be prescribed as necessary.

6.4. Administration

Patients will be given the CD19.CAR-aNKT product by intravenous injection over 1 to 10 minutes, and the line will be flushed with saline afterwards. The expected volume of product is between 1 and 50 mL.

6.5. Monitoring

Monitoring will be undertaken according to institutional standards for administration of blood products, with the exception that the injection will be given by a physician. Patients will be monitored for at least 2 hours post infusion.

6.6. Repeating Patient Treatment

In the event that patients do not develop progressive disease by the time of disease assessment, they can be eligible to receive another cycle of lymphodepleting chemotherapy (depending on blood count recovery) and/or infusion of T cells provided they continue to fulfill all treatment eligibility criteria (Section 5.0). In this scenario the treatment calendar will reset to Day 0 as the day of the repeat T cell infusion.

6.7. Post-Administration Patient Treatment

Patients will receive supportive care for acute or chronic toxicity, including blood components or antibiotics, and other interventions as appropriate. All treatments will be given at the Center for Cell and Gene Therapy, in Texas Children's Hospital or Houston Methodist Hospital. Patients will be carefully monitored by their treating physicians for evidence of incipient cytokine release syndrome (onset of fever, malaise and dyspnea within 3 weeks of administration) or graft-versus-host disease, and treated promptly if necessary (see Section 7.4 below). Patients who are not treated locally should stay in Houston, less than 1 hour away from the Texas Medical Center, for at least 4 weeks following T cell infusion.

7. Risks of This Study

Potential toxicities may be categorized as those related to (1) infusion of allogeneic cells, (2) retroviral transduction, (3) cross-reactivity with normal tissues, (4) tumor lysis syndrome/cytokine release syndrome and neurotoxicity, (5) secretion of IL-15, and (6) chemotherapy.

7.1. Co-Infusion of Allogeneic T Cells

In this study, we will be administering transduced allogeneic NKT cells. Given the invariant nature of their antigen receptor, there is no anticipated risk of alloreactivity. Although we will be using release criteria that include a specification for ≤ 5% T cells in the final product, there is the potential concern that these co-infused T cells could lead to graft-versus-host disease (GVHD).

We have extensive clinical experience with adoptive transfer of allogeneic hematopoietic

stem cell transplantation donor-derived virus-specific T-cells to treat a variety of post-transplant infections. These virus-specific T cell lines recognize up to 10% of a panel of 44 HLA disparate targets, indicating possible cross-reactivity with HLA-mismatched targets in vitro. Nonetheless, in 153 recipients, including 73 instances where there was an HLA mismatch, we saw no de novo acute GVHD after infusion of total cell numbers ranging from 5×10^6 to $1.35\times10^8/m^2$, and the incidence of GVHD reactivation was low and not significantly different in recipients of HLA-matched or mismatched T-cells.[80] Furthermore, we have administered third-party multivirus-specific T-cell (mVST) products that were cultured for 11 to 16 days while undergoing two rounds of stimulation against viral peptides. The PBMCs used for product manufacture were obtained from healthy donors and were not subjected to any cell selection. These products have been infused at a dose of $2\times10^7/m^2$ into 38 recipients who were HLA-mismatched in at least 1 to 6 loci. Despite this degree of HLA mismatch, only two occurrences of de novo GVHD (grade 1) were observed.[81] These data are consistent with the observations by others that there is a significant reduction in the frequency of allospecific T-cell precursors after T-cell culture in vitro,[82, 83] and such allospecific T cells are likely further diluted due to antigen-specific stimulation of therapeutic NKT cells in our study.[81]

Similar to the manufacture of mVST cells, NKT cells will be cultured with two rounds of stimulation by a specific antigen. Given the lack of alloreactivity of third-party mVST cells, expanded in culture for 11 to 16 days without any cell selection,[81] we believe the likelihood of our highly selected NKT cell product cultured for 15 to 20 days having alloreactivity is very limited. With our proposed starting cell dose of $1\times10^7/m^2$ CD19.CAR-aNKT cells, the total number of infused cells in a product with at least 90% NKT cell purity and 15% CAR expression would be $1\times10^7 \div 0.9 \div 0.15 = 7.4 \times 10^7/m^2$, which would contain a T cell dose of at most $3.7 \times 10^6/m^2$ ($0.05 \times 7.4 \times 10^7/m^2$, if ≤ 5% T cells in the product is used as a release criterion), which is below the $2\times10^7/m^2$ dose of allogeneic mVST cells that has been shown to be safe.

The CD19.CAR-aNKT product generated in our GMP lab and intended to be used in our protocol contains 0.04% T cells. Even with our maximum proposed cell dose of $1\times10^8/m^2$ CD19.CAR-aNKT cells, the total number of infused cells in that product (which has 99.83% NKT cell purity and 19.81% CAR expression) would be $1\times10^8 \div 0.9983 \div 0.1981 = 5.1 \times 10^8/m^2$, which corresponds to a T cell dose of $2.04 \times 10^5/m^2$ ($0.0004 \times 5.1 \times 10^8/m^2$) in the volume of product to be infused. This number is well below the $2\times10^7/m^2$ dose of allogeneic mVST cells that has been shown to be safe. Nonetheless, we will ensure that we will not infuse at any point product amounts that would result in administering more than $2\times10^7/m^2$ T cells to a patient.

Acute GVHD manifests with skin rash, diarrhea and liver abnormalities, for which hematological malignancy patients are routinely assessed. Mild forms of GVHD can be treated with observation or topical steroids. More severe forms require high dose systemic steroids (1-2 mg/kg/day of methylprednisolone or equivalent), which kill T lymphocytes. Acute GVHD will treated per SOP F08.03.XX.

7.2. Retroviral Transduction

Retroviral transduction results in new, random integrations in host cell DNA, which rarely may cause abnormal or uncontrolled proliferation. This effect is much more common with replication-competent retrovirus (RCR) where each cell receives multiple integrants. We will test all batches of vector supernatant with biological assays of RCR to exclude this possibility and test transduced cells and follow up samples from patients in accordance with FDA guidelines. Several publications have now reviewed extensive and long-term data on RCR monitoring in T cells immediately after gene modification with retroviral and

in subsequent follow-up samples from patients; no evidence for RCR has yet been found, providing important evidence for safety.[84-86]

Genotoxicity with retroviral vectors was seen in the initial trials transferring retroviral vectors to hemopoietic stem cells to correct inherited immunodeficiencies in the late 1990s and early 2000s. These studies demonstrated clinical activity in patients, but several years after treatment, some patients in the SCID trials, as well as patients treated for chronic granulomatous disease and Wiskott-Aldrich syndrome, developed acute leukemias due to activation of proto-oncogenes adjacent to proviral insertions.[87-89] These serious toxicities led to adoption of enhancer-deleted lentiviral or retroviral vectors for HSC clinical gene therapies. Up to now, these newer vectors have produced disease amelioration without genotoxicity.[90]

Our proposal should have a very different risk profile. The transduced cells will have no particular proliferative advantage over non-transduced NKT cells. Human gene-therapy studies of a chimeric receptor composed of the extracellular domain of CD4 and the intracellular domain of TCR-ζ (as in our study) retrovirally transduced into peripheral-blood T-cells to treat HIV have been performed.[91, 92] Large doses of transduced T-cells (in the order of 10^{10} cells) were administered and no leukemogenesis occurred. Although the inclusion of the CD28 endodomain in the CAR may enhance proliferation of transgenic NKT cells in response to the CD19 antigen, there is no reason to believe this effect will lead to uncontrolled NKT cell expansion. Ex vivo and published in vivo data using several artificial T-cell receptors show that CD28 sustains limited and temporary expansion over approximately 3-6 weeks.[93, 94] Thereafter, the cells maintain the expression of the transgene, but proliferate further only in the presence of both antigen and exogenous cytokines. Our recent clinical trials using CD19.CAR-T cells including the CD28 endodomain also indicate that these T cells expand significantly for only 2-3 weeks and then they decline without evidence of further expansion.[37]

To date more than 1000 patients have received genetically modified cells in clinical trials, including patients we have treated on our protocols – using retrovirally marked autologous marrow,[95] retrovirally marked EBV-CTLs,[96, 97] or CAR T-cells. In none of these has new malignancy caused by retroviral transduction been reported. However, there is a possibility that the vector could randomly integrate into a site that could lead to leukemogenesis.

Current intensive regimens to treat relapsed leukemia and lymphoma describe subsequent secondary malignancy rates in excess of 10%.[98] In light of this, the natural history and poor prognosis of advanced lymphoid malignancies, and given the entire previous experience with retroviral gene therapy, we feel that the risks of retroviral induced leukemogenesis are small and are justified in this patient group.

Because of the concern with spontaneous reversion to a replication-competent form of the vectors, the US Food and Drug Administration (FDA) published guidance for monitoring clinical vector lots, manufactured cell products, and patients post-infusion using biologic or PCR-based testing to detect RCR or RCL.[84] In the 25 years since this guidance was published, retroviral packaging cell line and vector designs have minimized the homology between vector and packaging cell sequences and have segregated packaging genes so that the generation of RCR is extremely unlikely.

In 2012, Bear et al.[99] published a multicenter review reporting that RCR screening using amplification in HEK293 cells and analysis with the S^+L^- focus assay were consistently negative from 30 master cell banks (MCBs) and 42 viral supernatant lots used in clinical trials at 4 centers. These vectors were used to generate 297 genetically-modified T cell

products, all of which were themselves RCR negative. RCRs were also absent in 629 patient follow-up samples.[99] A recent letter from Lyon et al.[85] updates the Baylor College of Medicine experience with 9 different retroviral vector lots tested for RCR. Again, all were negative. These vectors were used in 17 clinical trials to manufacture 266 T cell lines, all of which tested negative for RCR using a co-culture assay. A total of 549 patient samples from 220 patients infused with these products have been assayed, and all have been negative.[85]

The results from these studies provide compelling safety data attesting to the low risk of generating RCR with current retroviral PG13 packaging cells and vectors.[86]

7.3. Cross-Reactivity

With the important exception of hematopoietic stem cells, CD19 is expressed during all stages of B-cell differentiation. Direct hematotoxicity from targeting of CD19 expressing normal tissues by transduced cells is possible. In this eventuality, several therapeutic maneuvers are available. Corticosteroids in doses used to treat graft-versus host-disease will deplete the majority of circulating transduced cells. It is also possible to replace immunoglobulins, although it is also important to consider that many of these patients can also have long lasting B cell deficiency due to previous antibody therapy with rituximab, which is standard of care for these diseases, and not necessarily caused by the treatment proposed in this trial.

7.4. Cytokine Release Syndrome/Tumor Lysis Syndrome/Neurotoxicity

As described in Section 2.4.3 above, there have been several reported SAEs associated with cytokine release syndrome (CRS) in patients who received T cells expressing CARs containing additional costimulatory endodomains. We will collect serum before and after infusion for cytokine monitoring, and continue to monitor subjects for 2 hours after infusion. Patients will then be monitored by frequent telephone contact or physical examination for 3 weeks by their treating physicians for evidence of incipient CRS (onset of fever, malaise and dyspnea) and treated promptly. Management of CRS is described in more detail in SOP F05.11.XX and includes treatment options based on the clinical severity of the symptoms such as oxygen, inotropic agents, IL-6 receptor antibody (tocilizumab), and/or steroids.

Patients with highly aggressive lymphomas (e.g. Burkitt lymphoma) and a large tumor burden have a higher risk of tumor lysis syndrome and will be admitted for monitoring for this complication during the first 3 days after CD19.CAR-aNKT. During this period, they will receive prohylaxis with allopurinol and intravenous hydration; rasburicase will be used if indicated. Electrolyte abnormalities and potential renal dysfunction will be managed per standard practice.

There have been several reports of neurotoxicity (e.g. encephalopathy, somnolence, aphasia) after the infusion of CD19-specific CAR T cells.[41, 43, 51] Patients will be monitored closely as per study calendar and assessed for evidence of incipient neurotoxicity (mild somnolence, drowsiness or sleepiness) and treated promptly. Management will include steroids, anakinra, and anti-epileptics as detailed in SOP F 05.11.XX and described in the literature.[41, 43, 51]

7.5. IL-15 production by NKT cells

The systemic administration of IL-15 has been evaluated in a phase 1 study showing promising antitumor properties and acceptable safety profile at the lowest dose level (0.3µg/kg/day for 12 doses). However, higher systemic doses were associated with dose limiting toxicities of hypotension, thrombocytopenia and elevation of AST/ALT

enzymes.[100] CD19-CAR T cells engineered to express IL-15 have been tested in preclinical models to evaluate the effect of locally produced IL-15. These studies showed improved antitumor efficacy compared to T cells expressing CD19-CAR alone without reported toxicity in mice.[78, 101] Our own preclinical results demonstrate the beneficial effect of IL-15 on the antitumor properties of CD19.CAR-aNKTs. Our safety assessment showed no toxicity to murine tissues by histologic evaluation, undetectable systemic levels of IL-15 after adoptive transfer of CD19.CAR-aNKTs in mice, and a lack of autonomous, cytokine-independent growth of CD19.CAR-NKTs. Although these data support a low risk of additional toxicity from the incorporated IL15, the co-expression of IL-15 in tumor-redirected NKTs has not been tested in humans, except in our ongoing GINAKIT2 trial. Since patients eligible for the current study will have relapsed or refractory diseases, and given the apparent safety in preclinical testing, we believe that the potential benefits of co-expressing IL-15 with a CD19.CAR in NKTs outweigh the potential risks.

7.6. Chemotherapy

7.6.1 Cyclophosphamide

Cyclophosphamide is commercially available. A list of toxicities is attached in Appendix I.

7.6.2 Fludarabine

Fludarabine is commercially available. A list of toxicities is attached in Appendix I.

8. Patient Evaluation

8.1. Study Calendar

The detailed schedules for clinical monitoring are included below.

Study		Treatment							Follow-up				
	Pre-Day 0	Day 0	Day 7	Wk 2	Wk 3	Wk 4	Wk 6	M3	M6	M9	M12	Every 6 months × 4 years	Annually × 15 years (total)
Cy/Flu	X												
aNKT cell infusion		X											
Hx	X	X[$]	X	X	X[†]	X	X	X	X	X	X		X
PE	X	X	X	X	X	X	X						
Weight	X												
VS	X	X	X	X	X	X	X						
Pulse Ox	X												
Performance Status	X	X	X	X		X	X	X	X	X	X		X
Acute GVHD evaluation		X	X	X	X	X							
Pregnancy Test [1]	X												
HLA-typing (if missing)	X												
CBC d/p	X	X	X	X		X	X	X	X	X	X		X
Lytes/BUN/Cr	X	X	X	X		X	X	X	X	X	X		
AST/Bili/Alb	X	X	X	X		X	X	X	X	X	X		
LDH	X					X							
CRP	X*												
Imaging (see 8.5)	X					X[2]		X	X	X	X		

[1]Pregnancy testing will be done pre-chemotherapy in female patients of childbearing potential.
[2]Week 4-6.
[3]Week 8-12.
[4]See Section 8.7.1 on additional time points to be collected if transgene detected.
[5]This sample will be collected at any point, if a new malignancy develops.
[6]Week 1-6.
*and when clinically indicated
**also collected at 2-4 hours post infusion.
***see Section 8.7.5 below regarding HAMA.
[#]see Section 8.7.4 below regarding cytokine testing.
[†]These studies are optional.
[$]If stable, perform three times weekly until Day 28

Study		**Treatment**						**Follow-up**					
	Pre-Day 0	Day 0	Day 7	Wk 2	Wk 3	Wk 4	Wk 6	M3	M6	M9	M12	Every 6 months × 4 years	Annually × 15 years (total)
Bone marrow aspirate and biopsy (see 8.6)	X					X[2]		X[3]	X	X	X		
IgG/A/M	X							X	X	X	X		
Function and Persistence Studies	X**		X	X	X†	X	X	X	X	X	X	X[4]	X
Archive samples for RCR testing	X												X[5]
Cytokine testing#	X	X	X	X	X†	X	X						
HAMA testing***	X						X						
Viral serologies (if missing)	X												
Biopsy of accessible lymph nodes					X[6]								

[1]Pregnancy testing will be done pre-chemotherapy in female patients of childbearing potential.
[2]Week 4-6.
[3]Week 8-12.
[4]See Section 8.7.1 on additional time points to be collected if transgene detected.
[5]This sample will be collected at any point, if a new malignancy develops.
[6]Week 1-6.
*and when clinically indicated
**also collected at 2-4 hours post infusion.
***see Section 8.7.5 below regarding HAMA.
#see Section 8.7.4 below regarding cytokine testing.
†These studies are optional.
$If stable, perform three times weekly until Day 28

8.2. History and Physical Examination

History will be done pre-chemotherapy, or pre-infusion of CD19.CAR-aNKT cells and at 1, 2, 3 (optional), 4 and 6 weeks post infusion of aNKT cells, with particular emphasis in symptoms and signs of CRS, neurotoxicity and GVHD. History will then be taken every three months for a year, and then yearly for a total of 15 years. For safety, patients will be monitored 3 times weekly (with remote visits allowed) until the end of the fourth week post infusion of aNKT, with particular emphasis in symptoms and signs of CRS. Starting after week 4, patients who cannot visit the CAGT clinic may be contacted by the research nurse, coordinator or physician, instead.

8.3. Acute GVHD Evaluation

GVHD will be monitored for 4 weeks following infusion. Weekly GVHD organ stage scores, overall clinical grade, biopsy information for GVHD and relevant differential diagnosis will be recorded. The weekly score will encompass all information since the last assessment. Organ involvement, biopsy information, staging, differential diagnosis, and GVHD therapy will be documented in the medical record using the MAGIC criteria (Appendix III).

8.4. Routine Laboratory Investigations

8.4.1. The following routine laboratory investigations will be obtained as outlined in the study calendar:

8.4.1.1. CBC and differential

8.4.1.2. BUN, Creatinine, Na^+, K^+, Cl^-, CO_2

8.4.1.3. Bilirubin, Albumin, AST

8.4.1.4. LDH

8.4.1.5. CRP

8.4.2. While receiving chemotherapy, comprehensive metabolic profiles and complete blood counts with differential counts may be monitored as clinically indicated. If a patient develops CRS and is admitted, he/she will receive more frequent monitoring per the SOP F05.11.XX.

8.4.3. HIV and HTLV serologies will be obtained within 6 months before infusion, unless the patient has had a potential exposure or a recent clinical picture consistent with acute infection with either of these viruses.

8.4.4. Pregnancy test is required for female patients who have reached menarche unless they have no possibility of pregnancy (for example, post hysterectomy).

8.4.5. Immunoglobulins will be measured pre study and then every 3 months for a year. If patients still have CD19.CAR-aNKT cells detectable at a level of 1% of all peripheral blood nucleated cells or greater at any time study point, immunoglobulins will be measured at the following study follow up. If immunoglobulin levels decrease from baseline and the attending physician feels there is a significant risk of infection, the patient will be started on replacement therapy with IVIg.

8.4.6. For patients with a disease marker, such as t(14:18), we will collect data on detection during restaging performed for routine clinical care.

8.5. Diagnostic Imaging

For extramedullary disease, diagnostic imaging to document measurable disease and

response to therapy (PET, CT scans, MRI or nuclear imaging, as appropriate) will be obtained pre-infusion (within 4-6 weeks) and at 4-6 weeks following the infusion. The time may vary by 1-2 weeks due to scheduling constraints and may be done earlier if clinically indicated. The choice of imaging will depend on what studies have been most informative in following the patient's disease. If diagnostic imaging studies are performed at other times either during or after treatment on this study, that data will be collected and information gained will be used for this study. Additional imaging will be obtained at 3, 6, 9, and 12 months.

8.6. Diagnostic Bone Marrow Examination

For diseases involving the bone marrow, to document measurable disease and response to therapy, bone marrow aspirate (5 to 20 mL) and biopsy will be collected pre-infusion, at 4-6 weeks post infusion, and again at 8-12 weeks post-infusion. The material will be sent for routine pathology and will be used for tests described in Section 8.7. Additional bone marrow examinations will be obtained at 6, 9, and 12 months.

8.7. Tests of Function, Persistence and Safety

8.7.1. **Quantitative real-time PCR to detect retroviral integrants:** This analysis will be used to monitor function and persistence on peripheral blood and safety of transduced CD19.CAR-aNKT cells at time points indicated in the study calendar. If detection of CD19.CAR-aNKT cells is greater than 0.5% and there is persistent CD19.CAR-aNKT cell expansion 3 months after T-cell infusion, we will perform clonality studies (an extra 5 mL of blood). Further evaluations may be done based on clinical decisions.

8.7.2. **Replication Competent Retrovirus (RCR) Testing by PCR**: Samples for RCR testing by PCR will be collected for archival at a pre-treatment time point.

8.7.2.1. Should a new malignancy develop, RCR testing will be done on the sample collected pre-treatment and on an additional sample collected after the new malignancy has developed.

8.7.2.1.1. If any post-treatment samples are positive, further analysis of the RCR and more extensive patient follow-up should be undertaken in consultation with the FDA.

8.7.2.2. All negative RCR results will be reported to the FDA by way of the IND annual report.

8.7.2.3. Additionally, when the IND is renewed, the FDA will be notified of how many autopsies were requested, how many were obtained, and the results of those autopsies (as they relate to RCR).

8.7.2.4. Aliquots of cells and sera will also be archived for use in future studies for RCR as required by the FDA or RAC.

8.7.3. The following analysis may (depending on the total amount of PBMCs isolated at each time point) be obtained pre-infusion and 2-4 hours post infusion; at 1, 2, 3 (optional), 4 and 6 weeks post- aNKT cell infusion; and then at 3, 6, 9, and 12 months and yearly for a total of 15 years:

8.7.3.1. **Phenotype** to detect the presence of transgenic cells using a specific antibody recognizing the idiotype of the CAR, if the percentage of cells is expected to be greater than 0.1-1%.

8.7.3.2. **ELISPOT for cytokine release** using CD19-positive cells and CD19-negative target cells.

8.7.3.3. **Other functional assays**, such as in vitro reactivation of PBMCs, in patients on whom the appropriate reagents are available.

8.7.3.4. **Immunophenotyping**

8.7.4. **Cytokine level testing**
Serum from blood drawn for functional studies, as included in the study calendar, will be stored for measurement of cytokine levels should significant CRS occur. The cytokine panel study will include at a minimum IL-6, IFN-γ and IL-15. Serum collection may occur more often as clinically indicated, as per SOP F05.11.XX.

8.7.5. **HAMA**
Serum from blood drawn for functional studies on pre-week 0 and week 6 will be stored for measurement of human anti-mouse antibodies (HAMA). These studies will be performed only in the event of a suspected immunologic reaction.

8.7.6. **Blood Amount**
A maximum of the lesser of 60 mL or 2 mL/kg of body weight of blood will be drawn on any one day for these assays. If a patient's hemoglobin is ≤ 7.0 g/dL, on a previous evaluation at any of the evaluation times, the amount of blood drawn for the evaluation will be reduced and may be obtained over more than one venipuncture. If there is insufficient blood, these tests will be prioritized in the order given.

8.8. Other tissues

8.8.1. **Biopsy of accessible lymph nodes.** A biopsy will be done at 1-6 weeks after treatment. The sample will be used to assess for the presence of transduced peripheral blood aNKT cells in association with the tumor.

8.8.2. **Additional bone marrow aspirations and other biopsies**. While on study, if bone marrow trephine biopsy (apart from those specified per protocol) or tumor biopsy is required at any time after treatment, a sample of these will be used to assess presence of transduced peripheral blood aNKT cells in association with the tumor.

8.8.3. If the patient dies, an autopsy will be requested. If granted, tissue will be requested to assess presence of transduced cells.

9. Evaluation During Study

9.1. Follow-Up

9.1.1. Patients shall be evaluated (seen in clinic or contacted by research nurse) as outlined in study calendar Section 8.1. Additional visits or telephonic contacts will be obtained as clinically indicated.

9.2. Dose Limiting Toxicity (DLT)

9.2.1. For consistency, toxicity for all patients will be evaluated using the NCI Common Toxicity Criteria Scale (version 5.X located at http://ctep.cancer.gov) with the exception of CRS and neurological toxicities that are related to T-cell infusions. CRS and neurological toxicities will be graded according to the new ASTCT consensus grading (Appendix II). GVHD will be graded according to the MAGIC criteria[102] (Appendix III)

9.2.2. DLT will be defined as any of the following that is NOT (1) pre-existing, or (2) due to infection (to which patients with ALL, CLL and NHL are predisposed), or (3) due to underlying malignancy, and that may, after consultation with the FDA when

indicated, be considered possibly, probably, or definitely related to the study cellular product:

9.2.2.1. Development of GVHD that is:

9.2.2.1.1. Grade III-IV GVHD of any duration;

9.2.2.1.2. Grade II GVHD requiring treatment systemic steroids and does not improve to grade I within 7 days.

9.2.2.2. Non-hematologic DLT is one or more of:

9.2.2.2.1. any grade 4 non-hematologic toxicity (including CRS and neurotoxicity);

9.2.2.2.2. any grade 3 CRS that fails to return to grade 2 or less within 72 hours;

9.2.2.2.3. any seizure or grade 3 neurotoxicity;

9.2.2.2.4. any grade 3 non-hematologic toxicity involving vital organs (heart, lungs or central nervous system);

9.2.2.2.5. any grade 3 renal or hepatic toxicities that persist beyond 7 days;

9.2.2.2.6. any other grade 3 toxicities that persist beyond 72 hours;

9.2.2.2.7. any grade 2 to 4 allergic reactions to CD19.CAR-aNKT cell infusion.

9.2.2.3. Hematologic DLT is defined as any grade 4 hematologic toxicity that fails to return to grade 2 or baseline (whichever is more severe) within 14 days or within 28 days for patients with evidence of bone marrow disease.

9.2.3. Any grade 5 toxicity (with the exception of death due to progressive disease) occurring during the toxicity evaluation period will also be considered a DLT.

9.3. Additional FDA Reporting

9.3.1. All suspected unexpected serious adverse reactions (SUSARs) will be reported to the FDA as per SOP J02.06.XX.

9.3.2. Grade 3 and 4 expected reactions due to CRS and neurotoxicity are seen with the use of CAR-based immunotherapy.

9.3.2.1. Grade 3 cytokine release syndrome (CRS) infusion reactions and neurologic toxicity will only be reported to the FDA if they fail to return to grade 1 within 7 days.

9.3.2.2. Grade 4 CRS and grade 3 or 4 neurologic toxicities will be reported to the FDA in an expedited fashion.

9.3.3. All deaths occurring within 30 days of CD19.CAR-aNKT cells, regardless of attribution, will be reported to the FDA.

9.4. Halting rules

9.4.1. Temporary pausing of the study will take place to permit comprehensive assessment of any observed toxicity and causal relationship of a serious or unexpected adverse event to the CD19.CAR-aNKT cells.

9.4.2. Study accrual will be paused to allow consultation with the FDA for one or more of the following:

9.4.2.1. Any grade 4 toxicity related to CRS lasting more than 72 hours in 2 or more subjects;

9.4.2.2. Any death following CD19.CAR-aNKT cell infusion in the absence of

disease progression;

9.4.2.3. The first study subject at the starting dose level develops a DLT;

9.4.2.4. Any subject develops grade 4 neurotoxicity with cerebral edema;

9.4.2.5. Any subject develops grade III or IV GVHD.

9.5. Early Termination of Study and Modifications of Drug Dosages

9.5.1. The trial will stop when 6 patients have been treated at the MTD.

9.5.2. The only cell or drug dosage modifications allowed will be to ensure that patients receive less than the maximum number of allogeneic T cells/kg allowed at any dose level.

9.5.3. If the patient desires to withdraw from the study or if the physician feels that it is in the best interest of the patient, treatment will be discontinued.

9.6. Measurement of Effect

9.6.1. Although response is not the primary endpoint of this trial, patients with measurable disease will be assessed by standard criteria. Evaluations of tumor size will be performed within 4-6 weeks of the CD19.CAR-aNKT infusion. Additional imaging performed as part of standard clinical care will also be evaluated. All patients who receive an infusion will be evaluable for response.

9.6.2. **Definition of response for NHL**

This study will use the new Lugano Criteria.[103] The response criteria are defined below:

Response	Sites	PET-CT Based	CT Based
Complete (CR)		Complete metabolic response	Complete radiologic response (all of the following)
	Lymph nodes and extralymphatic sites	Score 1, 2, or 3 with or without a residual mass on 5PS†*	Target nodes/nodal masses must regress to ≤1.5 cm in LDi and no extralymphatic sites of disease
	Nonmeasured lesions	Not applicable	Absent
	Organ enlargement	Not applicable	Regress to normal
	New lesions	None	None
	Bone marrow	No evidence of FDG-avid disease in marrow	
Partial (PR)		Partial metabolic response	Partial remission (all of the following)
	Lymph nodes and extralymphatic sites	Score 4 or 5† with reduced uptake compared with baseline and residual mass(es) of any size	≥50% decrease in SPD of up to 6 target measurable nodes and extranodal sites: • when a lesion is too small to measure on CT, assign 5×5 mm as the default value • when no longer visible, 0×0 mm • for a node >5×5 mm, but smaller than normal, use actual measurement for calculation
	Nonmeasured lesions	Not applicable	Not applicable
	Organ enlargement	Not applicable	Not applicable
	New lesions	None	None

Response	Sites	PET-CT Based	CT Based
	Bone marrow	Residual uptake higher than uptake in normal marrow but reduced compared with baseline (diffuse uptake compatible with reactive changes from chemotherapy allowed)**	Not applicable
Stable disease (SD) or no response		No metabolic response	Stable disease
	Target nodes/nodal masses, extralymphatic sites	Score 4 or 5 with no significant change in FDG uptake from baseline at interim or end of treatment	<50% decrease from baseline in SPD of up to 6 dominant, measurable nodes and extranodal sites; no criteria for progressive disease are met
	Nonmeasured lesions	Not applicable	No increase consistent with progression
	Organ enlargement	Not applicable	No increase consistent with progression
	New lesions	None	None
	Bone marrow	No change from baseline	Not applicable
Progressive disease (PD)		Progressive metabolic disease	Progressive disease (requires at least 1 of the following)
	Individual target nodes/nodal masses	Score 4 or 5 with an increase in intensity of uptake from baseline and/or	PPD progression: An individual node/lesion must be abnormal with: • LDi >1.5 cm and • Increase by ≥50% from PPD nadir and • An increase in LDi or SDi from nadir (0.5 cm for lesions ≤2 cm; 1.0 cm for lesions >2 cm) In the setting of splenomegaly, the splenic length must increase by >50% of the extent of its prior increase beyond baseline (eg, a 15-cm spleen must increase to >16 cm); if no prior splenomegaly, must increase by at least 2 cm from baseline New or recurrent splenomegaly
	Extranodal lesions	New FDG-avid foci consistent with lymphoma at interim or end-of-treatment assessment	
	Nonmeasured lesions	None	New or clear progression of preexisting nonmeasured lesions
	New lesions	New FDG-avid foci consistent with lymphoma rather than another etiology (eg, infection, inflammation). If uncertain regarding etiology of new lesions, biopsy or interval scan may be considered	Regrowth of previously resolved lesions A new node >1.5 cm in any axis A new extranodal site >1.0 cm in any axis; if <1.0 cm in any axis, its presence must be unequivocal and must be attributable to lymphoma Assessable disease of any size unequivocally attributable to lymphoma
	Bone marrow	New or recurrent FDG-avid foci	New or recurrent involvement

Abbreviations: 5PS, 5-point scale; CT, computed tomography; FDG, fluorodeoxyglucose; IHC, immunohistochemistry; LDi, longest transverse diameter of a lesion; MRI, magnetic resonance imaging; PET, positron emission tomography; PPD, cross product of the LDi and perpendicular diameter; SDi, shortest axis perpendicular to the LDi; SPD, sum of the product of the perpendicular diameters for multiple lesions.
Measured dominant lesions: Up to six of the largest dominant nodes, nodal masses, and extranodal lesions selected to be clearly measurable in two diameters. Nodes should preferably be from disparate regions of the body

and should include, where applicable, mediastinal and retroperitoneal areas. Non-nodal lesions include those in solid organs (eg, liver, spleen, kidneys, lungs), GI involvement, cutaneous lesions, or those noted on palpation. **Nonmeasured lesions:** Any disease not selected as measured, dominant disease and truly assessable disease should be considered not measured. These sites include any nodes, nodal masses, and extranodal sites not selected as dominant or measurable or that do not meet the requirements for measurability but are still considered abnormal, as well as truly assessable disease, which is any site of suspected disease that would be difficult to follow quantitatively with measurement, including pleural effusions, ascites, bone lesions, leptomeningeal disease, abdominal masses, and other lesions that cannot be confirmed and followed by imaging. In Waldeyer's ring or in extranodal sites (eg, GI tract, liver, bone marrow), FDG uptake may be greater than in the mediastinum with complete metabolic response, but should be no higher than surrounding normal physiologic uptake (eg, with marrow activation as a result of chemotherapy or myeloid growth factors).

†PET 5PS: 1, no uptake above background; 2, uptake ≤ mediastinum; 3, uptake > mediastinum but ≤ liver; 4, uptake moderately > liver; 5, uptake markedly > liver and/or new lesions; X, new areas of uptake unlikely to be related to lymphoma.

*It is recognized that in Waldeyer's ring or extranodal sites with high physiologic uptake or with activation within spleen or marrow (eg, with chemotherapy or myeloid colony-stimulating factors), uptake may be greater than normal mediastinum and/or liver. In this circumstance, complete metabolic response may be inferred if uptake at sites of initial involvement is no greater than surrounding normal tissue even if the tissue has high physiologic uptake.

**If there are persistent focal changes in the marrow in the context of a nodal response, consideration should be given to further evaluation with MRI or biopsy or an interval scan

9.6.3. **Definition of response for patients with CLL**

This study will use the most recent IWG Criteria.[104] The response criteria are defined below:

Parameter	CR*	PR*	PD*
Group A			
Lymphadenopathy†	None > 1.5 cm	Decrease ≥ 50%	Increase ≥ 50%
Hepatomegaly	None	Decrease ≥ 50%	Increase ≥ 50%
Splenomegaly	None	Decrease ≥ 50%	Increase ≥ 50%
Blood lymphocytes	< 4000/μL	Decrease ≥ 50% from baseline	Increase ≥ 50% over baseline
Marrow‡	Normocellular, < 30% lymphocytes, no B-lymphoid nodules. (Hypocellular marrow defines CRi)	50% reduction in marrow infiltrate, or B-lymphoid nodules	
Group B			
Platelet count	> 100,000/μL	> 100,000/μL or increase ≥ 50% over baseline	Decrease ≥ 50% from baseline secondary to CLL
Hemoglobin	> 11.0 g/dL	> 11.0 g/dL or increase ≥ 50% over baseline	Decrease ≥ 2 g/dL from baseline secondary to CLL
Neutrophils‡	> 1500//μL	> 1500//μL or > 50% improvement over baseline	

Group A criteria define the tumor load, group B criteria define the function of the hematopoietic system (or marrow).
*CR (complete remission): all of the criteria have to be met, and patients have to lack disease-related constitutional symptoms; PR (partial remission): at least two of the criteria of group A plus one of the criteria of group B have to be met; SD is absence of progressive disease (PD) and failure to achieve at least a PR; PD: at least one of the above criteria of group A or group B has to be met.
†Sum of the products of multiple lymph nodes (as evaluated by CT scans in clinical trials, or by physical examination in general practice).
‡These parameters are irrelevant for some response categories.

9.6.4. Definition of response for patients with ALL or patients with bone marrow disease

Serial measurements of tumor burden may be performed either using absolute tumor cell numbers in marrow by routine H & E staining or percentage of cells positive for specific cytogenetic abnormalities and/or levels of measurable residual disease (MRD) by flow cytometry and molecular methods. Bone marrow biopsies will be done as dictated by protocol and as clinically indicated.

9.6.4.1. Complete response (CR)

- No circulating blasts or extramedullary disease:
  - No lymphadenopathy, splenomegaly, skin or gum infiltration, testicular mass or CNS involvement.
- Trilineage hematopoiesis and < 5% blasts in bone marrow
- Absolute neutrophil count > 1000/μL
- Platelet count > 100,000/μL
- No recurrence for 4 weeks

9.6.4.2. CR with incomplete blood count recovery (CRi)

- Meets all criteria for CR except platelet and/or neutrophil count

9.6.4.3. Progressive disease (PD)

- Increase of at least 25% in the absolute number of circulating or bone marrow blasts or development of extramedullary disease

9.6.4.4. Partial response (PR)

- Decrease of at least 50% in the percentage of bone marrow blasts but not meeting criteria for CR or CRi

9.6.4.5. Stable disease (SD)

- Not meeting criteria for any of the other categories

10. Statistical Considerations

10.1. Clinical Trial Design

This phase 1 dose-escalation trial is designed to evaluate the safety of CD19.CAR-aNKT cells in patients with refractory/relapsed B-cell NHL or leukemia (ALL or CLL). Each patient will receive one infusion of CD19.CAR-aNKT cells according to the following dosing schedule:

Dose level	Number of CD19.CAR-aNKTs
1	$1\times10^7/m^2$
2	$3\times10^7/m^2$
3	$1\times10^8/m^2$
4	$3\times10^8/m^2$

Patients will be enrolled into 2 parallel cohorts (cohort A, non-ALL; and cohort B, ALL). Dose escalation is guided by the Bayesian optimal interval (BOIN) design[105, 106] in order

to determine the maximum tolerated dose (MTD) of CD19.CAR-aNKT cells. Each cohort will undergo dose escalation independently. Dose-limiting toxicity (DLT) is defined in Section 9.2. Each treated patient will be followed for 4 weeks post the CD19.CAR-aNKT cell infusion for the evaluation of DLTs.

The BOIN design has algorithmic escalation/de-escalation rules like the traditional 3+3 design but allows for specification of various target toxicity rate, cohort size, and expanded accrual. With continued consistent toxicity monitoring, this design seeks to minimize incorrect escalation/de-escalation decision, thereby optimizing dose assignment for each patient. For a broad range of conditions, the BOIN design performs as well as the model-based modified continual reassessment method (CRM) designs in selecting the MTD, but has a lower risk of overdosing or under dosing.[107]

In this trial, three patients will be initially enrolled to the lowest dose level. When these patients are evaluable for DLT after their 4-week toxicity follow-up, we will assign the next cohort of 3 patients according to the decision rule of the BOIN design. The BOIN design is a type of 'interval design', in which dose choices are made according to pre-specified toxicity tolerance intervals. In general, with a target toxicity rate of 0.30 for this trial, we will escalate the dose if the observed toxicity rate at the current dose is <= 0.2365, or de-escalate the dose if the observed toxicity rate at the current dose is >=0.3585. If the observed toxicity rate is between 0.2365 and 0.3585, another cohort of patients will be treated at the current dose. For safety, no dose level will be skipped during dose escalation. Enrollment on the next dose level will wait until the completion of toxicity evaluation period following the T-cell infusion (4 weeks post infusion) for all treated patients enrolled at the current dose level. Dose escalation/de-escalation will continue until the recommendation is to assign the next cohort to a dose level that already has 6 patients treated. Dose escalation/de-escalation is executed according to the rules in the table below:

Dose escalation/de-escalation rule for the BOIN design

Number of patients treated at the current dose	3	4	5	6	7	8	9
Escalate if # of DLT ≤	0	0	1	1	1	1	2
Treat additional 3 patients on current dose if number of DLT =	1	1	NA	2	2	2	3
De-escalate if # of DLT≥*	2	2	2	3	3	3	4
Eliminate dose level and all higher dose from further use if number of DLT≥	3	3	4	4	5	5	5

**At the lowest dose level, if the recommendation is to de-escalate AND the dose level has not been eliminated from consideration, additional patients will be accrued at the same dose level; otherwise the trial will stop.*

"Eliminate" in the table means that we eliminate the current and any higher dose levels from the trial because they are overly toxic. Therefore we will prevent treating any future patients at these toxic dose levels. If the current dose is the lowest dose and the rule indicates dose de-escalation, we will treat additional patients at the current (i.e. lowest) dose unless the number of DLTs reaches the elimination boundary, at which time we will terminate the trial for safety. If the current dose is the highest dose and the rule indicates dose escalation, we will treat additional patients at the highest dose until 6 patients are already treated at the current (i.e. highest) dose.

Once dose escalation is finished and all patients are evaluable for DLT, we will determine the MTD based on isotonic regression as specified in Liu and Yuan,[105] specifically, the

dose for which the isotonic estimate of the toxicity rate is closest to the target toxicity rate. If there is no DLT that determine a MTD, the maximum dose level will be declared as the MTD. In case the lowest dose level is determined to be too toxic and the elimination boundary is reached, no MTD will be defined for the trial.

As mentioned, the cellular therapy infusions have been safe and we do not anticipate seeing any CD19.CAR-aNKT cell DLTs. Hence, upon completion of the dose escalation, we anticipate that a minimum of 3 patients will be treated with a total 6 of patients accrued at the MTD level. Depending on observed toxicities, we expect that at most 24 patients per cohort will be infused in the phase 1 trial.

10.2. Statistical Analysis

10.2.1. Safety Analysis of Adverse Event Data

All patients who received CD19.CAR-aNKT cell infusions will be included in the safety analysis. Adverse event data and corresponding toxicity grades 4 weeks after aNKT-cell infusions and during long-term follow-up will be summarized in the form of tables. Incidence tables will be generated to summarize incidence of patients reporting at least one episode of each specific adverse event, incidence of adverse events causing withdrawal and incidence of serious adverse events. The total number of episodes for each event reported (Frequency Table), the severity and attribution to study therapy of each episode reported (Severity Table and Attribution Table) will also be calculated.

Listings of adverse events by patients will include the time to onset, the duration of each event, the severity of each event, and the relationship of the event to study therapy, whether it was a serious event, and whether it caused withdrawal. Safety data will be summarized for the overall patient group and by dose levels.

If there is any death deemed possibly or probably related to CD19.CAR-aNKT cell infusion, the study will halt patient accrual, and the FDA would be notified in an expedited fashion; the data will undergo a thorough internal review and the outcome discussed with the FDA.

10.2.2. Safety Analysis of Laboratory Data

Laboratory data, which includes CBC, BUN, creatinine, and liver function tests, will be examined in different ways. Descriptive statistics (means, standard deviations, medians and ranges) at planned time points post CD19.CAR-aNKT cell infusion will be calculated. A scatter diagram depicting laboratory values at each time point for each patient will also be generated. In order to analyze changes in laboratory values, a shift table with Stuart-Maxwell chi-square analysis of the change in the normal range from pre-infusion to post infusion time points (using high, normal, low) will be performed. When appropriate, these tables will be collapsed and the McNemar's test applied in place of the Stuart-Maxwell test. These statistical tests will be primarily performed for the overall patient group.

10.2.3. Analysis of expansion and persistence of transduced aNKT cells.

The frequency of CD19.CAR-aNKT cells transduced with the vector will be summarized at pre and post-infusion time points using mean ± SD, medians and ranges to evaluate their expansion and persistence. Changes in these CD19.CAR-aNKT cells from infusion to each time point post-infusion will be assessed and compared using paired t-tests, or when appropriate, the Wilcoxon signed ranks test. These analyses will be performed in the overall patient group.

Plots of growth curves will be generated to graphically illustrate patterns of CD19.CAR-aNKT cell expansion. Longitudinal modeling techniques such as the random coefficient mixed model will be employed to model each of the repeatedly-measured CD19.CAR-aNKT cells. These models account for variation in individual-level intercepts and slopes over the follow-up time. Thus, we will be able to model proliferation of CD19.CAR-aNKT cells over time and estimate the magnitude of expansion or decline of CD19.CAR-aNKT cells. We will include dose level as an independent variable in the model to account for different doses received by patients.

Alternative strategies to analyze these outcomes include calculation of area under the curve over time for CD19.CAR-aNKT cell frequencies or piecewise longitudinal models based on apparent trends from plots of growth curves. Despite the small patient numbers, a data-dense study will be generated due to the repeat measurements on proliferation, immune function, and other parameters on each patient. The modeling strategies proposed here are amenable to these types of data but will however be considered exploratory and interpreted with caution due to limited study power.

Measures of immune function such as ELISPOT cytokine release will be analyzed using a similar strategy indicated above. The validity of the normality assumption on these data will be tested and appropriate transformations will be considered whenever indicated.

10.2.4. Analysis of anti-tumor activity

Although response is not the primary endpoint of this trial, we will summarize tumor response by calculating overall response rates along with exact 95% binomial confidence intervals. Tumor sites will be measured before and after aNKT cell therapy. Response will be evaluated as described in Section 9.6.

11. Off Study Criteria

11.1. Completion of all study specified procedures

11.2. Refusal of further study follow-up by patient or legal guardian

11.3. Loss to follow-up

11.4. Death

12. Reporting Requirements

12.1. Register all patients with Cell and Gene Therapy Research Nurse.

12.2. The following data will be captured:

12.2.1. Eligibility (at enrollment)

12.2.2. Pre-study (before beginning of study)

12.2.3. Concominant Medications

12.2.4. Adverse Events (including CRS and neurotoxicity)

12.2.5. Acute GVHD form

12.2.6. Off study

12.2.7. Death

12.3. Drug Toxicity and/or Adverse Reactions:

12.3.1. Adverse events will be collected as per SOP J02.05.XX (Routine Adverse Experience Reporting) and J 02.75.XX (AE Reporting for Cellular Therapy Studies). Data on all adverse experiences/toxicities regardless of seriousness must be collected for 4 weeks after the last dosing of the study product. The criteria listed in the NCI Common Toxicity Criteria Scale will be used in grading toxicity (Version 5.X located at http://ctep.cancer.gov) with the exception of CRS and neurological toxicities that are related to T-cell infusions. CRS and neurological toxicities will be graded according to Appendix II.

12.3.2. Serious adverse events will be collected and reported as per SOP J02.06.XX (Serious Adverse Experience and Unanticipated Problem Reporting). Reporting of serious adverse event related to gene transfer will continue for 15 years per SOP J02.51.XX (Long Term Follow Up for Gene Transfer Studies).

13. Informed Consent

13.1. All patients and/or their legal guardian must sign a document of informed consent consistent with local institutional and federal guidelines stating that they are aware of the investigational nature of this protocol and of the possible side effects of treatment. Further, patients must be informed that no efficacy of this therapy is guaranteed, and that unforeseen toxicities may occur. Patients have the right to withdraw from this protocol at any time. No patient will be accepted for treatment without such a document signed by him or his legal guardian. Full confidentiality of patients and patient records will be provided according to institutional guidelines.

13.2. Before acceptance of patients for this protocol, the investigator(s) will explain specifically the following to the patient and/or legal guardian:

13.2.1. The nature of a phase I study.

13.2.2. That the patient's tumor is resistant to conventional therapeutic agents, but there exists a chance that therapeutic benefits may be detected following treatment with CD19.CAR modified aNKT-cells, but that no benefit may be seen.

13.2.3. That blood samples will be obtained over a period of time during and after therapy.

13.2.4. That the patient and/or guardians are asked to sign a statement signifying that they have read and understand an account of the possible side effects of the agent and that the patient or guardians have the right to withdraw the patient from participation in the study at any time.

13.2.5. That the patient's participation in these studies is voluntary and that no financial remuneration is available for participating in this study, but that all necessary therapeutic measures will be provided should toxicity ensue, and that the records of the treatment of the patients will be kept confidential but may be reviewed by

representatives of the National Cancer Institute, Food and Drug Administration, or pharmaceutical manufacturers.

13.2.6. That a copy of the signed consent form will be furnished to the patient.

14. Clinical Trial Oversight and Monitoring

14.1. This protocol will be conducted in accordance with the Cell and Gene Therapy Monitoring Plan on file with the FDA.

14.2. This protocol will be monitored in accordance with the current Dan L Duncan Cancer Center Data Safety Monitoring Board Plan for investigator-initiated Phase I and II studies in the Center for Cell and Gene Therapy at Baylor College of Medicine.

14.3. The conduct of this clinical trial will be evaluated in accordance with the Texas Children's Cancer Center, and Center for Cell and Gene Therapy Quality Assurance Policy and Procedure Plan.

Appendix I

Potential Side Effects of Lymphodepleting Chemotherapy

Risks and side effects related to CYCLOPHOSPHAMIDE include those which are:

Likely	Less likely	Rare but serious
• Loss of appetite • Nausea • Vomiting • Fewer white blood cells • in the blood. • A low number of white blood cells, which may make it easier to get infections. • Hair loss • Decreased ability of the body to fight infections • Absence or decrease in the number of sperm, which may be temporary or permanent which may decrease the ability to have children	• Abnormal hormone function which may lower the level of salt in the blood • Abdominal pain • Diarrhea • Fewer red blood cells and platelets in the blood • A low number of red blood cells may make you feel tired and weak. • A low number of platelets may cause you to bruise and bleed more easily. • Bleeding and inflammation of the urinary bladder • Absence or decrease monthly periods which may be temporary or permanent and which may decrease the ability to have children • Temporary blurred vision • Nasal stuffiness with IV infusions • Skin rash • Darkening of areas of the skin and finger nails • Slow healing of wounds • Infections	• Heart muscle damage which may occur with very high doses and which may be fatal. • Abnormal heart rhythms • Damage and scarring of lung tissue which may make you short of breath • A new cancer or leukemia resulting from this treatment. • Damage or scarring of urinary bladder tissue • Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure, rapid heart rate chills and fever • Infertility, which is the inability to have children

Risks and side effects related to <u>FLUDARABINE (IV)</u> include those which are:

Likely (May happen in more than 20% of patients)	**Less Likely** (May happen in fewer than 20% of patients)	**Rare, but Serious** (May happen in fewer than 2% of patients)
• Low number of red blood cells (anemia) • Low number of white blood cells • Low number of blood platelets • Feeling tired • Nausea (feeling sick to your stomach) • Throwing up (vomiting) • Weak immune system • Pneumonia • Infection • Bleeding • Pain • Electrolyte imbalance	• Diarrhea • Mouth sores • Skin rash • Fever • Swelling of hands and feet • Numbness and tingling in hands and/or feet • Loss of appetite	• Changes in vision • Feeling nervous or anxious • Confusion • Cough • Difficulty breathing • Feeling weak • Severe brain injury which can lead to death • Kidney damage that could require dialysis • Coma • New (secondary) cancers

Appendix II

Grading of CRS and Neurological Toxicities

ASTCT CRS Consensus Grading Scale

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

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

*Fever is defined as temperature ≥38°C not attributable to any other cause. In patients who have CRS then receive antipyretics or anticytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.

†CRS grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a patient with temperature of 39.5°C, hypotension requiring 1 vasopressor, and hypoxia requiring low-flow nasal cannula is classified as grade 3 CRS.

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

ASTCT Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS) Grading Scale

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 conciousness†	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 nonconvulsive 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
Elevated 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 grade 3 ICANS.

N/A indicates not applicable.

*A patient with an ICE score of 0 may be classified as grade 3 ICANS if awake with global aphasia, but a patient with an ICE score of 0 may be classified as grade 4 if unarousable.

†Depressed level of consciousness should be attributable to no other cause (eg, 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.

***Immune Effector Cell-Associated Encephalopathy (ICE) score**

- **Orientation:** orientation to year, month, city, hospital: 4 points
- **Naming:** ability to name 3 objects (eg, point to clock, pen, button): 3 points
- **Following commands:** ability to follow simple commands (eg, “Show me 2 fingers” or “Close your eyes and stick out your tongue”): 1 point
- **Writing:** ability to write a standard sentence (eg, “Our national bird is the bald eagle”): 1 point
- **Attention:** ability to count backwards from 100 by 10: 1 point

Appendix III

Grading of GVHD[102]

MAGIC Grading Scale

Stage	Skin (active erythema only)	Liver (bilirubin)	Upper GI	Lower GI (stool output/day)
0	No active (erythematous) GVHD rash	<2 mg/dL	No or intermittent nausea, vomiting, or anorexia.	Adult: <500 mL/day or <3 episodes/day Child: <10 mL/kg/day or <4 episodes/day
1	Maculopapular rash <25% BSA	2-3 mg/dL	Persistent nausea, vomiting, or anorexia.	Adult: 500-999 mL/day or 3-4 episodes/day Child: 10-19.9 mL/kg/day or 4-6 episodes/day
2	Maculopapular rash 25-50% BSA	3.1-6 mg/dL		Adult: 1000-1500 mL/day or 5-7 episodes/day Child: 20-30 mL/kg/day or 7-10 episodes/day
3	Maculopapular rash >50% BSA	6.1-15 mg/dL		Adult: >1500 mL/day or >7 episodes/day Child: >30 mL/kg/day or >10 episodes/day
4	Generalized erythroderma (>50% BSA) *plus* bullous formation and desquamation >5% BSA	>15 mg/dL		Severe abdominal pain with or without ileus or grossly bloody stool (regardless of stool volume)

Overall clinical grade is based on most severe target organ involvement:

Grade 0: No stage 1-4 of any organ

Grade I: Stage 1-2 skin without liver, upper GI, or lower GI involvement

Grade II: Stage 3 skin and/or stage 1 liver and/or stage 1 upper GI and/or stage 1 lower GI

Grade III: Stage 2-3 liver and/or stage 2-3 lower GI, with stage 0-3 skin and/or stage 0-1 upper GI

Grade IV: Stage 4 skin, liver, or lower GI, with stage 0-1 upper GI

15. REFERENCES

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