RELY-30: Phase I Study of Relapsed CD30 Expressing Lymphoma Treated with CD30 CAR T Cells

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TABLE OF CONTENTS

RELY-30 CHECKLIST FOR PATIENT ELIGIBILITY FOR PROCUREMENT

PATIENT ID _____ **PATIENT NAME** _____

YES	NO	VALUE/DATE	
Any "**NO**" answers will make a patient **ineligible** for study participation.			
			Diagnosis of relapsed/refractory HL or NHL.
			CD30 positive tumor (can be pending at this time) as assayed in a CLIA certified Pathology Laboratory.
			Hgb ≥7.0(may be transfused value)
			Karnofsky or Lansky score of > 60%
			Informed consent explained to, understood by and signed by patient or guardian. Patient or guardian given copy of informed consent.

YES	NO	
Any "**YES**" answers will make a patient **ineligible** for study participation.		
		Active infection with HIV or HTLV (can be pending at this time)
		Active bacterial fungal or viral infection.

Signature of MD _____ **Date** _____

RELY-30 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 relapsed/refractory HL or NHL: see 4.3.1 for more details and specify what category applies
			CD30 positive tumor as assayed in a CLIA certified Pathology Laboratory
			Recovered from all acute non-hematologic toxic effects of all prior chemotherapy
			Adequate pulmonary function with FEV1, FVC and DLCO ≥50% of expected corrected for hemoglobin.
			Age 16 to 75 for the first three patients on a dose level; thereafter, if no DLT, patients aged 12 to 75 can be treated on that dose level
			Bilirubin 1.5 times or less than upper limit of normal
			AST 3 times or less than upper limit of normal
			Estimated GFR > 70 mL/min
			EKG shows no significant arrhythmias
			Pulse oximetry of > 90% on room air
			Karnofsky or Lansky score of > 60%
			Available autologous T cells with ≥15% expression of CD30-specific CAR determined by flow cytometry
			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.

(continued on the following page)

YES	NO	
Any "**YES**" answers will make a patient **ineligible** for study participation.		
		Currently receiving any investigational agents or received any tumor vaccines within the previous six weeks
		Received anti-CD30 antibody-based therapy within the previous 4 weeks.
		Subjects with rapidly progressive disease, defined as kinetic failure to previous chemotherapy.
		Bulky disease (defined as a ≥10-cm mass or **mediastinal** disease with a transverse diameter exceeding 33% of the transthoracic diameter),
		History of hypersensitivity reactions to murine protein-containing products
		Pregnant or lactating
		Tumor in a location where enlargement could cause airway obstruction
		Current use of systemic corticosteroids at a dose equivalent to 0.5 mg/kg/day of prednisone or higher.
		Active hemorrhagic cystitis
		Active bacterial, viral or fungal infection
		Symptomatic cardiac disease (NYHA Class III or IV disease)

Signature of MD _____ **Date** _____

1.0 Objectives

1.1. Primary Objective

To evaluate the safety of one dose of autologous activated T lymphocytes (ATL), genetically modified to express an artificial chimeric antigen receptor (CAR) that targets the CD30 molecule (CD30.CAR) and also contains the CD28 endodomain, in patients with CD30+ refractory/relapsed Hodgkin lymphoma (HL) or non-Hodgkin lymphoma (NHL) after lymphodepleting chemotherapy.

1.2. Secondary Objectives

To measure the survival of CD30.CAR transduced ATL *in vivo*.

To measure the anti-tumor effects of CD30.CAR transduced ATL in patients with CD30+ refractory/relapsed Hodgkin lymphoma (HL) or non-Hodgkin lymphoma (NHL).

2.0 Background and Rationale

2.1. Hodgkin's lymphoma (HL)

HL has an incidence of about 2.4 per 100,000 per year.[1] Modern radiotherapy and/or chemotherapy regimens have dramatically improved the cure rate of patients with HL. However, despite the identification of clinical prognostic factors, and the optimal use of primary and secondary treatments, HL remains fatal for more than 15% of patients,[1] and even in patients who are cured, the morbidity of therapy is substantial and long lasting.[2] New therapeutic agents are required therefore not only to further reduce mortality but also to alleviate morbidity.[2] In about 40% of cases of HL, tumor cells express viral latency proteins of Epstein Barr Virus (EBV) such as LMP-1 and 2. However, the great majority of HL do not expressed viral proteins, while they almost invariably express the CD30 antigens.[3] This marker is routinely used for the diagnosis of HL.

2.2. CD30+ Non Hodgkin's lymphoma (NHL)

Other types of lymphoma also express the CD30 antigen. Anaplastic large cell lymphoma (ALCL) is a subtype of peripheral T-cell lymphoma (PTCL) first described in 1985. ALCL represents a distinct category of large cell lymphomas defined by a strong expression CD30 on all or most neoplastic cells.[4]

CD30+ lymphoproliferative disorders of the skin (CD30+ LPD) represent a well-defined spectrum of primary cutaneous T-cell lymphomas which have been recognized as distinct entities in recent lymphoma classifications.[5] Lymphomatoid papulosis and anaplastic large-cell lymphoma share the expression of CD30 antigen as a common phenotypic hallmark but differ in regard to their clinical and histological features as well as their biologic behavior.[5] Treatment with conventional dose chemotherapy following relapse usually fails to cure. High dose chemoradiotherapy followed by hematopoietic stem cell transplantation, however, may represent an effective therapy in these patients.[6]

$CD30^+$ B-cell lymphomas have been observed and they are most commonly diffuse large B-cell lymphomas. In addition, occasional to numerous $CD30^+$ B cells have been described in sporadic cases of follicular lymphoma (FL), resembling the pattern seen in reactive tonsils and lymph nodes.[7]

CD30 may be present on activated B or T cells, but not resting mature or precursor B or T cells. Benign reactive $CD30^+$ cells tend to be large, immunoblastic-appearing cells located at the periphery of germinal centers.[7]

2.3. **Immunotherapy for HL and NHL**

Because of the association between EBV in approximately 40% of cases of HL, and the expression of viral latency proteins by these tumor cells, we have previously assessed the feasibility of the adoptive transfer of EBV-specific cytotoxic T lymphocytes (EBV-CTL) for patients with EBV-related HL.[8] In a phase I clinical trial in patients with active HL, we showed that EBV-CTL can successfully be generated from the peripheral blood of the majority of patients even when in clinical relapse.[8] We administered EBV-CTL to 14 patients treated for relapsed EBV+ HL.[9] Gene marking studies showed that infused effector cells could further expand by several logs in vivo, contribute to the memory pool (persisting up to 12 months), and traffic to tumor sites. Clinically, EBV-CTL were well tolerated, could control type B symptoms (fever, night sweats, and weight loss), and had antitumor activity. After CTL infusion, five patients were in complete remission at up to 40 months two of whom had clearly measurable tumor at the time of treatment. One additional patient had a partial response, and five had stable disease.[9]

Because in EBV-positive HL only a limited number of EBV derived antigens[10] (EBNA1, LMP1 and LMP2) are expressed, follow up studies have focused on targeting LMP1 and LMP2 with CD8+ T cells, since EBNA1 is mainly presented on MHC class II molecules.[11] The results of a clinical trial infusing LMP2 and LMP1-specific CTLs (LMP-CTLs) was very promising. Twenty-eight of 29 high-risk or multiple-relapse patients receiving LMP-CTLs as adjuvant therapy remained in remission at a median of 3.1 years after CTL infusion. None subsequently died as a result of lymphoma, but nine succumbed to complications associated with extensive prior chemoradiotherapy, including myocardial infarction and secondary malignancies. Of 21 patients with relapsed or resistant disease at the time of CTL infusion, 13 had clinical responses, including 11 complete responses. T cells specific for LMP as well as nonviral tumor-associated antigens (epitope spreading) could be detected in the peripheral blood within 2 months after CTL infusion, but this evidence for epitope spreading was seen only in patients achieving clinical responses.[12, 13] However, because only a proportion of HL and NHL tumors are EBV-related,[10] the identification of new target antigens is required in order for CTL immunotherapy approach to be an option for all patients.

2.4. **CD30 as a Target Antigen**

CD30 is a transmembrane glycoprotein and is a member of the tumor necrosis factor receptor superfamily.[7] CD30 plays a role in regulating the function or proliferation of normal lymphoid cells. Members of the tumor necrosis factor (TNF)/TNF-receptor (TNF-R) superfamily coordinate the immune response at multiple levels. For example, TNF, LTα, LTβ and RANKL provide signals required for lymphoid neogenesis, CD27, OX-40, 4-1BB and CD30 deliver costimulatory signals to augment immune responses, while pro-apoptotic members such as TNF, CD95L and TRAIL may contribute to the termination of the response.[7] CD30 plays a role in regulating the function or proliferation of normal lymphoid cells. Ki-1 (CD30) antigen expression has been used to identify anaplastic large cell lymphoma and Reed-Sternberg (RS) cells in Hodgkin's disease.[7] Indeed, CD30 is expressed on virtually all HRS cells, and is therefore a potential target for developing both antibody based immunotherapy and cellular therapies.[7] Brentuximab vedotin, an antibody-drug conjugate that targets CD30 is approved for the treatment of CD30-positive HL.[14] However, because of the short duration of the clinical effects, other strategies are desirable.[3]

2.5. **Chimeric Antigen Receptors (CARs)**

Since HL and NHL are both apparently sensitive to the cellular immune response (graft versus lymphoma effect) and antibody treatment, there is interest in combining both approaches through the generation of artificial CARs. Chimeric receptors are usually 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.[15] This scFv is then attached to the transmembrane and cytoplasmic portion of T Cell Receptors (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 have exhibited specific lysis via the perforin/granzyme pathways, as well as cytokine secretion upon exposure to tumor cells expressing the cognate target antigen.[15, 16] 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.[15, 17] 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 αβTCRs of T lymphocytes. Target cell recognition by αβTCRs 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.[17] Moreover, many tumors down-regulate expression of MHC molecules and/or have dysfunctional antigen processing machinery, so that the target antigenic epitopes for αβTCR 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. Moreover, CARs can recognize non-protein antigens, unlike conventional αβTCRs.[15, 17]

2.6. **Extending the Survival of CAR-redirected T Cells**

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*.[17] A number of factors likely contribute to these differences but a major contribution is the inability of CAR-engagement alone to recapitulate the co-stimulatory events that follow the physiologic engagement of the native αβTCR. Full activation and proliferation of T cells requires not only TCR engagement (first signal) but also co-stimulation provided by antigen presenting cells (APCs, second signal) and cytokines (third signal).[17] A multiplicity of these costimulatory receptor-ligand and cytokine signals is required, in an optimal temporal and spatial sequence. 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. 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. To compensate for the lack of costimulation following CAR engagement,[17] costimulatory signaling domains (CD28, OX40, 41BB, etc) have been incorporated as part of the CAR itself. Studies using polyclonal activated T lymphocytes expressing these novel "second generation" CARs are currently open at several institutions.[17]

We have constructed and tested a CAR targeting CD30 in preclinical studies and shown that T-lymphocytes engineered to express this receptor are redirected to kill $CD30^+$ HL cell lines.[18, 19] This CAR also incorporates the CD28 co-stimulatory endodomain (from now on this construct will be called CD30.CAR). We have later developed a clinical trial using this construct to express the CD30.CAR in activated T lymphocytes (ATL). We had anticipated that CD28 should provide an adequate co-stimulation to CAR-modified T lymphocytes, consistent with observations from our own and other clinical trials in patients with B-cell malignancies treated with redirected T cells that target the CD19 antigen and also contain the

CD28 endodomain.

In our previous study we therefore expressed the antigen binding domain of a CD30 mAb as part of a CAR on T cells, coupled to the CD28 and ζ chain endodomains, in an effort to ensure prolonged persistence, active penetration of tumors and activation of multiple lytic components of the immune system. We undertook an initial analysis of our phase I dose escalation study of activated autologous CD30.CAR-T cells (CD30.CARTs) infused in patients with relapsed/refractory EBV-negative CD30+ HL or NHL. We manufactured CD30.CARTs for 18 patients using retroviral transduction.[20] Starting from a median of 2.4×10^7PBMCs (range 3.6×10^6 to 4.9×10^7), we obtained 9.0×10^8 CD30.CARTs (range 2.8×10^8 to 2.9×10^9) in 15±3 days of culture, with a transduction efficiency of 89%±1%. The cell products comprised >99% T cells and phenotypic analysis showed 58%±29% $CD8^+$ T cells, with a majority of them being effector T cells ($CD45RO^+$ 94%±7%). ^{51}Cr-release cytotoxicity assays confirmed that patients' CD30.CARTs lysed a CD30+ tumor line, HDLM-2 (60%±13% killing at a 20:1 effector:target ratio), with negligible effects on CD30-negative target cells (<5% killing). During cell manufacture, 3 patients became ineligible due to rapid worsening of their performance status and 1 patient was not infused because his tumor was subsequently shown to be CD30-negative.

Nine patients (7 with HL and 2 ALCL) have received CD30.CARTs. Eight of these had relapsed or progressed after treatment with brentuximab. Two patients were treated on dose level (DL) 1 (2×10^7 $CD30.CAR^+$ T cells/m^2), 2 patients on DL2 (1×10^8) and 5 patients on DL3 (2×10^8). None of the patients received any conditioning regimen before CART infusion. CART infusions produced no attributable adverse events; in particular, no patient had evidence of cytokine release syndrome. The molecular signal from CARTs, assessed by Q-PCR in peripheral blood, peaked at 1 week following infusion, but decreased to near background by 4 weeks post infusion. The signal level was dose dependent, with an mean of 7020 copies/μg DNA in patients treated on DL3, in whom CAR-T cells were detectable by flow cytometry in the peripheral blood (~5% of PBMCs), versus 60 copies/μg for DL1.

At 6 weeks after treatment, 1 patient had a CR, 1 patient had a very good PR, and 4 patients had stable disease (persisting for 1½ to 8 months), while 3 patients had disease progression.[20] Having completed the dose escalation and found that DL3 is safe we now wish to evaluate if expansion and clinical activity can be improved by lymphodepletion.

2.7. **The Role of a Lymphodepleted Environment**

It is now well established that expansion of lymphocytes *in vivo* is subjected to homeostatic mechanisms that control their total numbers. Therefore, it has become apparent that lymphopenia will facilitate expansion of infused T cells. Given the nature of the conditioning regimens, which are myeloablative and lymphodepleting, lymphopenia will always be present immediately after stem cell transplantation. However, other subjects may benefit not only from induction of lymphopenia before T cell infusion but also from the depletion of inhibitory cell subsets in the patient and their tumors. Thus, response rates appear much higher in studies in which high doses of cyclophosphamide and/or fludarabine preceded infusion of CAR-T cells.[21, 22] We now therefore hypothesize that T cells engrafted with a CAR targeting the CD30 molecule will expand and survive longer after appropriate lymphodepletion.

2.8. **Our Study Hypothesis**

The major hypothesis of this study is that repeat dosing of ATL expressing the CD30.CAR infused after lymphodepleting chemotherapy is safe for therapy of CD30+ HL and CD30+ NHL and that these cells will persist in these patients long enough to potentially control disease. In support of our proposal, clinical trials of CD30 monoclonal antibodies (Mabs) (conjugated with

immunotoxins or radioisotopes) have produced clinical responses in patients with advanced/refractory HL .[3] However, these responses are often modest and short-lived, as monoclonal antibodies are limited by the transient persistence and poor bio-distribution in tumors.[3] Clinical studies using T cells redirected with CARs have shown some clinical benefits[23] and our trial in patients with B-cell lymphoma receiving CD19-CAR-specific T cells indicated that these cells expand better *in vivo* when the CAR incorporates the CD28 endodomain.[24]

2.9. **Testing This Hypothesis**

To test this hypothesis we plan to give one infusion of CD30.CAR-bearing ATL following lymphodepleting chemotherapy to human subjects with relapsed/refractory HL or CD30+ NHL. We will monitor the persistence of these infused cells in the peripheral blood of the patients and whenever lymph nodes are accessible at the tumor site as well, by measuring the level of the transgene by both phenotypic and molecular analyses. Currently, we can regularly detect a single transduced cell in 50,000 peripheral blood mononuclear cells by real-time PCR amplification. Moreover, because gene persistence and expression may not correlate with antitumor effector function, we will also determine the functionality of the peripheral blood T cells *ex vivo*.

2.10. **Safety of Targeting the CD30 Antigen in Patients with HL and CD30+ NHL**

Monoclonal antibodies targeting CD30 have been associated with minimal toxicity, primarily related to the chemotherapy agent conjugated to the antibodies. There are currently more than 50 clinical trials using CAR-transduced T cells targeting surface antigens expressed in hematological malignancies and solid tumors.[25] Encouraging results have been seen in CD19+ malignancies and the main adverse event has been cytokine release syndrome.

3.0 Construction of CD30 Vectors

The single chain antibody (scFv) targeting the CD30 molecule was cloned by Dr. Abken.[18] The human IgG1 immunoglobulin heavy constant region (hinge-CH_2CH_3 regions) was added to provide a spacer region between the scFv and the ζ and CD28ζ endodomain (CD30.CAR).[26, 27] This spacer region also guarantees the detectability of the CAR on transduced T cells by FACS analysis using a specific monoclonal antibody. The CAR.CD30 was then cloned into the retroviral vector SFG (provided by R.C. Mulligan, Cambridge, Massachusetts) that is a Moloney murine leukemia (Mo-MuLV) virus-based vector. A schematic representation of the retroviral construct is shown in **Fig. 1**.

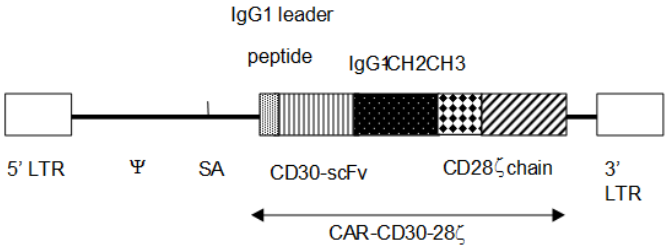

Fig. 1 Schematic representation of SFG.CD30.CAR.

4.0 Procurement and Treatment Eligibility Criteria

4.1. Procurement Inclusion

Referred patients will initially be consented for procurement of blood for generation of the transduced ATL. Eligibility criteria at this stage include:

1. Diagnosis of relapsed/refractory HL or NHL.
2. CD30 positive tumor as assayed in a CLIA certified Pathology Laboratory (result can be pending at this time)
3. Hgb ≥7.0 (may be a transfused value)
4. Informed consent explained to, understood by and signed by patient/guardian. Patient/guardian given copy of informed consent.
5. Karnofsky or Lansky score of > 60%

4.2. Procurement Exclusion Criteria

1. Active infection with HIV or HTLV (can be pending at this time).
2. Active bacterial fungal or viral infection

4.3. Treatment Inclusion Criteria

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

1. Diagnosis and clinical course falling into one of the following categories:

Hodgkin lymphoma	Refractory to second line chemotherapy
	Relapsed or progressive after high dose therapy/autologous stem cell transplantation
	Relapsed or progressive after treatment with brentuximab or a checkpoint inhibitor
Aggressive non-Hodgkin lymphoma	Refractory to second line chemotherapy
	Relapsed or progressive after high dose therapy/autologous stem cell transplantation
ALK-negative anaplastic T cell lymphoma or other peripheral T-cell lymphoma	Refractory to first line chemotherapy
	Relapsed after first line therapy (possibly including high dose therapy/autologous stem cell transplantation)

ALK-positive anaplastic T cell lymphoma	Refractory to second line therapy
	Relapsed after second line therapy

2. CD30-positive tumor as assayed in a CLIA certified Pathology Laboratory.

3. Age 16 to 75 for the first three patients on a dose level; thereafter, if no DLT, patients aged 12 to 75 can be treated on that dose level.

4. Bilirubin 1.5 times or less than the upper limit of normal.

5. AST 3 times or less than the upper limit of normal.

6. Estimated GFR > 70 mL/min.

7. Pulse oximetry of > 90% on room air

8. EKG shows no significant arrhythmias

9. Karnofsky or Lansky score of > 60%.

10. Available autologous T cells with ≥15% expression of CD30CAR determined by flow-cytometry.

11. Recovered from all acute non-hematologic toxic effects of all prior chemotherapy.

12. Adequate pulmonary function with FEV1, FVC and DLCO ≥ 50% of expected corrected for hemoglobin.

13. 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.

14. Informed consent explained to, understood by and signed by patient or guardian. Patient or guardian given a copy of the informed consent form.

4.4. **Treatment Exclusion Criteria**

1. Currently receiving any investigational agents or received any tumor vaccines within the previous six weeks.
2. Received anti-CD30 antibody-based therapy within the previous 4 weeks.
3. Subjects with rapidly progressive disease, defined as kinetic failure to previous chemotherapy.
4. Bulky disease (defined as a 10 cm mass or **mediastinal** disease with a transverse diameter exceeding 33% of the transthoracic diameter).
5. History of hypersensitivity reactions to murine protein-containing products.
6. Pregnant or lactating.
7. Tumor in a location where enlargement could cause airway obstruction.
8. Current use of systemic corticosteroids at a dose equivalent to 0.5 mg/kg/day of prednisone or higher.
9. Active hemorrhagic cystitis.
10. Active bacterial, viral or fungal infection.
11. Symptomatic cardiac disease (NYHA Class III or IV disease).

5.0 Generation of Transduced Cells.

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

5.1. **Source Material.**

Up to 240 mL (in 2 collections) of peripheral blood will be obtained according to the procurement consent. In patients with low (CD3 count as assayed by flow cytometry less than 200/uL) T-cell count in the peripheral blood, a leukopheresis may be performed to isolate sufficient T cells. The parameters for pheresis will be 2 blood volumes.

5.2. **Activated T lymphocytes.**

ATL will be generated using our previously validated SOP. Briefly, PBMC will be activated with anti-CD3 and anti-CD28 antibodies on day 0, and then fed with IL-7 and IL-15 on day 1.[23]

5.3. **Retroviral Production.**

A retroviral producer line has been generated for the construct. A master cell-bank of producer cells has been generated and tested to exclude production of replication competent retrovirus and infection by Mycoplasma, HIV, HBV, HCV and others. Producer lines are grown to confluence. Supernatant is then harvested, filtered, aliquoted and rapidly frozen and stored at -80°C. 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.

5.4. **Transduction.**

ATL are transduced on day 3-4 after initiation as described previously using recombinant Fibronectin fragment CH-296 (Retronectin^{TM}, Takara Shuzo, Otsu, Japan) coated plates or

bags. Virus is attached to retronectin by incubating producer supernatant in coated plates or bags. Cells are then transferred to virus coated plates or bags.

5.5. **Ex vivo expansion.**

After transduction transgenic ATL will be expanded feeding them with IL-7 and IL-15 twice a week to reach sufficient numbers as previously described.[23] After transduction a small number of cells will be removed to evaluate for transduction efficiency using flow cytometry and Q-PCR.

5.6. **Freezing.**

When sufficient number for cell infusion(s) is achieved, cells will be collected and frozen following previously validated SOPs. Cells will be also tested for cytotoxicity against CD30+ tumor cells and CD30 negative cell lines to check receptor function. All lines will be checked for identity, phenotype and microbiological culture and cryopreserved prior to administration according to SOPs. The results will be reviewed by QA prior to issuing a certificate of analysis.

5.7. **Testing**

Products that meet study specific release criteria, as detailed on the Certificate of Analysis, will be infused as per Section 6.3.

If a positive sterility testing result is reported after the product was infused, the FDA and other relevant parties would be notified as per our manufacturing SOP B01.03.XX (Product Quality Assurance Program and Release and Return of Clinical GMP/GTP Products) and our clinical research SOP J02.06.XX (Serious Adverse Experience and Unanticipated Problem Reporting). Our management of such a situation is further described in our SOP F05.09.XX (Management of Culture Positive Cell Therapy Products).

6.0 Treatment Plan

6.1. **Dosing.**

Three dose levels will be evaluated based on safety data from our current study of CD30 CAR T cells. Cohorts of three to six patients will be enrolled at each dose level (see section 13). The dose escalation is guided by the modified continual reassessment method. Each patient will receive one infusion of CAR modified T cells according to the following dosing schedule:

Dose Level One

Day 0
2×10^7 cells/m^2

Dose Level Two

Day 0
1×10^8 cells/m^2

Dose Level Three

Day 0
2×10^8 cells/m^2

The first three patients on a dose level will be aged 16 to 75; thereafter, if no DLT, patients

aged 12 to 75 can be treated on that dose level.

The dose is based on the number of CD30.CAR-expressing cells. In our previous study the highest dose was 2 x 10^8 cells/m^2 and we did not reach a MTD. With a lower limit of CAR positivity at 15%, even a larger patient (2.0 m^2) would receive only 5.33 x 10^9 total cells, which would still be in the lower range of infused cells as reported by Johnson et al.[28]

There will be a gap of 4 weeks between the first and second patient on each dose level.

In addition, in accordance with the rationale described in Section 2.7:

(a) Unless post autologous transplant, patients will receive three daily doses of cyclophosphamide (Cy: 500mg/m^2/day) together with fludarabine (Flu: 30mg/m^2/day) to induce lymphopenia, finishing at least 48 hours before T cell infusion, but no later than 2 weeks prior to infusion of the cells. 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, intravenous hydration and anti-emetics should also be provided following local institutional guidelines.

(b) Patients post autologous stem cell transplantation will receive T cell infusion starting at least 14 days after the date of autologous stem cell transplant, unless there is clear evidence of relapse. In this case, T-cell infusion can occur at any time after autologous stem cell transplant. No lymphodepleting chemotherapy will be given to these patients.

6.2. **Premedication.**

Patients may be premedicated with Benadryl (diphenhydramine) up to 1mg/kg IV (max 50mg) and Tylenol (acetaminophen) 10 mg/kg po (max 650mg), especially if they have a history of reactions to blood products. Steroids should be avoided given their detrimental effect on the survival of the infused T cells. Anti-emetics in appropriate dosage for each patient will be prescribed as necessary.

6.3. **Cell Administration.**

Patients will receive one infusion of T-cell product, after lymphodepleting chemotherapy per Section 6.1. CAR+ ATL will be given by intravenous injection over 1-10 minutes through either a peripheral or a central line on Day 0. The expected volume will be 1-50 mL per infusion.

6.4. **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 3 hours post infusion.

6.5. **Post-administration Patient Treatment.**

Patients will receive supportive care for acute or chronic toxicity, including blood components or antibiotics, and other intervention as appropriate. All treatments will be given at the Center for Cell and Gene Therapy, in Texas Children's Hospital or Houston Methodist Hospital. Ideally, patients should not receive other antineoplastic agents for at least 6 weeks post T-cell infusion (for purposes of evaluation). However, patients with progressive disease may receive other therapy if needed at the discretion of their attending physician.

6.6. Additional Doses of CD30.CAR ATL.

At the discretion of the investigator, if patients with active disease have stable disease or a response (see Section 12) at week 8 or on subsequent evaluations, they are eligible to receive up to 6 additional treatments of CD30.CAR ATLs at 8 to 12 week intervals, each of which will consist of the same cell number or less than their initial CD30.CAR-specific T-cell injection. Patients will not be able to receive additional doses of T-cells until the initial safety profile is completed at 6 weeks following the last CD30.CAR ATL infusion. Patients will be monitored after CD30.CAR ATL infusion as outlined in Section 8.0, using the study calendars included in Section 8.1. Patients who receive additional treatments will have a 6-8 week safety evaluation that will be identical to the evaluation after the first treatment. The decision to administer lymphodepleting chemotherapy with new treatments will be at the discretion of the PI, and will take clinical condition (including recovery from toxicities of prior treatment) and ability to tolerate chemotherapy into account.

7.0 Risks of this study

Potential toxicities may be categorized as those related to: infusion of T cells; retroviral transduction; cross-reactivity; cytokine release syndrome and neurotoxicity; and chemotherapy.

7.1. Infusion of autologous T-cells

In this study we will be administering transduced autologous peripheral blood ATL so there will no risk of alloreactivity. Many previous studies have infused much larger numbers of autologous T cells that have been activated ex vivo with no adverse effects.[29, 30]

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 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.[31-34]

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.[35, 36] 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.[37, 38]

Our proposal should have a very different risk profile. Extensive animal experiments have been conducted using genetically modified T cells exposed to retroviral or lentiviral vectors and, in sharp contrast with hematopoietic stem cells, none of these studies reported tumorigenic events.39 Other long-term clinical data suggest that highly differentiated cells such as T lymphocytes may be less susceptible to transformation compared to hematopoietic

stem cells. Thus far, hundreds of patients have received genetically modified T-cells in clinical trials, including patients we have treated on our protocols using retrovirally marked EBVSTs[9, 40-45] or T cells transduced with chimeric antigen receptors.[23, 24, 46] In none of these has malignancy caused by retroviral transduction been reported.

Current intensive regimens to treat relapsed lymphoma describe subsequent secondary malignancy rates in excess of 10%.[47] In light of this, the natural history and poor prognosis of advanced lymphoma, 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.

7.3. **Cross-reactivity**

CD30 can be expressed by activated T cells and NK cells. Direct toxicity to these cells due to targeting of their CD30 molecules by transduced cells is possible. In case of this eventuality a number of therapeutic maneuvers are available. Corticosteroids in doses used to treat graft-versus host-disease (GvHD) will deplete the majority of circulating transduced cells.

CD30 is a 120 kDa type I cell surface glycoprotein and its extracellular domain shares sequence similarity with other tumor necrosis factor (TNF)/nerve growth factor (NGF) receptor superfamily. We chose to target CD30 since this antigen is only expressed on cells of hematopoietic origin. It is not detected on cells of the peripheral blood or on resting lymphocytes, but it is only present on a subpopulation of physiologically activated T cells and on thymic medulla.[7] The CD30 knock out mouse shows impaired thymic negative selection of T lymphocytes but this does not compromise the development of efficient immune responses.[48] We previously showed that CD30.CAR-redirected T cells did not impair the generation of virus specific CTLs ex vivo[49] and CD30 is not expressed in normal tissues like lung and fibroblasts (**RAC#1003-1034**). In addition, given these concerns, we monitored antiviral immunity in CD30.CART recipients treated on our previous study. The frequency of T cells responding to CMV, adenovirus, influenza virus and EBV (assessed using stimulation with viral peptides in IFNγ ELIspot assays) remained unchanged by treatment.[20]

CD30 is also expressed on first trimester decidua so there may be a risk of miscarriage in females of child-bearing potential if the cells persist long term. To evaluate this risk we will add a question about miscarriage to our annual follow up evaluation in these patients and report any such events in our annual IND report.

7.4. **Cytokine Release Syndrome and Neurotoxicity[50-52]**

There have been several reported SAEs associated with cytokine release syndrome (CRS) in patients who received T cells expressing CARs containing additional costimulatory endodomains. Our study subjects will receive initial doses of T cells that associated with CRS. We will collect serum before and after infusion for cytokine monitoring, and continue to monitor subjects for 3 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 F 05.11.XX and includes treatment options based on the clinical severity of the symptoms such as oxygen, inotropic agents, IL-6 receptor antibody (4-8 mg/kg),TNF-α antibody (5-10 mg/kg), and/or steroids (1-2 mg/kg/day of methylprednisolone or equivalent).

There have been several reports of neurotoxicity (e.g. encephalopathy, somnolence, aphasia) after the infusion of CD19-specific CAR T cells. 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 IL-6 receptor antibody (4-8 mg/kg), and/or steroids (1-2 mg/kg/day of methylprednisolone or equivalent), and anti-epileptics as described in the literature.

7.5. **Immunogenicity**

Because it has murine components, the CD30.CAR could, in theory, elicit an immune response. This could either lead to quick clearance of the cellular product after repeat administrations or give rise to anaphylactic reactions after repeat administration. In light of this, detection of human anti-mouse antibody (HAMA) was mandated by our current CD30.CAR protocol. None of the patients developed significant levels of HAMA or anaphylactic reactions even after repeat infusions of CD30.CAR T cells. Therefore, we will not assess HAMA formation by default. Nonetheless, serum from blood drawn for functional studies on week 0, week 6 and prior to each additional dose will be stored for measurement of human anti-mouse antibodies (HAMA) in the event of a suspected immunologic reaction.

7.6. **Chemotherapy**

Cyclophosphamide

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

Fludarabine

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

8.0 Patient Evaluation

8.1. Study Calendar

The detailed schedules for clinical monitoring are included below.
For patients with lymphodepletion, post stem cell transplantation:

Study	T-Cell Therapy							Follow-up					
	Wk 0	Wk 1	Wk 2	Wk 3	Wk 4	Wk 6	Wk 8	M 3	M 6	M 9	M 12	Every 6 M x 4 yrs	Annually x 15 yrs (total)
ATL infusion	X												
Hx	X	X	X	X**	X	X	X	X	X	X	X		X
PE	X	X	X	X**									
Performance Status	X		X					X	X	X	X		
Pregnancy Testing[1]	X												
Pulmonary function tests	X												
CBC d/p	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		
CT/MRI/PET	X						X†						
EKG	X												
Thyroid function	X							X			X		
Function and Persistence Studies	X*	X	X	X**	X	X	X	X	X	X	X	X[3]	X
RCR by PCR and archive samples	X[2]												
Quantitative PCR	X*	X	X	X**	X	X		X	X	X	X	X[3]	X
Cytokine Testing****	X***	X	X										

[1] Pregnancy testing will be done pre-infusion of chemotherapy in female patients of childbearing potential
[2] Samples for RCR will be collected at baseline and if the patient develops a new malignancy.
[3] See Section 8.6 on additional timepoints to be collected if transgene detected.
* Also collected at 3-4 hours post infusion
** These 3 week studies are optional
*** Serum collected before and after infusion
**** Samples are collected but testing is only done if the patient develops CRS.
† 6-8 weeks post infusion
NOTE: Follow-up will start over per this calendar if the subject receives additional T cell infusions.

8.2. **Lymphodepletion Study Calendar**

Study	Treatment								Follow-up					
	Chemo-therapy	T-Cell Therapy												
	Pre-Wk 0	Wk 0	Wk 1	Wk 2	Wk 3	Wk 4	Wk 6	Wk 8	M 3	M 6	M 9	M 12	Every 6 M x 4 yrs	Annually x 15 yrs (total)
Cy/Flu	X													
ATL infusion		X												
Hx	X	X	X	X	X**	X	X	X	X	X	X	X		X
PE	X		X	X	X**									
Performance Status	X			X					X	X	X	X		
Pregnancy Test [1]	X													
Pulmonary function tests	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	X		
AST/Bili/Alb	X	X	X	X		X	X	X	X	X	X	X		
CT/MRI/PET	X							X†						
Thyroid Function	X								X			X		
EKG	X													
Function and Persistence Studies	X	X*	X	X	X**	X	X	X	X	X	X	X	X[3]	X
RCR by PCR and archive samples	X[2]													
Quantitative PCR	X	X*	X	X	X**	X	X		X	X	X	X	X[3]	X
Cytokine Testing****	X***	X*	X	X										

[1] Pregnancy testing will be done pre-chemotherapy in female patients of childbearing potential
[2] Samples for RCR will be collected at baseline and if the patient develops a new malignancy.
[3] See Section 8.6 on additional timepoints to be collected if transgene detected.
* Collected at 3-4 hours post infusion
** These 3 week studies are optional
*** Serum collected prior to first chemo day
**** Samples are collected but testing is only done if the patient develops CRS.
† 6-8 weeks post infusion.
NOTE: Follow-up will start over per this calendar if the subject receives additional T cell infusions.

8.3. Routine Laboratory Investigations

The following investigations will be obtained pre-chemotherapy, pre-ATL infusion and at 1, 2, 4, 6 and 8 weeks post day 0 infusion, and then at 3, 6, 9 and 12 months:

1) CBC and differential,
2) BUN, creatinine, Na, K, Cl, CO2
3) Bilirubin, SGOT, albumin.
4) Pregnancy test required for female patients of childbearing potential (pre-chemotherapy and pre-infusion only).
5) Thyroid function will be monitored at baseline, month 3 and one year in view of changes seen in patients receiving CD30 antibody.
6) Serum or plasma for cytokine testing will be obtained pre and post infusion as well as Weeks 1 and 2. Cytokine testing on these samples will only be conducted if patients develop CRS.

Lymphodepleting chemotherapy plus CD30.CAR T cells:
While receiving chemotherapy, comprehensive metabolic profiles and complete blood counts with differential counts may be monitored more frequently, as medically indicated.

Post CD30.CAR T-cell infusion:
Patient will be monitored as outlined in the Study Calendar. If a patient develops CRS and is admitted, he/she will receive more frequent monitoring per the SOP F5.11.XX

8.4. Diagnostic imaging

Diagnostic imaging to document measurable disease and response to therapy (PET, CT scans, MRI, nuclear imaging) pre-infusion and at 6-8 weeks following the day 0 infusion. The time may vary by 1-2 weeks due to scheduling issues 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.

8.5. Tests of Function, Persistence and Safety

The following investigations will be obtained per the time points in the study calendar. The analyses will be used to monitor function and persistence in peripheral blood and safety of transduced T-cells at time-points indicated in the study calendar.

8.5.1 Replication competent retrovirus (RCR) testing by PCR. A blood sample for RCR testing will be collected and archived pre study.

8.5.1.1. An additional blood sample will be collected and run for RCR testing if the patient develops a new malignancy.

8.5.1.2. If a post treatment sample is positive, the assay will be run on the pre study sample. In addition, further analysis of the RCR and more extensive patient follow-up should be undertaken in consultation with the FDA.

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

8.5.1.4. 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).

8.5.1.5. Aliquot of cells and serums will also be archived for use in future studies for RCR as required by the FDA or RAC.

8.6. Quantitative real-time PCR

To detect retroviral integrants will be collected at pre study, 3-4 hours post infusion, 1, 2, 3 (optional), 4, and 6 weeks post day 0 cell infusion and then at 3, 6, 9, and 12 months, every 6 months for 4 years and then yearly for a total of 15 years. PCR to detect retroviral integrant clonality and integrant locus will also be done if the transgene is detected at >0.5% at 3 months. If detection of CART CD30 T cells is greater than 0.5% and there is persistent CART CD30 T cell expansion 3 months after the 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. Functional assays

Functional assays such as in vitro reactivation of PBMCs in patients on whom the appropriate reagents are available.

8.8. Immunophenotyping

8.9. HAMA

Serum from blood drawn for functional studies on week 0, week 6 and prior to each additional dose will be stored for measurement of human anti-mouse antibodies (HAMA). These studies will be performed in the event of a suspected immunologic reaction.

8.10. Blood Amount

A maximum of the lesser of 50 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 less than or equal to 7.0 g/dl 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 necessary. If there is insufficient blood RCR testing and Quantitative real-time PCR will be the first priorities.

8.11. Other tissues

- If biopsy of accessible lymph-nodes is required at any time during the study, a sample of this will be used to assess presence of transduced peripheral blood T-cells in association with the tumor.
- Bilateral bone marrow aspirations and biopsies. If bone-marrow trephine biopsy or tumor biopsy is required at any time during the study, a sample of this will be used to assess presence of transduced peripheral blood T-cells in association with the tumor.
- 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 Interval

Patients shall be evaluated (seen in clinic or contacted by research coordinator) as outlined in study calendar section 8.0. Additional visits will be obtained as clinically indicated.

9.2. Dose Limiting Toxicity

DLT will be defined as any of the following that may, after consultation with the FDA, be considered possibly, probably, or definitely related to the study cellular products.

- Any Grade 5 event
- Non-hematologic dose-limiting toxicity is any Grade 3 or Grade 4 non-hematologic toxicity that fails to return to Grade 2 within 72 hours
- Grade 2 to 4 allergic reaction to T cell infusion.
- Hematologic dose limiting toxicity 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
- Grade 3 and 4 expected reactions due to CRS and neurotoxicity are seen with the use of CAR-based immunotherapy. 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. Grade 4 CRS and neurologic toxicities will be reported to the FDA in an expedited fashion.

10. Early Termination of Study and Modifications of Drug Dosages

10.1. The trial will terminate when a minimum of 12 patients are treated with six patients accrued at the current MTD. Depending on patient availability, a maximum of 18 patients can be accrued into this Phase I trial (see section 13).
10.2. Therapy for an individual patient will be terminated if a dose limiting toxicity has been observed.
10.3. If the patient/parent 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.
10.4. If a patient dies within 60 days of CD30 CAR T cell infusion of any cause except disease progression, accrual will be paused to allow consultation with the FDA.

11. Measurement of Effect

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 6-8 weeks of the CTL infusion. Additional imaging performed as part of standard clinical care will also be evaluated

12. Measurement of Disease

This study will use the new Lugano Criteria.[53]

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

Response	Sites	PET-CT Based	CT Based
	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
	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

Response	Sites	PET-CT Based	CT Based
	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

13. Statistical Considerations

13.1. Clinical Trial design

This Phase I dose-escalation trial is designed to evaluate the safety of autologous CD30.CAR T-cells. Each patient will receive one infusion of CAR modified T cells according to the following dosing schedule:

Dose Level One

Day 0
1×10^7 cells/m^2

Dose Level Two

Day 0
1×10^8 cells/m^2

Dose Level Three

Day 0
2×10^8 cells/m^2

Dose escalation is guided by the modified continual reassessment method (mCRM) in order to determine the maximum tolerated dose (MTD) of transduced T-cells. Dose-limiting toxicity (DLT) is defined in **Section 9.2**. For this study, MTD is defined as the maximum dose level that has at most 20% probability of inducing DLT.

To reduce the probability of treating patients at unacceptable toxic dose levels, we employ modifications to the original CRM.[54] The modified CRM has been shown in the literature to be superior to the standard 3+3 method designed for the targeted MTD probability 33.3%. Specifically, the modifications are: (1) the first patient starts at the lowest dose level, (2) more than one patient can be treated at the same dose level, (3) there will be no jumps over a dose level and the dose is escalated according to the pre-specified levels, and (4) if a patient experiences a DLT, then there is no dose escalation for the immediate next patient. The modified CRM phase-I design has been studied extensively in the literature. Compared to the 3+3 design, mCRM provides better estimates of the MTD, affords smaller number of patients accrued at lower and more likely ineffective dose levels, and treats more patients at the MTD level.

In this trial, three patients will be initially enrolled to the lowest dose level, and mCRM will be implemented with 3-6 patients treated at each dose level. Each treated patient will be followed for 6 weeks post the T-cell infusion for the evaluation of DLTs. A decision on dose escalation will be made after at least 3 patients have completed their 6-weeks toxicity follow-up. Depending on DLT data at each dose level, a maximum 18 patients will be accrued for dose escalation, where we will enroll a total of 6 patients at the MTD dose level. During the trial, real-time monitoring of patient toxicity outcome will be performed in order to estimate the dose-toxicity curve.

The dose escalation is guided by the model-based mCRM, assuming a logistic model for the probability of toxicity $p(\text{tox}|d)=[1 + \exp(0.41 - \theta \log\{(d_a+d_b)/\alpha\})]^{-1}$, where (d_a+d_b) is the total number of injected T cells ($4\text{x}10^8$, $6\text{x}10^8$, $8\text{x}10^8$, respectively), $\alpha=16\text{x}10^8$ is a scale constant, and the unknown parameter θ is assumed to have a non-informative uniform prior distribution. Based on our previous trials, we expect a shallow dose-toxicity curve for the doses proposed in this trial. Although we are confident that the dosing range is safe, we use a model that is conservative in assuming a potentially, slightly higher prior predictive probability of DLT, i.e., based on the model, the prior predictive probability is 12.3%, 16.5%, 21% for the three dose levels, respectively.

Based on the observed toxicity data from the subjects, we will update θ by computing its posterior distribution. The predictive probability of DLT under the current dose level and the next dose level are evaluated. DLTs that occur during the toxicity evaluation period following the T-cell infusion (6 weeks post infusion) will be used for the CRM calculations to determine the predictive probability, so enrollment on the next dose level will wait until the completion of toxicity evaluation period for all treated patients enrolled at the current dose level.

If the predictive probability of the DLT of the current and the next dose level is ≤ 20%, then 3 patients will be enrolled at the next dose level. If the probability of DLT of the next dose level is > 20% but at the current dose level is ≤ 20%, then three more patients will be enrolled at the current dose level. If the probability of DLTs at the current dose level is > 20%, the dose

will be de-escalated to the highest dose level with predictive probability of DLT ≤ 20%. The trial will be stopped if the probability of DLT of all dose levels is more than 20% after 6 patients have been studied at the current dose level. The procedure continues until the dose has reached the last dose level or stopped. If the probability of DLT under at the lowest dose level is > 20%, then the trial will be stopped and no MTD will be declared and there will be no phase II study. If the trial reached the highest dose level, and after three patients, the probability of DLT is ≤ 20%, then a total 6 will be evaluated at the highest dose level.

The final MTD is defined as the highest dose level with toxicity probability lower than or equal to the target 20%. As mentioned, the T-cell infusions are safe and we do not anticipate seeing any T-cell-related 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. If there are indeed one or more DLT events, we expect that at most 18 patients will be infused in the phase-I trial dose escalation stage.

After the dose escalation stage of the trial reaches the MTD level, we will expand the cohort at the MTD levels to accrue a maximum 12 patients in each of the four possible disease settings: (1) Hodgkin lymphoma, (2) aggressive non-Hodgkin B cell lymphoma, (3) ALK-negative anaplastic T cell lymphoma or other peripheral T-cell lymphoma, and (4) ALK-positive anaplastic T cell lymphoma. The expansion cohort will include all patients studied at the MTD levels. The primary goal of the expanded cohort is to further study the safety profiles of CAR-T cells in each of the disease settings, but only with lymphodepleting chemotherapy.

In the expansion stage of the trial, the sample size is 12 patients for each disease setting. We expect the safety profiles among the 4 disease settings to be similar and the risk of toxicities due to CAR-T cells to be very low. The goal of the expansion cohort is to study whether there are excessive DLTs. For this purpose, a DLT rate above 30% is considered excessive, while a rate of 5% or below is considered acceptable. We will monitor the toxicity continuously so the trial can halt accrual early if the toxicity rate is excessive using sequential boundaries. The accrual will be halted for the respective disease group if the number of DLTs is equal to or exceeds the boundaries (see the table below). This is a Pocock-type stopping boundary[55] that yields the probability of early stopping at 4.2% when the true DLT rate is equal to the acceptable rate 5%, and the probabilities of early stopping is high at 79% if the true DLT rate is equal to 30%.

Number of patients	1	2	3	4	5	6	7	8	9	10	11	12
Boundary	-	2	2	2	2	2	3	3	3	3	3	3

We expect to treat at most 48 patients in the expansion cohort and the maximum total sample size for the study is 66. If there is any death deemed possibly or probably related to the CD30.CAR-T cell infusion, the study will halt patient accrual for the respective disease group, 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.

13.2. Statistical Analysis

Safety Analysis of Adverse Event Data

All patients who received CD30.CAR T-cell infusions will be included in the safety analysis. Adverse event data and corresponding toxicity grades six weeks after T-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 displayed.

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.

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 pre-infusion, and at 1, 2, 4, 6 weeks post- T-cell infusion will be calculated. Laboratory data collected at 3-month intervals for the first year will also be summarized. 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 are 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.

Analysis of expansion and persistence of transduced T cells.

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

Plots of growth curves will be generated to graphically illustrate patterns of T-cell expansion. Plots will be generated to depict patterns of survival and expansion of T cells for each of the two vectors. Longitudinal modeling techniques such as the random coefficient mixed model will be employed to model each of the repeatedly-measured T 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 T cells over time and estimate the magnitude of expansion or decline of T 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 T 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, etc. 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.

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 T cell therapy. Response will be evaluated as described in section 12.

14. Off Study Criteria

14.1. Completion of study specified procedures.

14.2. Lost to follow-up

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

14.4. Death

15. Reporting Requirements

15.1. Register all patients with Cell and Gene Therapy Research Coordinator.

15.2. Enter all patients by phoning or e-mailing Dr. Ramos or Dr. Heslop.

15.3. The following data will be collected:

- Eligibility (at enrollment)
- On study (before beginning of study)
- Off study (up to one month after the study is stopped)
- Adverse Events
- CRS Adverse Events (as applicable)
- Neurotoxicity Adverse Events (as applicable)

16. Drug Toxicity and/or Adverse Reactions

16.1. Adverse events will be collected as per SOP J 02.05.XX and J 02.75.XX. Data on all adverse experiences/toxicities regardless of seriousness must be collected for documentation purposes only for 6 weeks after the last dosing of the study drug/biologic. The criteria listed in the NCI Common Toxicity Criteria Scale will be used in grading toxicity (Version 4.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.

16.2. Serious adverse events will be collected and reported as per SOP J 02.06.XX. Reporting of serious adverse event related to gene transfer will continue for 15 years per J 02.51.XX. At the suggestion of the FDA on June 12, 2019, mild (grade 1 or 2) CRS will no longer require the expedited 15 day reporting required by CFR 21 Part 312.32 (outlined in SOP J 02.05.XX). Mild (grade 1 or 2) CRS, even when brief hospitalization for observation and/or treatment is required, is now considered an expected, self-limited event associated with CD30-CAR T cell infusions.

17. Informed Consent

17.1. 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.

17.2. Patients may be asked to volunteer for other appended or ancillary protocols which investigate other questions such as issues of supportive care, the use of hematopoietic growth factors, management of infectious diseases and/or their prophylaxis, and pharmacokinetics studies. Such protocols will not be implemented without the review and approval of the Institutional Review Board. In circumstances where such protocols are approved, a separate informed consent document, consistent with the above guidelines, will be obtained.

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

17.4. The nature of a Phase I study.

17.5. 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 CD30.CAR modified T cells, but that no benefit may be seen.

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

17.7. That the patient and/or parents 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 parents have the right to withdraw the patient from participation in the study at any time.

17.8. 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.

17.9. That a copy of the signed consent form will be furnished to the patient and/or parents. If there is conflict between the parent’s and child’s wishes, patients will have an opportunity to discuss the proposal with a representative from the social services or psychology departments, who will serve as a patient advocate and who should also witness the consent. All consent procedures will be performed with a child–life specialist present. This person will help insure the minor patient understands the study according to their capabilities.

18. Clinical Trial Oversight and Monitoring

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

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

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 <u>CYCLOPHOSPHAMIDE</u> 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

CRS Grading Scale

Grade	Symptoms
1	• Symptoms are not life threatening and require symptomatic treatment only (e.g. fever, nausea, fatigue, headache, myalgia, malaise)
2	• Symptoms require and respond to moderate intervention • Oxygen requirement <40% or hypotension responsive to fluids or • low dose of one vasopressor or Grade 2 organ toxicity
3	• Symptoms require and respond to aggressive intervention • Oxygen requirement ≥ 40% or hypotension requiring high dose or multiple vasopressors or • Grade 3 organ toxicity or Grade 4 transaminitis
4	• Life-threatening symptoms • Requirements for ventilator support or Grade 4 organ toxicity (excluding transaminitis)
5	• Death

Neurological Toxicity Grading Scale

Grade	Symptoms
1	• Somnolence-mild drowsiness or sleepiness • Confusion-mild disorientation • Encephalopathy-mild symptoms • Dysphasia-not impairing ability to communicate
2	• Somnolence-moderate, limiting instrumental ADL* • Confusion-moderate disorientation, limiting instrumental ADL* • Encephalopathy-limiting instrumental ADL* • Dysphasia-moderate impairing ability to communicate spontaneously
3	• Somnolence – obtundation or stupor • Confusion-severe disorientation, limiting self-care-ADL* • Encephalopathy-limiting self- care ADL* • Dysphasia-severe receptive or expressive characteristics, impairing ability to read, write or communicate intelligibly
4	• Life-threatening Consequences of Grade 3 Toxicities • Urgent Intervention Indicated • Mechanical Ventilation
5	• Death

*ADL: activities of daily living

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