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Investigational Agent: Autologous T cells Transduced with Lentiviral Vector Expressing CD20-specific Chimeric Antigen Receptor Gene for the treatment of Refractory B Cell Non-Hodgkin Lymphomas ("CD20 CAR T cells")

Study Regimen: Cyclophosphamide, cyclophosphamide plus fludarabine, or bendamustine chemotherapy followed by CD20 CAR T cell infusion

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Investigator Statement:

I have carefully read Protocol 9738 entitled "A Phase I/II Study to Evaluate the Safety of Cellular Immunotherapy Using Autologous T cells Engineered to Express a CD20-Specific Chimeric Antigen Receptor for Patients with Relapsed or Refractory B Cell Non-Hodgkin Lymphomas" ("Protocol") version date 01/06/2023.

I agree to carry out my responsibilities in accordance with the Protocol, applicable laws, and regulations (including 21 CFR Part 312), Good Clinical Practice: Consolidated Guidance (ICH-E6), and applicable policies of Fred Hutch.

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1 INTRODUCTION AND BACKGROUND

1.1 Introduction

More than 70,000 new cases of non-Hodgkin Lymphoma (NHL) are diagnosed each year in the USA, and more than 19,000 patients die of this group of diseases annually.[1] Aggressive B cell lymphomas such as diffuse large B cell lymphoma (DLBCL) are the most common subset, accounting for 35-40% of NHL.[2] The majority of patients with aggressive B-NHL are successfully treated with combination chemotherapy, but a significant proportion relapse or have refractory disease, and the outcome of these patients is poor. Approximately 30% of such cases can be salvaged with autologous stem cell transplant (ASCT), but the majority die of progressive disease.[3] Indolent forms of NHL, including follicular lymphoma (FL), marginal zone lymphoma, lymphoplasmacytic lymphoma, and small lymphocytic lymphoma account collectively for ~40% of all cases of NHL,[4] and are also incurable with available therapies, except high dose chemoradiotherapy with hematopoietic cell transplantation (HCT).[5-7] Mantle cell lymphoma (MCL) is a distinct subtype defined by clinical, morphologic, immunophenotypic, cytogenetic, and molecular characteristics and accounts for 5-10% of NHL cases.[2,8,9] The International Lymphoma Study Group determined that MCL has the worst failure-free survival (11% at 5 years) and overall survival (27% at 5 years) of any type of B-cell NHL.[2] No curative treatment is available for advanced MCL, with the possible exception of allogeneic stem cell transplantation.[10-12] Chronic lymphocytic leukemia is the most common leukemia in the Western world and despite the introduction of several novel therapeutic agents, still remains incurable.[13] Furthermore, treatment of high-risk CLL remains an unmet clinical and research need and allogeneic stem cell transplant is still considered standard of care for selected patients.[14] Since many patients with relapsed B-NHL lack histocompatible donors or are not suitable candidates for stem cell transplantation, innovative new treatments are urgently needed. This proposal will develop and test a new immunotherapeutic approach for relapsed B-NHL using autologous T lymphocytes that have been genetically modified with a novel lentiviral vector to express a chimeric antigen receptor (CAR) recognizing the CD20 antigen.

Cellular immunotherapy using adoptive T cells expressing a CAR specific for tumor-associated B-cell antigens CD19 or CD20 has emerged as a potent therapy for B lymphoid malignancies. The CD20 antigen has proved to be an effective target for antibody therapy in treating variety of NHLs. It is expressed at a high density on the surface of more than 95% of B-NHL. It is minimally shed, internalized, or modulated upon antibody binding. It usually continues to be expressed on B-NHL lymphoma cells despite repetitive therapy with rituximab, an anti-CD20 antibody. Unfortunately, many patients who initially benefit from rituximab develop resistance, while others never respond. Our group has conducted two proof-of-concept phase I clinical trials that demonstrated the feasibility, safety, and minimal toxicity of treating patients with relapsed or refractory B-NHLs with autologous T cells that had been genetically modified to express an anti-CD20 CAR via plasmid DNA transfection. However, several limitations of the initial approach became apparent during the conduct of these trials: 1) the low transfection efficiency achieved with electroporation as a gene transfer method, which required antibiotic selection and prolonged in vitro expansion, 2) suboptimal cell surface expression of the CAR on T cells (<10,000 molecules per cell), 3) the long time interval required to expand genetically modified T cells to sufficient numbers to perform

therapeutic infusions (2-4 months), 4) T cell exhaustion resulting from the prolonged culture times and multiple restimulation cycles, and 5) short in vivo persistence of infused T cells (a few weeks). To address these limitations, we have generated and successfully tested a lentiviral based vector containing a third-generation, fully human anti-CD20 CAR and a truncated CD19 (trCD19) selectable marker. The goal of this phase I/II study is to develop and test this immunotherapeutic approach for patients with relapsed or refractory B-NHL.

1.2 Background

1.2.1 Conventional chemotherapy for B-cell lymphomas

Induction therapy for B-NHL generally consists of a combination of an anti-CD20 antibody (rituximab) and chemotherapy (CHOP, bendamustine, DA-EPOCH, HyperCVAD, or other regimens). R-CHOP is curative of approximately 60% of patients with advanced stage DLBCL, but patients who are refractory to or relapse after R-CHOP have poor outcomes. Indolent lymphomas, including follicular, marginal zone, small lymphocytic and lymphoplasmacytic lymphomas typically present in elderly patients with disseminated disease. Despite their clinically "indolent" nature initially, these lymphomas progress inexorably to death, which occurs at a median of 10-15 years after diagnosis. With the exception of a small fraction of patients (10-15%) with truly localized involvement, the majority of indolent lymphoma patients do not achieve long-term durable remissions with conventional treatment regimens, and there is no current consensus among experts on the best treatment approach for this group of diseases.[15] Studies have not demonstrated a curative potential for patients treated with currently tested conventional chemotherapeutic agents including single alkylating agents, bendamustine monotherapy or combinational regimens such as R-CVP, or R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone).[15] Mantle cell lymphoma is typically treated in younger patients with an intensive high-dose cytarabine-containing induction regimen (such as Nordic regimen or alternating R-CHOP/rituximab, dexamethasone, high-dose Ara-c, and cisplatin [R-DHAP]) followed by autologous HCT in first remission.[16,17] Older patients with MCL are typically treated with either rituximab plus bendamustine or rituximab, bortezomib, cyclophosphamide, doxorubicin, and prednisone (VR-CAP).[18,19] The response rates for these regimens are high, but relapse is inevitable. Historically, chemoimmunotherapy regimens have been used in treatment of CLL. Fludarabine, cyclophosphamide and rituximab (FCR), bendamustine and rituximab (BR), and chlorambucil and obinutuzumab were some of the more commonly utilized regimens until the introduction of more recent novel agents.[13,20] Despite long-term remissions from the FCR regimen in patients with normal TP53, the regimen is associated with a 5-7% risk of secondary myeloid malignancies (AML/MDS).[21] More importantly, and in a number of head-to-head randomized trials, novel agents (ibrutinib, acalabrutinib, and venetoclax) have been shown to be superior compared to chemoimmunotherapy in both first-line and relapsed settings. [22-29] As a result, chemotherapy is only a reasonable option for selected patients in the first line setting and is rarely used in previously treated patients.[20] All investigators agree on the necessity of developing better treatment regimens for patients with B-cell lymphomas.

1.2.2 High dose therapy with autologous stem cell transplantation (ASCT) for relapsed/refractory B-NHL

High dose therapy with autologous stem cell rescue is commonly employed for treatment of patients with relapsed lymphomas of all types. The Parma trial demonstrated an overall survival benefit for patients with relapsed or refractory aggressive NHL that was responsive to salvage chemotherapy who underwent high-dose therapy with autologous stem cell transplantation compared with standard chemotherapy alone.[30] More recent studies in the rituximab era indicate that higher cure rates with induction therapy have resulted in worse outcomes for patients who fail frontline treatment. Patients who develop progressive disease within 12 months of R-CHOP have a 3-year progression-free survival (PFS) of only 23%.[31] ASCT is frequently employed in the treatment of MCL as consolidation of frontline therapy in younger patients, and appears to have resulted in improved overall survival in this disease. For patients with relapsed indolent B-NHL, a small randomized trial showed that ASCT led to an improvement in PFS and suggested an overall survival benefit as well, although this was performed in the pre-rituximab era.[32] It is important to note that despite these improvements in disease control, ASCT trials for MCL and indolent NHL often fail to demonstrate convincing plateaus on the survival curves, and even these high dose regimens are generally not considered to be curative.[33-36]

1.2.3 Allogeneic stem cell transplantation for relapsed/refractory B-NHL

Several studies have examined the role of allogeneic transplantation in relapsed/refractory B-NHL.[5,6,12] This treatment approach, which relies on an immunologic graft-versus-lymphoma effect mediated by alloreactive donor T cells, has the potential to lead to cures. However, many B-NHL patients are not candidates for this therapy due to advanced age, poor performance status, or lack of a suitable HLA matched donor. Furthermore, allogeneic stem cell transplantation is associated with high rates of morbidity and non-relapse mortality due to acute and chronic graft-versus-host disease (GVHD) and infection, limiting the applicability of this approach.

1.2.4 Targeted small molecule therapies for B-NHL

Recent improvements in the understanding of lymphoma biology has led to the development of several new small molecule agents targeting proteins involved with active signaling pathways in malignant B cells. Inhibitors of B cell receptor pathway proteins such as Bruton's tyrosine kinase (BTK), phosphotidylinositol-3 kinase (PI3K), and spleen tyrosine kinase have proven to be very active in this group of diseases. Ibrutinib, a BTK inhibitor, has shown unprecedented activity for a single agent in relapsed MCL, with an overall response rate of 68% and median PFS of 13 months in this setting.[37] Idelalisib, a PI3K inhibitor, led to a 57% response rate and median PFS of 11 months in heavily pre-treated patients with indolent B-NHL.[38] Venetoclax, a Bcl-2 inhibitor that is approved for CLL/SLL, has also shown promising single agent activity in relapsed or refractory MCL, with a response rates of 75%.[39] These therapies are often safe and well-tolerated, however rare serious complications such as intracranial bleeding can occur with ibrutinib, and fatal pneumonitis and hepatitis may occur with idelalisib. Although the drugs in this new class are valuable additions to the armamentarium of lymphoma therapies, they are not curative, require indefinite therapy, and are very costly.

1.2.5 Novel therapeutic agents for CLL/SLL and role of cellular therapy (allogeneic transplant and CAR-T therapy)

Treatment of chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL) has been revolutionized since the introduction of novel therapeutic agents including BTKis (ibrutinib and acalabrutinib), PI3Kis (idelalisib and duvelisib), and the Bcl-2 inhibitor, venetoclax. Chemotherapy-free regimens are currently standard of care for the majority of CLL patients and all high-risk patients with an abnormal TP53 (del17p or a mutated TP53). [13,20,22,24,26-28,40,41] Despite these advancements, clinical outcomes of patients who progress on a BTKi or venetoclax are poor, and treatment options are limited for this population. Cellular therapy, namely allogeneic stem cell transplant, is recommended at least as a consideration for fit patients with available donors who have disease progression on either a BTKi or venetoclax. This is consistent with guidelines published by the American Society of Transplant and Cellular Therapy (ASTCT) as well as by the European Bone Marrow Transplant (EBMT) and the European Research Initiative for CLL (ERIC).[14,42,43] Despite the efficacy (long-term PFS ~40%), allogeneic transplant is associated with a high rate of treatment related mortality ranging from 10-25%. Therefore, other cellular therapy approaches with high efficacy but less toxicity are needed. [42,44]

Studies using CD19-targeted CAR-Ts have shown promising results in treatment of high-risk CLL, including in patients with abnormal TP53 and those with prior disease progression on a BTKi and/or venetoclax. Experience from the Fred Hutch group using a second generation CAR with 4-1BB co-stimulatory domain, showed a 1-year progression-free survival of 38% and 50% when CAR-T was administered with or without concurrent ibrutinib, respectively.[45,46] Also, an ongoing multicenter study using lisocabtagene maraleucel (liso-cel) for high-risk CLL patients showed high response rates (CR 45% and PR 36%).The same promising signal was observed in patients who had disease progression on BTKi and venetoclax (double refractory), with CR rate of 67% and PR rate of 22%. The rate of elimination of measurable residual disease as measured by flow cytometry with a sensitivity of 10^{-4} (uMRD4) was 75% and 65% in all and 87% and 75% in double-refractory patients in the blood and bone marrow, respectively.[47] The uMRD status after CAR-T seems to be a strong predictor of long-term remissions based on the studies from Fred Hutch. [45,46]

1.2.6 CAR-T therapy for lymphoma patients with CNS involvement

Primary and secondary CNS lymphomas are considered unmet clinical needs.[48] Despite the recent advancement in treatment of lymphoma, none of the recently approved drugs for B-NHL are effective or available for patients with CNS lymphoma.[49-51] Outcome of patients with primary CNS lymphoma with poor response to first-line treatment or with relapsed/refractory disease remains extremely poor. Patients with secondary CNS lymphoma also have inferior clinical outcomes compared to patients without CNS involvement.[52] Most importantly, secondary CNS involvement by lymphoma commonly excludes patients from clinical trials testing novel investigational treatments.

Patients with CNS involvement were excluded from the ZUMA-1 and JULIET trials, that led to the approval of axicabtagene ciloleucel and tisagenlecleucel, respectively.[53] Therefore, the FDA label for those two products does not include patients with active CNS disease and the published clinical experience with axicabtagene ciloleucel and tisagenlecleucel in treatment of CNS lymphoma patients is based on small case series. Despite this limitation, an increasing number of publications confirm the safety and efficacy

of CAR-T products in patients with active CNS lymphoma. Frigault et. al. reported on a case series of 8 patients who received tisagenlecleucel for secondary CNS lymphoma at the FDA approved standard dose. Only 1 patient had grade 1 neurotoxicity, and activity was demonstrated in those heavily pretreated patients.[54] Based on these results the group at the Massachusetts General Hospital (MGH) designed and conducted a phase I/II study of tisagenlecleucel for primary CNS lymphoma. Twelve patients were treated on the protocol and only 1 patient developed grade 3 ICANS and other AEs (CRS and ICANS) only occurred at grade 1-2. There was no treatment-related deaths and 3 of 12 patients had ongoing complete remissions. Authors also showed persistent CAR-T cells in blood and trafficking to CSF. [55]Ghafouri and colleagues also reported safety and efficacy of axicabtagene ciloleucel in 5 patients with secondary CNS lymphoma. They reported no CAR-T related deaths and they only observed grade 1-2 CRS and grade 3 ICANS. 4 of 5 pts showed response although responses were not durable.[56]

Unlike ZUMA-1 and JULIET trials in the TRANSCEND NHL 001 study, the pivotal registration trial that led to the approval of lisocabtagene maraleucel for DLBCL, patients with secondary CNS involvement were eligible for the study and comprised 3% of the study population. [57]Patients received CAR-T at the same dose other patients on the study. Neurological events occurred in 2 patients and both patients developed grade 3 ICANS. Of note, no patients developed CRS and no patients died from neurological events. Of 7 patients, 6 were evaluable for efficacy and all 3 achieved a CR.

There is more limited data on primary CNS lymphoma. Investigators at the City of Hope medical center reported on 5 patients with primary CNS lymphoma who received an investigational CD19 targeted CAR-T[58] They only observed grade 1-2 CRS and ICANS. In this phase 1 trial, 2 dose levels were tested and there was no difference in rate of grade 2 CRS between the 2 dose levels. Importantly, 3 of 5 patients achieved a CR after treatment.

Overall and despite the limited clinical experience, there does not seem to be concerning safety signal when CAR-T is used for treatment of patients with active CNS lymphoma. Additionally, the observed responses justify including these patients in investigational studies.

1.2.7 Immune-based therapies targeting CD20

CD20 is a non-glycosylated, tetrapass, 35 KD B-cell lineage-specific phosphoprotein that is expressed in high, homogeneous density on the surface of more than 95% of B-NHL[59]. CD20 is minimally shed, internalized, or modulated upon antibody binding.[60] Importantly, CD20 continues to be expressed on the lymphoma cells of most patients with relapsed B-NHL despite repetitive rituximab treatments.[61,62] Thus, loss of CD20 expression is not a major contributor to treatment resistance. These attributes of CD20 make it a particularly attractive molecular target for immune-based therapies.

1.2.8 Humoral immunotherapy with anti-CD20 Abs

The availability of monoclonal anti-CD20 antibodies has revolutionized the management of patients with $CD20^+$ B-NHL. Over the past 15 years, the safety and efficacy of the anti-CD20 antibody rituximab has resulted in its use in virtually all patients with B-NHL.[63] Unfortunately, despite the fact that CD20 is an excellent target for antibody-mediated humoral immunotherapy, many patients who initially benefit from rituximab develop resistance while others never respond.[63] Newer anti-CD20 antibodies have been

developed, including obinutuzumab and ofatumumab. Obinutuzumab is a humanized type II antibody with glycoengineered Fc regions designed to enhance antibody-dependent cellular cytotoxicity, and is approved for newly diagnosed CLL and also follicular lymphoma that has relapsed after rituximab.[64,65] Ofatumumab is a type I fully human anti-CD20 antibody that is approved for several indications for CLL patients.[66,67] Ofatumumab binds to both the small and large extracellular loops of CD20 at a non-overlapping epitope compared with rituximab, whereas obinutuzumab binds an overlapping epitope with rituximab, albeit in a different orientation.[68]

1.2.9 Cellular Immunotherapy with adoptive antigen-specific T cells

Adoptive T cell therapy, involving the *ex vivo* selection and expansion of antigen-specific T cells, provides a means of augmenting antigen-specific immunity without the *in vivo* constraints that can accompany vaccine-based strategies. Many preclinical studies have focused on testing this approach in treating a variety of cancers as well as viral diseases. Its feasibility and efficacy have been demonstrated in studies with viral antigen-specific T cells against HIV, CMV, EBV, and other viral diseases,[69,70] though its success in cancer is still far from satisfactory. Major obstacles include difficulties in identifying and characterizing common tumor-specific or tumor-associated antigens and peptides, the inability of transferred T cells to expand and migrate to desired sites, host immune tolerance, tumor immune evasion and limitation of therapeutic efficacy to a highly selected group of patients due to MHC restriction.[71,72]

1.2.10 Chimeric antigen receptor (CAR) T cell therapy

Eshhar et al.[73,74] developed an alternative strategy for adoptive T cell immunotherapy that involves redirecting the specificity of cytotoxic T cells in an MHC-independent fashion via genetic modification with engineered receptor constructs. This method allows targeting tumor cell surface antigens that are unavailable to endogenous T cell receptors due to poor immunogenicity, clonal deletion, or immune tolerance during T cell development. In other words, this approach does not require pre-existing anti-tumor immunity. CARs are constructed by fusing a gene encoding the antigen binding region of an Ab against a tumor-associated antigen with a gene responsible for activating signal transduction of T cells such as the ζ chain of the CD3 molecule. This fusion gene product is delivered into T cells and equips them with newly redirected specificity to the desired target. The biggest advantage of this approach is that one receptor construct can theoretically be used to treat any patient with a tumor expressing the target antigen.

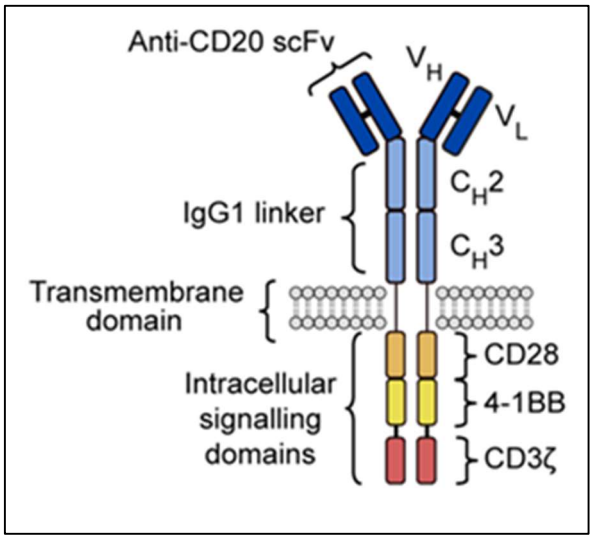


Figure 1.1: Diagram of a 3rd generation CD20-specific chimeric antigen receptor

An emerging group of CARs for targeting human tumors including breast cancer, colon cancer, renal cancer, prostate cancer, neuroblastoma, and lymphoma have been described.[71,75,76] *In vitro* studies have shown that both $CD4^+$ and $CD8^+$ effector T cell functions can be triggered via these receptors and result in

antigen-specific lysis of tumor targets and production of cytokines such as IL-2, TNF-α, and IFN-γ.[71,75,77] Several studies have now been reported using CD19-targeting CARs to treat relapsed or refractory B cell lymphoid malignancies, with very promising results (see below).

1.2.11 T cells with CD20 specific CAR

Michael Jensen, in his work at the City of Hope, was the first to document the feasibility of engineering an anti-CD20 CAR plasmid construct, its ability to transfect T cells, its anti-CD20 specific tumor activity *in vitro*, the ability to expand transfected $CD8^+$ effector T cells to large numbers, and the tolerability of infusing such cells into humans.[78-80]

In a collaborative effort, our group and Dr. Jensen's group have confirmed the potent CTL activity of T cells genetically modified to express a 1st generation anti-CD20 scFvFc-ζ CAR, derived from the Leu16 murine anti-human CD20 antibody, for both lymphoma cell lines and freshly isolated lymphoma cells from patients with NHL.[81] Our group also demonstrated the ability to generate large numbers of T cell clones and to infuse the cells safely into lymphoma patients.[82] Our group successfully completed a proof-of-concept phase I clinical trial which demonstrated the feasibility, safety, and minimal toxicity of treating patients with relapsed B-NHL with autologous T cells that have been genetically modified to express an anti-CD20 CAR via plasmid transfection.[83] An improved version of a plasmid-based CAR with anti-CD20 specificity, co-stimulatory domains from CD28 and CD137, and a SP163 translational enhancer (a so-called "3rd generation" CAR) was generated in our laboratory and tested *in vitro*. T cells transfected with this vector expressed 2 to 3 fold higher levels of surface anti-CD20 CAR, enhanced cytokine secretion, and improved proliferation when compared with 1st generation plasmid.[84] Additionally, faster *ex-vivo* expansion was observed when transfected T cells were cultured in bulk mixtures instead of after limiting dilution cloning.[84]

1.2.12 Phase I clinical trials using autologous T cells transfected with anti-CD20 scFvFc-ζ CAR

Our laboratory previously conducted two phase I clinical trials testing adoptive immunotherapy with CD20-specific CAR^+ T cells. In the first trial, T cells were transfected with a CD20-specific 1st generation CAR plasmid.[83] Therapeutic numbers of transfected T cells expressing the CAR and exhibiting CD20-specific effector activity were generated in 7 out of 10 patients. Seven patients completed a total of 20 T cell infusions. All toxicities were grade 2 or less and were due to IL-2 injections. No adverse events were associated with T cell infusions. No cellular immune responses to the CAR or NeoR gene products were detected, although two patients subsequently developed human anti-mouse antibodies (HAMA). Modified T cells were detectable ranging from 5 to 65 days after infusions by a quantitative PCR (Q-PCR) technique. The *in vitro* expansion durations ranged from 5 to 8 re-stimulation cycles. One partial remission was observed out of 5 patients with measurable disease. Four patients experienced stable disease for 3, 5, 12, and 48 months. Two additional patients without evidence of disease remained progression-free for 3 and 13 months.

A second phase I clinical trial using autologous T cells transfected with an improved 3rd generation anti-CD20 CAR containing CD28 and 4-1BB co-stimulatory domains for patients with relapsed or refractory $CD20^+$ indolent NHL and mantle cell lymphoma was published by our group in 2012.[85] In this study, 4

patients with B-NHL were enrolled, and 3 were infused sequentially with 10^8, 10^9, and 3.3 x 10^9 T cells/m^2, followed by low-dose subcutaneous IL-2 (250,000 IU/m^2 every 12 hours for 14 days). Treatment was well tolerated, although one patient developed transient, reversible infusional symptoms of fever, orthostatic hypotension, and hypoxemia. There were no other adverse events attributable to the nine infusions of T cells. In this second trial, infused T cells were detectable in the blood of patients for up to 1 year after treatment by Q-PCR, albeit at low levels. No cellular or humoral immune responses against the transgene were detected. Genetically modified T cells trafficked to tumor sites including lymph nodes (LN) and/or bone marrow (BM) in all 3 patients, as documented by biopsies. Anti-tumor activity of the regimen was suggested by an objective partial remission observed in one patient lasting 9+ months. The other two patients had no evaluable disease at the time of therapy and remained NED for 12 and 24 months after therapy. The patient who achieved a partial remission developed a late near-complete response approximately 2 ½ years after the infusions, and remains in remission more than 5 years after treatment. Another patient underwent an additional infusion of CAR T cells after relapse and now remains in complete remission more than 4 ½ years later.

1.2.13 Advantages of using a lentiviral mediated gene transfer approach

Despite numerous efforts to augment the plasmid-based gene transfer approach, persistent problems have hindered further exploitation of this method. These difficulties include low transfection efficiency (<0.1%), suboptimal cell surface expression of the CAR (<10,000 molecules per cell), and the long expansion duration (2 to 4 months) required to achieve sufficient numbers of genetically modified T cells.[83,84] Retrovirus-mediated gene transfer has been embraced by the field of gene therapy because of its ability to provide higher transduction efficiencies, higher levels of target gene expression, and more rapid *in vitro* selection and expansion. Among the currently available retroviral vectors, self-inactivating (SIN) lentiviral vectors may have advantages over gamma-retroviral vectors in several areas, including resistance to gene silencing and a potentially safer integration site profile.[86-88]

To improve upon the results of our previous clinical trials, we transferred the anti-CD20 CD28-41BB CAR to a self-inactivating 3rd-generation lentiviral vector[89] and made several additional modifications to optimize efficacy in pre-clinical models. Recently published data from our center revealed that cellular immune rejection of the CAR transgene is a frequent cause of poor persistence of CD19-targeted CAR T cells, with immunogenic epitopes mapping to the murine single-chain variable fragment (scFv).[90] We therefore replaced our murine scFv with one derived from the fully human 1.5.3 monoclonal antibody,[91] with the expectation that it will reduce CAR immunogenicity and thus diminish the need for intensive lymphodepletion.

Data from our laboratory and others have demonstrated the importance of intercellular distance with respect to CAR T cell triggering,[83,92,93] suggesting that for a given target epitope, an ideal extracellular spacer length exists for each CAR.[94] CD20 has a membrane-proximal epitope, and we and others have tested extracellular spacer domains of varying lengths and found that a long spacer is required for optimal T cell function.[95 and unpublished data] However, full length spacers derived from human IgG Fc domains, such as the one used in our CAR construct, can bind Fcγ receptors and trigger activation-induced cell death.[96] We therefore modified the IgG1 Fc spacer region to abrogate binding to Fcγ receptors.[96]

The question of which intracellular signaling domain(s) to include in CARs is controversial, with different groups favoring different costimulatory domains. We tested 41BB-only, CD28-only, and CD28-41BB constructs and found that the CD28-41BB CAR led to superior activity in mouse xenograft experiments compared with 41BB-only and CD28-only constructs. This is consistent with our previous studies with murine-derived scFvs that showed superior proliferation and cytokine secretion of a 3rd generation CD20-targeted CAR compared with 1st or 2nd generation CARs.[84] These data led us to select this construct for clinical testing (**Figure 1.2**).

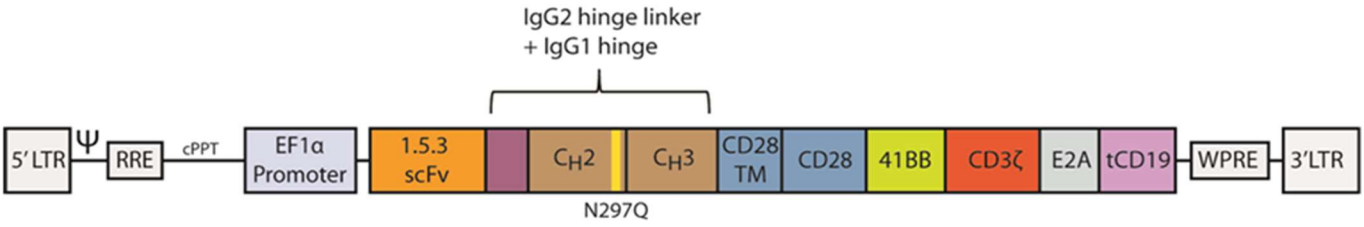


Figure 1.2: Lentiviral vector encoding bicistronic 3rd generation fully human CAR

The vector also encodes a non-functional truncated cell surface human CD19 (tCD19) separated from the CAR cassette by a self-cleavable E2A element that ensures coordinate expression of both proteins. The truncation of tCD19 abrogates all conserved tyrosine residues that serve as phosphorylation sites,[97] but retains the extracellular epitopes recognized by anti-CD19 antibodies. The tCD19 facilitates tracking of CAR T cells *in vivo*, and could potentially be used as a target for anti-CD19 antibodies or antibody-drug conjugates to eliminate CAR T cells if uncontrolled toxicity occurs.

The genes are expressed under the control of a truncated EF1α promoter. A synthetic WPRE sequence modified to remove the protein X open reading frame exists downstream of the 2 transgenes and proximal to the 3' polyadenylation signal to further enhance the expression of transgenes. In vitro, 1.5.3-based CARs function as well or better than those derived from the Leu16 or 1F5 murine anti-CD20 antibodies, and are capable of eradicating established lymphoma xenografts in NSG mice, with cure rates of approximately 80% (**Figure 1.3** and data not shown).

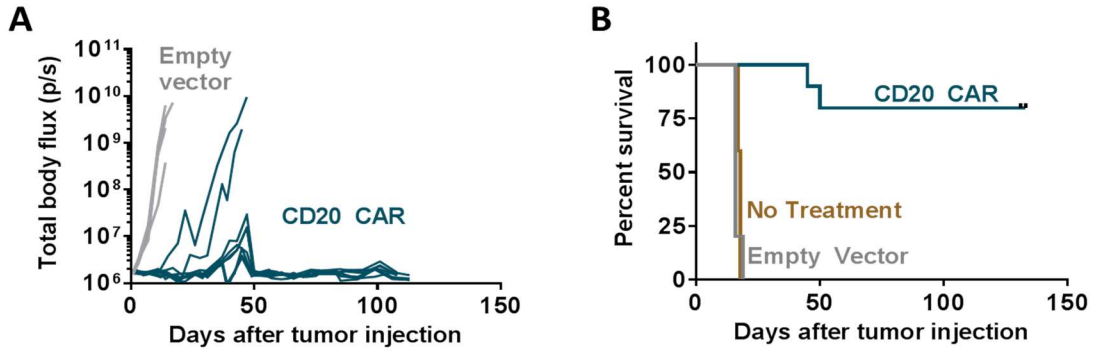


Figure 1.3: In vivo antitumor effect of fully human CD20-specific CAR. NOD-SCID-$\gamma^{-/-}$mice were injected i.v. with Raji-ffLuc tumor cells, followed 2 days later by i.v. injection of T cells transduced either with the 1.5.3-NQ-28-BB-ζ CAR or an empty vector. A) Tumor burden over time as assessed by bioluminescence imaging. B) Kaplan-Meier plot of overall survival.

T cells from healthy donors transduced with this lentivirus have demonstrated far superior surface CAR

expression than we were previously able to achieve using plasmid DNA transfection methods. The lentiviral transduction efficiency is 20-75%, compared to <0.1% with electroporation. These transduced T cells readily exhibited CD20-specific cytolytic toxicity when tested by standard chromium release assays using several different CD20 expressing lymphoma cell lines (data not shown).

1.2.14 Effect of rituximab on CD20 CAR T cell function

The proposed use of CD20-targeted CAR T cell therapy in a patient population that will have been uniformly previously treated with rituximab and/or other anti-CD20 antibodies raises two concerns. The first is that the cause of rituximab resistance could also confer resistance to the CAR T cells targeting the same antigen. The mechanism of resistance to rituximab is poorly understood, but only infrequently involves CD20 mutation or loss of CD20 expression.[98,99] Preclinical data from our lab indicate that CD20 CAR T cells retain cytolytic activity against rituximab-refractory cells and are capable of rejecting rituximab-refractory tumors in vivo.[100] The second concern is that residual levels of rituximab in the serum could block binding of the CD20 CAR to its target epitope, which is shared by rituximab, and thereby impair CAR T cell activity. We conducted extensive in vitro and in vivo tests on the impact of various levels of rituximab on CD20 CAR T cell function and found that levels of rituximab typically found in the serum of patients who have undergone recent rituximab-containing salvage therapy only minimally impaired the function of CD20 CAR T cells in vitro, and there was no negative impact of rituximab administered prior to CAR T cells in a xenograft mouse model (**Figure 1.4**).[100]

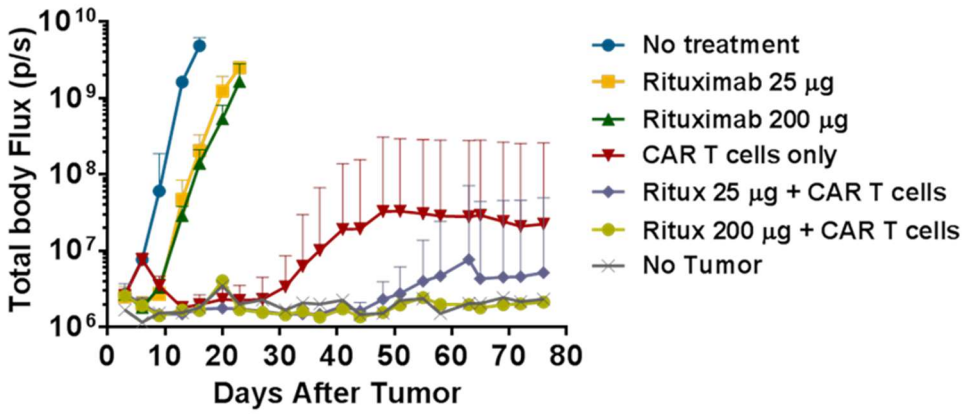


Figure 1.4. Effect of residual rituximab on CAR T cell activity in vivo. NOD-SCID-$\gamma c^{-/-}$ mice were injected with rituximab-refractory Raji-ffLuc tumors i.v., followed 5 days later by rituximab 25 or 200 µg i.p. Third-generation CD20-CAR T cells were administered 1 day after rituximab and tumor bioluminescence was measured twice weekly.

1.2.15 Clinical trials using CD19-targeted CAR T cells to treat B cell malignancies

Several groups have conducted clinical trials testing CD19-specific CAR T cells as therapy for relapsed or refractory B lymphoid tumors. The most responsive disease subtype appears to be acute lymphoblastic leukemia (ALL), with complete response rates of 80-90% in multiple studies.[90,101,102] The response rates for NHL and chronic lymphocytic leukemia have been lower, in the 50-70% range, with complete remissions in the 40-50% range.[103-105] Since the follow-up of most patients is still short, the durability of responses is still not yet well established, but a few patients have been reported to have long-term remissions without additional therapy.

A hallmark of CAR T cell activity has been cytokine release syndrome (CRS), marked by fever, hypotension, elevated serum levels of cytokines as well as LDH, ferritin, and CRP.[27,90,101] This can resemble septic shock clinically, and some patients require vasopressor support in the intensive care unit (ICU). The severity of CRS correlates closely with tumor burden, particularly in patients with ALL.[90] This complication can be managed by intervening with tocilizumab (anti-IL-6 receptor antibody) and/or dexamethasone. Another sequela of CAR T cell therapy, particularly in patients who develop severe CRS, is neurotoxicity, including delirium, hallucinations, seizures, and coma. Patients at high risk of CRS and neurotoxicity are often treated prophylactically with anticonvulsant therapy. The mechanism of neurological manifestations following CAR T cell therapy is poorly understood but symptoms are fully reversible in the vast majority of patients.

1.2.16 Adoptive therapy using defined T cell subsets

To date, most groups conducting CAR T cell clinical trials have generated T cell products from unselected patient-derived PMBC. Since patients have variable ratios of T cell subsets, particularly after treatment with various lymphotoxic regimens, the composition of the infused cell products in these patients has been highly variable, with some patients receiving products consisting of > 95% $CD4^+$ cells, and others with > 95% $CD8^+$ T cells.[85,101,103,104,106] The Darcy group in Australia showed that a 1:1 infusion of $CD4^+$ to $CD8^+$ CAR T cells led to the most effective antitumor activity in mouse models.[107] Subsequently, the Riddell Laboratory performed detailed studies evaluating various $CD4^+$ and $CD8^+$ T cell subsets, and found that adoptively transferred cells derived from central memory T cells have a unique ability to expand and persist in vivo and respond to antigen re-challenge,[108] and that combinations of $CD4^+$ and $CD8^+$ cells, particularly $CD8^+$ central memory cells, led to the most effective antitumor activity in a xenograft mouse model.[96] Other groups have also demonstrated that $CD4^+$ T cells are known to enhance the proliferation, survival, effector function, and anti-tumor potency of $CD8^+$ T cells.[91,107,109] and in some models $CD4^+$ cells alone can mediate a cytotoxic effect and in vivo anti-tumor activity.[110]

On this clinical trial, we observed poor ex-vivo expansion of the $CD8^+$ cell cultures for 6 of the first 8 patients for whom a cell product was generated. We hypothesized that the reason for the poor $CD8^+$ cell growth is lack of $CD4^+$ cell help inherent with culturing each subset in isolation, and that combining $CD4^+$ and $CD8^+$ cells into a single culture, mirroring the physiologic state, would improve $CD8^+$ cell growth. We subsequently conducted extensive pre-clinical tests evaluating the co-culture of $CD4^+$ and $CD8^+$ cells, and found that $CD8^+$ cells cultured together with $CD4^+$ cells have significantly better ex vivo expansion, less exhausted phenotype, higher cytokine secretion, and improved anti-tumor function in a mouse lymphoma model, compared with $CD8^+$ cells grown separately.[26] We also found that while a precise 1:1 ratio cannot be achieved in the same way possible with separate $CD4^+$ and $CD8^+$ expansion, initiating the cell cultures at a defined CD4:CD8 ratio led to a more consistent CD4:CD8 ratio in the final cell product than what has historically been reported in CAR T cell trials using unselected T cells.[111]

1.2.17 Phase I/II clinical trial with CD19-targeted cells using defined T cell subsets.

In an ongoing clinical trial at Fred Hutch, Drs. Maloney, Turtle, and Riddell have generated T cell products using a 1:1 ratio of $CD4^+$ to $CD8^+$ T cells, with some patients receiving unselected $CD8^+$ T cells and others receiving CD8 central memory T cell-derived cells.[90,105] In this study, 103 patients have been treated to

date, including 37 patients with ALL, 50 with NHL, and 16 with CLL. Of 29 evaluable ALL patients, 27 (93%) achieved a complete remission. The defined subset approach facilitated analyses that defined response and toxicity relationships with cell dose as well as rates of T cell expansion. Toxicities were also dependent on the tumor burden, particularly in ALL. For patients with lymphoma, the overall response rate was 63%. For patients conditioned with cyclophosphamide +/- etoposide, the complete response rate was only 1/12, but among patients who were treated with fludarabine-containing lymphodepletion regimens (see below), the CR rate improved to 50%, with an overall response rate of 73%. It should be noted that this regimen was not without toxicities. Four of 32 NHL patients and 7 of 30 ALL patients developed severe cytokine release syndrome requiring ICU-level care, and approximately 50% of patients had mild-moderate cytokine release syndrome. Neurotoxicity was also prevalent, with 15 of 30 ALL and 28% of NHL patients developing grade ≥ 3 toxicities.

One limitation of this study was initially short persistence of the infused CAR T cells, due to host cellular immune responses mounted against transgenic peptides. Epitope mapping studies indicated that immune responses were predominantly against epitopes derived from the murine scFv domain. The addition of fludarabine to the conditioning regimen has significantly reduced the incidence of transgene rejection and has resulted in improved CAR T cell expansion and persistence.[90]

1.2.18 The role of lymphodepletion in promoting proliferation and expansion of genetically modified T cells in vivo.

Studies in mice and humans have revealed an intricate homeostatic system that controls the numbers of T lymphocytes in the body.[112,113] Though details of this feedback regulatory system are still under investigation, concentrations of IL-2, IL-7, IL-15, and perhaps IL-21 appear to be important.[81,113] As a result of this feedback homeostatic regulation, the proliferation, persistence, and expansion of adoptively transferred anti-tumor T cells is restricted in the presence of large numbers of endogenous T cells which compete for these trophic T cell cytokines. Conversely, expansion of infused T cells is augmented if endogenous T cells are depleted prior to T cell therapy, presumably due to availability of higher concentrations of IL-2, IL-7, IL-15, and IL-21.[114,115] One such approach is chemotherapy-mediated lymphodepletion prior to adoptive T cell infusion.[115-117] Specifically, the homeostatic system and the tumor microenvironment may be modulated to be more favorable for adoptively transferred tumor targeted T cells through lymphodepleting chemotherapy with cyclophosphamide and/or fludarabine.[90,115-119] Reduction of endogenous $CD4^+CD25^+$ regulatory T cells is an additional expected advantage, since Treg cells suppress the anti-tumor effects of adoptively transferred effector T cells. Investigators at the NCI have demonstrated impressive persistence of tumor-specific T cells for many months after using a lymphodepletion regimen of cyclophosphamide prior to infusion of *ex vivo* expanded tumor infiltrating T cells isolated from melanoma patients.[120]

In the ongoing CD19 CAR T cell trial at Fred Hutch, Drs. Maloney and Turtle initially used lymphodepletion with high-dose cyclophosphamide (CY) with or without etoposide. Initially, patients who were re-treated with a second infusion of T cells had poor expansion of T cells, and this was found to be to the result of cellular immune responses directed against the transgene. Consequently, fludarabine was added to the regimen, with most patients receiving a regimen of CY 60 mg/kg followed by 3 days of fludarabine 25

mg/m^2/day. Patients who were lymphodepleted with fludarabine-containing regimens had significantly higher IL-7 and IL-15 levels in the serum, better persistence and expansion of CAR T cells, and better complete response rates as noted above. Furthermore, patients who received 2nd infusions of CAR T cells also had good expansion and persistence and the rate of anti-transgene immune responses appeared to be significantly lower.

1.2.19 Summary

We therefore plan to test autologous T cells modified with a fully human 3rd-generation anti-CD20 CAR lentiviral vector, manufactured using CD4$^+$ to CD8$^+$ T cells combined at a defined ratio at culture initiation, in patients with relapsed or refractory lymphoma and CLL patients.

2 OBJECTIVES

2.1 Primary Objective

To estimate the maximum tolerated dose (MTD) of adoptive cellular immunotherapy using *ex vivo* transduced and expanded autologous T cells expressing a 3rd-generation fully human CD20-specific chimeric antigen receptor (CAR) in patients with relapsed or refractory CD20$^+$ B-NHL or CLL/SLL.

2.2 Secondary Objectives

2.2.1 To evaluate the safety and toxicity associated with CD20 CAR T cell infusions.

2.2.2 To evaluate preliminary antitumor activity of adoptively transferred T cells in patients with measurable tumor burden prior to treatment as measured by overall response rate (ORR) and complete remission (CR) rate.

2.2.3 To evaluate progression-free survival (PFS) and overall survival (OS) among patients treated with adoptively transferred CD20-specific T cells.

2.3 Tertiary Objectives

2.3.1 To evaluate baseline tumor characteristics predictive of response or treatment resistance.

2.3.2 To evaluate mechanisms of treatment failure in patients who relapse or do not respond, including both T cell- and tumor-related factors.

2.3.3 To evaluate the duration of in vivo persistence of adoptively transferred CD20-specific T cells.

2.3.4 To evaluate the trafficking of transferred CD20-specific T cells to tumor sites, lymph nodes, and bone marrow, and to evaluate their immunophenotypes.

2.3.5 To assess development of endogenous anti-tumor responses and epitope spreading.

2.3.6 To evaluate the development of host immune responses against the CD20 CAR T cells in patients.

3 SUBJECT SELECTION

Please note: All potential study candidates will have an initial clinic visit and will sign a screening consent that includes all the screening procedures described in section 8.1.,

including tumor biopsy. The following eligibility criteria will be evaluated after the screening stage, prior to study enrollment, which occurs just prior to leukapheresis (as defined in Section 4.1.3).

3.1 Inclusion Criteria

3.1.1 Patients must have B-cell non-Hodgkin lymphoma or chronic lymphocytic leukemia/small lymphocytic lymphoma. Eligible lymphoma subtypes include (but are not limited to): mantle cell, follicular, lymphoplasmacytic, marginal zone, transformed indolent B cell lymphoma (including transformed CLL), or diffuse large B cell lymphoma that has relapsed after a response to at least one prior therapy regimen or is refractory to prior therapy. Patients with mantle cell lymphoma must have previously been treated with a BTK inhibitor and have either had disease progression, intolerance, or exposure to the drug for at least 3 months. Patients with CLL/SLL are eligible if they had disease progression or intolerance to BTKis and/or a BCL-2 inhibitors. They are also required to have been treated with the other agent for at least 3 months (i.e. patients with progression/intolerance to BTKi need to be treated with a BCL-2 inhibitor for at least 3 months, and patients with progression/intolerance to BCL-2 inhibitor need at least 3 months of exposure to a BTKi).

Patients with de novo DLBCL must meet one of the following criteria:

a. Biopsy-proven refractory disease after a frontline regimen containing both an anthracycline and rituximab or other anti-CD20 antibody (i.e. “primary refractory”), where any disease recurring within 6 months of completion of the regimen is considered refractory.
b. Relapsed or refractory disease after at least one of the following:
    i. At least 2 lines of therapy (including at least one with an anthracycline and anti-CD20 antibody)
    ii. Autologous stem cell transplant
    iii. Allogeneic stem cell transplant

3.1.2 Patients with large cell lymphoma transformed from indolent lymphomas are eligible if previously treated with anthracycline-containing regimen for either the indolent or large cell histology.

3.1.3 Patients with CNS lymphoma need to meet one of the following criteria:
Primary CNS lymphoma:

a. Progressive disease after 3 cycles or an inadequate response after at least 4 cycles of a high-dose methotrexate (MTX) containing regimen in the opinion of the treating physician, OR
b. Not eligible for MTX therapy due to comorbidities or tolerance issues per treating physician, OR
c. Recurrent disease after an initial response to MTX-based treatment.

Secondary CNS lymphoma

a. An inadequate response to at least 2 cycles of a MTX containing regimen, OR
b. Recurrent disease after an initial response to MTX-based treatment.

3.1.4 Patients must be 18 years of age or older, of any gender, race or ethnicity.

3.1.5 Patients must be capable of understanding and providing a written informed consent.

3.1.6 Negative serum pregnancy test within 2 weeks before enrollment for women of childbearing potential, defined as those who have not been surgically sterilized or who have not been free of menses for at least 1 year.

3.1.7 Fertile male and female patients must be willing to use an effective contraceptive method before, during, and for at least 4 months after the CAR T cell infusion.

3.1.8 Patients must have a Karnofsky performance status (KPS) of ≥ 60%. Patients with CNS lymphoma with KPS of ≥ 50% are eligible if performance status is low because of the active lymphoma.

3.1.9 Confirmation of diagnosis by internal pathology review of initial or subsequent biopsy or other pathologic material at Fred Hutch/UW/HMC.

3.1.10 Evidence of CD20 expression by immunohistochemistry or flow cytometry on the tumor specimen obtained with the biopsy performed with screening. If the CD20 expression on the screening tumor biopsy is unclear or could not be assessed due to technical reasons, CD20 expression on a concomitant tumor specimen (such as marrow biopsy or circulating tumor cells) may be used to satisfy this requirement. For CLL/SLL and lymphoplasmacytic lymphoma/Waldenström macroglobulinemia (WM) patients, a peripheral blood or bone marrow specimen will suffice to document CD20 expression for eligibility purposes. For patients with CNS lymphoma, a screening tumor biopsy is not required and evidence of CD20 expression can be documented from a previous biopsy.

3.1.11 Patients must have acceptable organ function, as defined as:

a. Serum creatinine ≤ 2.5.

b. Total bilirubin ≤ 3.0 mg/dL (except in patients with a confirmed Gilbert's syndrome by genetic test), AST and ALT ≤ 5x the upper limit of normal.

c. Adequate pulmonary function, defined as ≤ grade 1 dyspnea and SaO_2 ≥ 92% on room air. If PFTs are performed based on the clinical judgment of the treating physician, patients with FEV1 ≥ 50% of predicted and DLCO (corrected) of ≥ 40% of predicted will be eligible.

d. Adequate cardiac function, defined as left ventricular ejection fraction (LVEF) of ≥ 50% as assessed by echocardiogram or MUGA scan, or LVEF of 45-49% and clearance by a cardiologist.

3.1.12 Measurable disease that can be accurately measured in at least one dimension as ≥ 1.5 cm with CT, ultrasound, or MRI techniques. Extranodal disease that is measurable by FDG-PET imaging only will also be allowed. Note that if an excisional biopsy was performed that removed the sole site of measurable disease, the patient will not be eligible for leukapheresis and generation of CAR T cell product.

3.1.13 For patients with CNS lymphoma, a lesion ≥ 1 cm on brain or spine MRI is required.

3.1.14 Patients with WM without radiologic evidence of disease are eligible if they have measurable disease, as defined by serum monoclonal M-spike of ≥ 0.5 g/dL.

3.2 Exclusion Criteria

3.2.1 Active autoimmune disease requiring systemic immunosuppressive therapy.

3.2.2 Patients requiring corticosteroid therapy at a dose of 15 mg or more per day of prednisone or the equivalent. Pulsed corticosteroid dose for disease control is acceptable.

3.2.3 Patients who are HIV seropositive.

3.2.4 Women who are pregnant or breastfeeding.

3.2.5 Significant cardiovascular diseases within the past 6 months including uncontrolled congestive heart failure (>NYHA class II), myocardial infarction, unstable angina, or uncontrolled arrhythmia.

3.2.6 History of severe immediate hypersensitivity reaction to cyclophosphamide or fludarabine.

3.2.7 History or presence of clinically relevant non-lymphoma central nervous system pathology, including seizures that are uncontrolled on anticonvulsant therapy (≥ 1 seizure in the last year), paresis, aphasia, stroke, severe brain injuries, dementia, Parkinson disease, cerebellar disease, or psychosis.

3.2.8 Treatment with any investigational agent on a different clinical trial within 4 weeks prior to enrollment, unless the patient is documented to be unresponsive to the therapy and at least 3 half-lives have elapsed prior to enrollment.

3.2.9 Treatment with any anti-CD19 or anti-CD20 antibody or antibody-drug conjugate therapy within 4 weeks before enrollment.

3.2.10 Previous treatment with CD19-targeted CAR T cells that has resulted in ongoing B cell aplasia at the time of enrollment. Patients that demonstrate recovery of normal B cells (≥ 20 B cells/µl) by flow cytometry at any point 28 days or later after CD19 CAR T cell infusion will be considered to have functional loss of CD19 CAR T cells and are potentially eligible.

3.2.11 Patients with systemic disease without radiologic evidence of CNS involvement and with isolated CSF involvement detectable by flow cytometry are eligible for enrollment in systemic disease cohorts if neurologically asymptomatic. CSF involvement is not an exclusion for patients enrolled as primary or secondary CNS lymphoma.

3.2.12 Presence of active acute or chronic GVHD.

3.2.13 Uncontrolled active infection (bacterial, fungal, viral, mycobacterial) not responding to treatment with intravenous antibiotics, antiviral or antifungal agents.

3.2.14 Patients with concurrent known additional malignancy that is progressing and/or requires active treatment. Exceptions include squamous or basal cell carcinoma of the skin and low grade prostate carcinoma (Gleason grade ≤ 6). Maintenance anti-hormone therapies for breast or prostate cancers are allowed and are not considered active treatment.

3.2.15 Patients with blood or platelet transfusion within 1 week prior to signing Consent A, or with platelets < 50,000/mm^3, neutrophils < 750/mm^3, or hemoglobin < 8.5 g/dL, unless the cytopenias are considered by the treating physician to be largely due to marrow involvement by their B-cell malignancy.

3.3 Eligibility for Lymphodepletion Chemotherapy

3.3.1 Absence of uncontrolled active infection (bacterial, fungal, viral, mycobacterial) not responding to treatment with antibiotics, antiviral agents, or antifungal agents.

3.3.2 Absence of active autoimmune disease requiring ongoing systemic immunosuppressive therapy.

3.3.3 Negative serum pregnancy test within 2 weeks before lymphodepletion chemotherapy for women of childbearing potential, defined as those who have not been surgically sterilized or who have not been free of menses for at least 1 year.

3.3.4 No treatment with any investigational agent on a different clinical trial between enrollment and lymphodepleting chemotherapy.

3.3.5 Patients must have acceptable organ function, as defined as:

a. Serum creatinine ≤ 2.5.
b. Total bilirubin ≤ 3.0 mg/dL, AST and ALT ≤ 5x the upper limit of normal.
c. Adequate pulmonary function, defined as ≤ grade 1 dyspnea and SaO_2 ≥ 92% on room air. If PFTs are performed based on the clinical judgment of the treating physician, patients with FEV1 ≥ 50% of predicted and DLCO (corrected) of ≥ 40% of predicted will be eligible.
d. Adequate cardiac function, defined as left ventricular ejection fraction (LVEF) of ≥ 50% as assessed by echocardiogram or MUGA scan, or LVEF of 45-49% and clearance by a cardiologist. If subject receives cardiotoxic chemotherapy after enrollment, repeat echocardiogram or MUGA is required to reestablish eligible LVEF.

3.3.6 Patients must have a Karnofsky performance status of ≥60%. Patients with CNS lymphoma with KPS of ≥ 50% are eligible if performance status is low because of the active lymphoma.

3.3.7 Measurable disease that can be accurately measured in at least one dimension as ≥ 1.5 cm with CT, ultrasound, or MRI techniques. Extranodal disease that is measurable by FDG-PET imaging only will also be allowed. Note that if an excisional biopsy was performed that removed the sole site of measurable disease, the patient is not eligible for lymphodepletion and CAR T cell infusion. Measurable disease can be based on the imaging study done during the screening unless the patient received treatment in the interim, in which case imaging should be repeated. For patients with CNS lymphoma, a lesion ≥ 1 cm on brain or spine MRI is required. Patients with WM without radiologic evidence of disease are eligible if they have measurable disease, as defined by serum IgM level ≥ 0.5 g/dL.

3.3.8 Patients must require no corticosteroid therapy or dose of less than 15 mg per day of prednisone or the equivalent. Pulsed corticosteroid dose for disease control is acceptable until the day before the start of lymphodepletion.

3.3.9 Patients must have no active acute or chronic GVHD.

4 STUDY DESIGN

This is a phase I/II, open-label, non-randomized study. We plan to treat 50 DLT-evaluable patients with $CD20^+$ relapsed or refractory B-cell NHL or CLL/SLL. Each treated patient will undergo a tumor biopsy, leukapheresis, lymphodepletion chemotherapy, and adoptive transfer of autologous T cells modified with a CD20-CAR.

4.1 Overview of the Screening and Enrollment Process

4.1.1 Following arrival to the immunotherapy service, patients will have an arrival conference with the Immunotherapy Attending Physician, who will provide an overview of the treatment process, and

then patients will sign the screening consent that includes screening evaluations, restaging, bone marrow biopsy, and tumor biopsy (see **Section 8.1**).

4.1.2 If patients are determined to be ineligible or do not proceed to leukapheresis, they will be documented as a screen failure per **Section 10.1**.

4.1.3 If, after undergoing screening evaluations, patients meet all eligibility criteria outlined in **Sections 3.1 and 3.2** they will have a data review conference during which they will sign the consent form for leukapheresis, lymphodepleting chemotherapy, and T cell infusion (Consent A), at which point they will be considered enrolled. They will then proceed with leukapheresis and generation of a CD20 CAR T cell product. If a patient is unable to collect cells or generation of T cells is unsuccessful, they will be considered off-study.

4.1.4 Prior to lymphodepleting chemotherapy patients should have another brief conference with the attending physician, who will re-review the risks of chemotherapy and T cell infusions that were discussed with the patient at the data review conference. No written consent will be obtained at this conference unless re-consent is required.

4.1.5 Once patients meet criteria outlined in **Section 3.3**, they will proceed with lymphodepletion chemotherapy and T cell infusion as outlined in **Section 7**.

5 INFORMED CONSENT OF SUBJECTS

Subjects will be referred to the Fred Hutch by their primary care hematologists or oncologists for consideration of this protocol and will be completely evaluated. The protocol should be discussed thoroughly with the subject and other family members if appropriate, and all known and potential risks should be described. The procedures (including tumor biopsies, leukapheresis, lymphodepletion, and T cell infusion) and alternative forms of therapy should be presented as objectively as possible, and the risks and hazards explained. Consent will be obtained using forms approved by the Institutional Review Board of the Fred Hutchinson Cancer Center (Fred Hutch). Two consent forms will be used: 1) a screening consent to collect serum specimens for screening eligibility testing (HIV, and hepatitis B and C), perform restaging studies, obtain a blood sample to screen for circulating T cells, a tumor biopsy and bone marrow biopsy, and 2) a main study consent form that describes the research study procedures and risks for leukapheresis, lymphodepletion chemotherapy, T cell infusion, and study-related evaluations. A summary of the clinic visit detailing what was covered should be dictated for the medical record.

6 SUBJECT REGISTRATION

Potentially eligible subjects will be identified and registered into the system by the Clinical Coordinators Office (CCO) / Intake Office and assigned a UPN (Unique Patient Number). The CCO will register enrolled subjects on to the protocol through the Data Management Office.

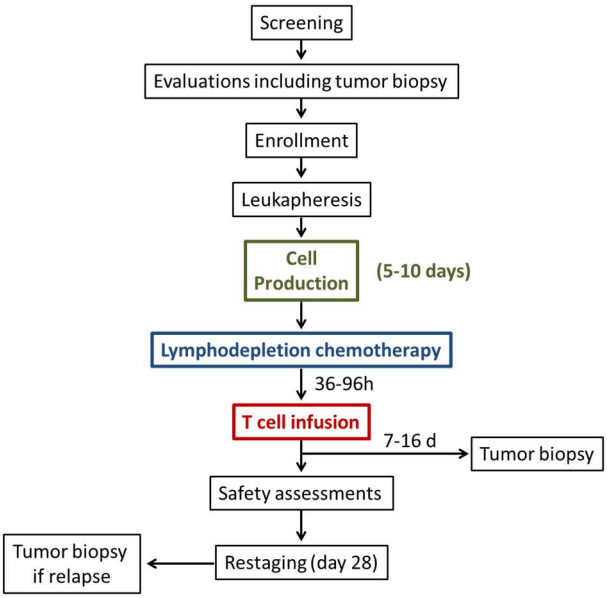
7 PLAN OF TREATMENT

7.1 Overview

As described in **Figure 7.1**, patients who sign the screening consent will undergo screening evaluations, including a tumor biopsy and restaging studies. Eligible patients will be enrolled into the study and

undergo leukapheresis. The PBMCs obtained by leukapheresis will be sorted to yield CD4 and CD8 T cells, which will be activated, transduced with CD20 CAR lentivirus, and expanded in the Good Manufacturing Practices (GMP)-compliant Cell Processing Facility at Fred Hutch. Prior to CAR T cell infusion, subjects will receive lymphodepletion chemotherapy intravenously (i.v.) ending 36-96 hours prior to T cell infusion. At 7-16 days following T cell infusion, patients will be asked to undergo a second tumor biopsy (if safe and feasible).

Figure 7.1 Study Schema



7.2 Leukapheresis

Each patient will undergo a leukapheresis procedure to obtain a sufficient quantity of peripheral blood mononuclear cells (PBMCs) to generate the CAR T cell product. Corticosteroid therapy (> 10 mg/day of prednisone or equivalent) should be avoided within 4 days of leukapheresis. The leukapheresis procedure will be performed by Fred Hutch staff, using standard operating procedures for obtaining PBMCs. Should a technical issue arise during the procedure or in the processing or manufacturing of the CAR T-cell product, or insufficient CD20 CAR T cells be manufactured for the prescribed CAR T cell dose, a second apheresis procedure may be performed.

Patients ineligible for a vein-to-vein apheresis may elect to have a percutaneous central venous catheter placed to permit this collection. Patients ineligible for apheresis who have a hematocrit of at least 38% and a total non-malignant (normal) lymphocyte count of > 2000/mcl may undergo phlebotomy of 400 ml of blood to obtain PBMCs necessary for generation of the CAR T cells. This approach would only be taken in patients that would be enrolled at dose levels 0 (1×10^5 $tCD19^+$ cells/kg), 1 (3.3×10^5 $tCD19^+$ cells/kg) and 2 (1×10^6 $tCD19^+$ cells/kg).

The leukapheresis or phlebotomy product will be delivered to the Cell Processing Facility (CPF) at the Fred Hutch and undergo CD4 and CD8 cell selection, processing, and quality release testing according to the procedures described under the Investigational New Drug (IND) application associated with this study. PBMCs from the leukapheresis or cell selection products may be archived for research.

7.3 Generation of CD20 CAR cells

Patient-derived apheresis PBMC will be selected for $CD4^+$ and $CD8^+$ T cells, transduced with purified lentivirus encoding the CD20 CAR and truncated CD19 transduction marker, and expanded under Good Manufacturing Practice (GMP) conditions. The specific methods for generating the T cell product and the release tests are outlined in the IND application to the U.S. Food and Drug Administration (FDA). Modifications to the IND will be submitted to the FDA. A single T cell product consisting of combined $CD4^+$ and $CD8^+$ T cells will be prepared for each infusion according to protocols established in the GMP Cell Processing Facility and will be formulated to provide a cell product at the specified cell dose of CAR^+ T cells, defined as the number of cells expressing the truncated CD19 transduction marker, which is expressed coordinately with the CAR in the vector. Cell products may either be infused fresh or following cryopreservation. Quality control and release testing will be performed on the CAR T cell product prior to its release for patient infusion. Investigational studies assessing phenotypic and functional status will also be performed on aliquots of expanded CAR T cells. Additionally, if cryopreserved T cells are available and deemed not to be needed for clinical use (for example, because a patient has died, or has already received an infusion, or is ineligible for infusion), these cells may be used for research purposes provided the patient has allowed use of "leftover tissues" for research in the consent form.

7.3.1 Products that cannot be formulated to meet target cell dose

If a T cell product cannot be formulated to meet the target dose because of low transduction efficiency or suboptimal cell growth, the cell product should be infused at or as close to possible to the specified total CAR T cell dose. The minimum acceptable number of cells that may be infused is 5×10^4 $tCD19^+$ cells/kg. Patients receiving cell products that do not meet ≥ 85% of the target dose specification will not be considered evaluable for the safety analysis in the assigned dose cohort for the purposes of the CRM design (as described in **Section 12**) to escalate to the next dose level (though a DLT occurring in such a patient would still count towards dose de-escalation—see **Section 12.4**), and a replacement subject will be added to the cohort. However, if such a patient receives a dose that meets the specification for a lower dose level, they would be considered evaluable for inclusion of the safety analysis for that dose level.

7.4 Cytoreductive chemotherapy

Patients may receive treatment after leukapheresis to control disease during production of CD20 CAR T cells. Decisions regarding chemotherapy in this interval should be discussed with the study PI. Treatment with any investigational agent on a clinical trial or with any agent targeting CD19 or CD20 is not allowed.

7.5 Lymphodepletion chemotherapy

Patients will receive lymphodepleting chemotherapy, which will be scheduled to be completed 36-96 hours prior to the anticipated infusion of CD20 CAR T cells. The goals of administering chemotherapy are

to provide lymphodepletion to facilitate survival of transferred T cells, and to reduce the tumor burden prior to infusion of CD20 CAR T cells. As outlined in the statistical considerations (**Section 12**), patients were initially treated with a single dose of cyclophosphamide (CY) i.v. 1 g/m^2. However, the response rate with CY only in the first 4 patients treated on this protocol was inadequate in order to continue this regimen (as per **Table 12.3**). The lymphodepleting regimen was changed to CY + fludarabine, as outlined in **Table 12.3**. Patients should be treated with cyclophosphamide 300 mg/m^2 IV on days 1-3 and fludarabine 30 mg/m^2 IV on days 1-3 and should be scheduled for T cell infusions starting 36-96 hours after the last dose of chemotherapy. For patients with impaired renal function, please refer to the fludarabine package insert for dose modifications. Alternative lymphodepletion regimens can be considered after discussion with the PI if CY + fludarabine is considered clinically inappropriate (such as in the case of prior allergy, drug intolerance or other medically significant reason) or if one or both drugs are not available. A preferred alternative lymphodepleting regimen is bendamustine as a single agent given at the dose of 90 mg/m^2/day for 2 days, typically on days -4 and -3 before CAR-T infusion.

Bendamustine is a chemotherapeutic agent with both alkylating and purine analog activities. Bendamustine has been utilized as one of the options for lymphodepletion (LD) before autologous CAR-T therapy. In the JULIET study, which was a registration trial that led to the approval of tisagenlecleucel for patients with diffuse large B-cell lymphoma after two prior lines of therapy, 20% of patients received bendamustine for lymphodepletion.[121] It was given at the dose of 90 mg/m^2 for 2 days on days -4 and -3 before CAR-T infusion. Response to CAR-T treatment was not different based on the type of lymphodepleting regimen (cyclophosphamide and fludarabine [CY-Flu] versus bendamustine). Similarly, the ELARA trial for patients with relapsed refractory follicular lymphoma allowed for the use of bendamustine as one of the lymphodepletion options.[122] Bendamustine is currently included in the prescribing information for tisagenlecleucel.[123]

In a multicenter retrospective study, investigators at the University of Pennsylvania, Oregon Health and Science University, and the Medical University of Vienna compared the safety and efficacy of CAR-T therapy with tisagenlecleucel between patients who received CY-Flu vs. bendamustine for lymphodepleting regimen.[124] They reported similar efficacy between the two groups. They also observed lower rates of cytokine release syndrome, neurotoxicity, and hematologic toxicities with bendamustine. There were also fewer episodes of infections, febrile neutropenia, and post-CAR-T hospitalization in the bendamustine cohort. The authors reported that the regimen was commonly used in their practices.

In 2021 and with the unexpected national shortage of fludarabine in the United States, the use of bendamustine as the lymphodepleting regimen was recommended by a panel of experts to avoid delay in delivering potentially life-saving treatments to patients in need of CAR-T therapy.[125] In a single-institution study conducted by the Stanford group, bendamustine was used for lymphodepletion before axicabtagene ciloleucel. The results of this study will be presented at the Annual meeting of the American Society of Hematology (ASH) in December 2022. Per verbal communication with the study's principal investigator, CAR-T expansion was comparable to what is seen in the post CY-Flu setting and there was no concerning signal for safety or efficacy.

7.6 CAR T cell infusion

There must be at least a 36-hour interval between the last dose of chemotherapy and the T cell infusion.

On the day of scheduled T cell infusion the patients should undergo a clinical evaluation and a clinical determination for appropriateness to proceed with CAR-T cell administration.

Patients who receive lymphodepleting chemotherapy but are deemed not clinically appropriate for the T cell administration or who were unable to undergo CAR-T cell infusion due to CAR-T cell manufacturing or release issues may be eligible to receive the T cell infusion outside the 36-96 hour window, for up to 14 days after the end of the 36-96 hour window, if clinical concerns and problems with manufacturing or release criteria are resolved. Discussion with the study PI is required for dose delay or need for repeat lymphodepletion chemotherapy. Patients who receive CAR-T cell infusion more than 14 days after lymphodepletion will not be considered DLT-evaluable for the purposes of dose escalation.

7.7 CD20 CAR T cell dose

Dosing will be based on transduced cell number, as determined by tCD19 expression. The starting dose will be dose level 1 (3.3 x 10^5 $tCD19^+$ cells/kg), and dose escalation/de-escalation will occur as described under **Section 12.4**.

Table 7.2: Dose Levels to be used (see **Section 12.4** for further explanation of dose escalation)

Dose level	CD20 CAR T Cell Dose
0	1 x 10^5 $tCD19^+$ cells/kg (± 15%)
1 (starting dose)	**3.3 x 10^5 $tCD19^+$ cells/kg (± 15%)**
2	1 x 10^6 $tCD19^+$ cells/kg (± 15%)
3	3.3 x 10^6 $tCD19^+$ cells/kg (± 15%)
4	1 x 10^7 $tCD19^+$ cells/kg (± 15%)

These cell doses are lower than the doses used by us and others in previous T cell protocols with CARs and in the same range as those in current CAR trials led by Drs. Riddell, Turtle, and Maloney.[83,126] (Our two previous CAR trials administered three infusions per patient with escalating doses of 10^8, 10^9 and 3.3 x 10^9 cells/m^2 given 2-5 days apart.) The current trial utilizes lower cell doses for safety reasons since the T cells used in this trial are expected to be more potent due to higher CAR expression and shorter in vitro incubation times. Cell dose modifications may be required for toxicities or technical difficulties.

7.8 Cell administration

Each patient will receive a single intravenous infusion of CD20 CAR T cells between 36-96 hours following completion of lymphodepleting chemotherapy. At least the first patient treated at each dose level will be admitted to the Institute of Translational Health Sciences Clinical Research Center at the University of Washington Medical Center for the T cell infusion.

Vital signs: All patients will be monitored during the T cell infusion. Vital signs (including oxygen saturation) should be recorded before infusion, approximately every 15 minutes during the infusion, and approximately hourly for 2 hours after the infusion. Subjects will remain in the infusion unit for a minimum of 2 hours following infusion, or until resolution of any infusion-related toxicity deemed to pose a significant risk to the study subject as an outpatient.

Infusion rate: Each cell infusion should be administered intravenously over approximately 20-30 minutes, adjusted as needed to comply with guidelines for endotoxin limits for parenteral drugs (≤ 5 EU/kg/hour). The infusion rate can also be adjusted if subjects experience mild infusion-related adverse events (grade 2 or lower).

7.9 Recommended Supportive Care, Additional Treatment, and Monitoring

Patients at risk for tumor lysis syndrome based on the judgment of the treating physician should receive prophylactic measures per institutional or clinical standards. Supportive care for the management of cytokine release syndrome is detailed in **Section 9.1.3**. Transfusion of red blood cells or platelets, and/or use of myeloid growth factors is permitted per institutional or clinical standards. Administration of daily G-CSF after T cell infusion until absolute neutrophil count is > 1000/µl should be considered.

The use of prophylactic or empiric antibiotics such as trimethoprim/sulfamethoxazole for pneumocystis pneumonia prophylaxis, broad spectrum antibiotics, antifungals, or antiviral agents is permitted per institutional standards.

The following should be considered for infection prophylaxis, as clinically indicated:

1) Antibiotic (such as a quinolone): while neutropenic.
2) Antifungal: start fluconazole on the day of CAR T cell infusion and continue for approximately 21 days.
3) Antiviral: start before chemotherapy and continue for 3-6 months after CAR T cell Infusion.
4) Pneumocystis jiroveci pneumonia (PJP) prophylaxis (e.g. trimethoprim/sulfamethoxazole): start approximately day 21 after CAR T cell infusion and continue until 3-6 months after infusion.

Patients with a history of seizures or history of CNS involvement by lymphoma should receive prophylactic treatment with anticonvulsants (levetiracetam or similar) following CAR T cell infusion for approximately 4 weeks.

8 PATIENT EVALUATION

8.1 Screening evaluations (should be performed within 30 days of enrollment unless otherwise specified)

Please note that results of tests and procedures conducted as per standard of care purposes prior to screening may be used for research purposes if conducted within the protocol-defined window.

The screening consent and HIPAA authorization must be signed before any non-standard of care evaluations are performed.

8.1.1 Medical history and physical examination, including prior therapies and response to therapy, if known, and Karnofsky performance status.

8.1.2 Laboratory tests, including:

a. CBC, differential, platelet count
b. Basic metabolic panel
c. Hepatic function panel with LDH
d. Fred Hutch (PSBC) Donor Battery Panel (should be performed within 30 days of leukapheresis)
e. β2 microglobulin level
f. Serum ferritin
g. Serum CRP
h. Uric acid
i. Phosphate
j. Serum IL-6
k. Quantitative serum immunoglobulin levels (IgG, IgA, IgM)
l. Serum protein electrophoresis and immunofixation (for patients with WM)
m. Prothrombin time (PT), partial thromboplastin time (PTT)
n. Serum pregnancy test for females of childbearing potential within 14 days of enrollment

8.1.3 Confirmation of diagnosis by internal pathology review of initial or subsequent biopsy or other pathologic material at the Fred Hutch/UW/HMC

8.1.4 Baseline chest x-ray

8.1.5 Baseline pulse oximetry and documentation of O_2 saturation on room air

8.1.6 Baseline 12-lead EKG

8.1.7 Echocardiogram or MUGA scan

8.1.8 Flow cytometric analysis for B cell quantitation and screening for circulating lymphoma cells/minimal residual disease (MRD) to Fred Hutch Hematopathology.

8.1.9 A CT scan (preferably diagnostic quality) and, if possible, a PET scan should be performed to evaluate disease status. If a PET/ CT is not performed then the CT scan must be of diagnostic quality. Imaging studies may be omitted in patients who have had recent imaging within 42 days before enrollment AND have not received anti-tumor therapy in the interim. Imaging should be performed prior to tumor biopsy (**Section 8.1.13**) in patients without palpable adenopathy. For patients with palpable adenopathy, the PET-CT scan may be performed after leukapheresis unless needed to confirm the presence of measurable disease.

8.1.10 MRI of the brain and spine with and without contrast (for CNS lymphoma patients only)

8.1.11 A unilateral bone marrow biopsy and aspirate should be performed, with pathology and flow cytometry. PCR for IgH clonality and/or FISH and other molecular studies may be performed as indicated by the disease. This may be omitted if another bone marrow biopsy was performed within 42 days of enrollment AND the patient has not received anti-tumor therapy in the interim.

8.1.12 Lumbar puncture with CSF evaluation is required for any patient with a history of CNS disease or signs or symptoms of CNS or epidural disease. This may be omitted if a lumbar puncture performed within 42 days of enrollment did not show evidence of CNS disease.

Research samples:

8.1.13 RESEARCH tumor biopsy (within 30 days prior to enrollment):
Patients will be asked to undergo either an imaging-guided biopsy or excisional biopsy. Radiologic-guided biopsies should be core needle biopsies, if possible the equivalent of 6-8 cores, 1 cm in length, using a 16-18 gauge needle, as well as 2-4 fine-needle aspirates (FNAs). All biopsy cores (or excisional biopsy tissue) and FNAs should be placed in RPMI medium and sent directly to the Fred Hutch pathology lab for processing via a dedicated STAT courier. A portion of the tissue will be submitted for clinical evaluation and assessment of CD20 expression by flow cytometry and/or immunohistochemistry (IHC), and the remainder will be used for correlative research assays such as gene expression profiling and analysis of immune cell subsets by flow cytometry. If adequate tissue is obtained, archival tumor cells may also be taken and cryopreserved for future studies. The tumor biopsy is optional for patients with primary or secondary CNS lymphoma, and for CLL/SLL and WM patients who have bone marrow or peripheral blood involvement.

8.1.14 Lumbar puncture (for primary and secondary CNS lymphoma patients). Clinical samples for cell count and flow cytometry should be collected, as well as a 2 ml research sample that should be sent to the Till Lab (D3-367).

8.1.15 Immunophenotyping of CD3, CD4, and CD8 T cells: A 10 ml blood sample (in EDTA [lavender top] tube) should be sent to the Till Lab.

8.1.16 PBMC Baseline Samples: A 60 ml blood sample (in EDTA [lavender top] tube) for research should be sent to the Specimen Processing/Research Cell Bank lab at the Fred Hutch for PBMC isolation and cryopreservation. These cells will be used as PBMC baseline samples for comparison to post-treatment samples for all cellular correlative tests, including CAR T cell persistence and immunophenotype by flow cytometry or Q-PCR, B cell reconstitution (IGH, IGK, IGL, Bcl-2) or TCR-β deep sequencing (Adaptive Biotechnologies) assays, assessment of anti-transgene and anti-tumor immune responses.

8.1.17 Baseline serum: A 20 ml blood sample (serum separator tube) should be sent to the Specimen Processing/Research Cell Bank lab at the Fred Hutch to be used as serum baseline to assess anti-transgene and anti-tumor immune responses. These tests may be performed depending on tumor response and CAR T cell persistence.

8.1.18 Serum cytokine levels: A 10 ml blood sample (serum separator tube) should be obtained for measurement of serum cytokine levels and sent as soon as possible to the Specimen Processing/Research Cell Bank lab at the Fred Hutch.

8.1.19 PBMC Archive Specimens: A 30 ml blood sample (in EDTA [lavender top] tubes) should be sent to the Specimen Processing/Research Cell Bank lab at the Fred Hutch for processing and cryopreservation as PBMC archive specimens for future correlative tests.

8.1.20 Serum archival specimens: A 10 ml blood sample (serum separator tube) should be sent to the Specimen Processing/Research Cell Bank lab at the Fred Hutch for processing as serum archival specimens for future correlative tests.

8.1.21 Bone marrow aspirate: If a bone marrow biopsy is performed, then an additional 5-10 ml of marrow aspirate should be obtained and sent in EDTA [lavender top] tubes to the Specimen Processing/Research Cell Bank Lab at the Fred Hutch, as archival tissue for tumor cells and as baseline for assessing CAR T cell migration to tumor sites. For patients with CLL/SLL or WM who forego a tumor biopsy, an additional 3 ml bone marrow aspirate sample should be obtained in an EDTA [lavender top] tube and sent to the Till Lab (D3-367).

8.1.22 Other biopsy or sample: If biopsy or sampling of tissues other than bone marrow (i.e. CSF, pleural fluid, etc.) is performed for clinical indications, then additional tissue may be obtained during the same procedure and sent to the Specimen Processing/Research Cell Bank Lab at the Fred Hutch for research studies. Please discuss the planned procedure with the study PI.

8.1.23 RCL Testing: A 10 ml (EDTA/lavender top) blood sample should be collected as baseline for RCL testing and sent to the Specimen Processing Lab/ Research Cell Bank Lab at the Fred Hutch.

8.2 Evaluations prior to lymphodepleting chemotherapy (should be performed within 1 week of initiation of lymphodepleting chemotherapy unless otherwise specified)

8.2.1 Interval history and physical exam (with documentation of Karnofsky performance status).

8.2.2 Laboratory evaluations:

a. CBC, differential, and platelets
b. Basic metabolic panel
c. Hepatic function panel with LDH
d. Serum ferritin
e. Serum C-reactive protein (CRP)
f. Uric acid
g. Phosphate
h. Serum protein electrophoresis with immunofixation (for WM patients who received interim therapy since baseline assessments)
i. Serum pregnancy test for females of reproductive potential within 14 days prior to starting lymphodepleting chemotherapy

8.2.3 Research Labs:

PBMC Archive Specimens: A 30 ml blood sample (in EDTA [lavender top] tubes) should be sent to the Specimen Processing/Research Cell Bank lab at the Fred Hutch for processing and cryopreservation as PBMC archive specimens for future correlative tests.

8.2.4 Repeat imaging (CT or PET/CT, or brain/spine MRI for CNS lymphoma patients) is required in patients who received interim cytoreductive treatment. This can be performed any time after the completion of treatment and before initiation of lymphodepletion chemotherapy.

8.2.5 Repeat assessment of LVEF (echocardiogram or MUGA) is required in patients who received cardiotoxic treatment with potential effect on the ejection fraction after enrollment. This can be performed any time between completion of the cardiotoxic therapy and initiation of lymphodepletion.

8.3 Evaluations prior to T cell Infusion

8.3.1 Interval history and physical exam.

8.3.2 Blood draw for laboratory studies:

a. CBC, differential, and platelets
b. Basic metabolic panel
c. Hepatic function panel with LDH
d. Uric acid
e. Phosphate
f. Serum ferritin
g. Serum CRP
h. Serum IL-6 level
i. DIC panel without platelets (PT, PTT, fibrinogen, D-dimer)

8.3.3 Research Labs:

a. Serum cytokine levels: A 10 ml serum separator tube should be sent as soon as possible to the Fred Hutch Specimen Processing/Research Cell Bank lab for cryopreservation, to be used for measurement of serum cytokine levels.

8.4 Evaluations following T cell infusion

The following evaluations will be performed after the T cell infusion.

8.4.1 Record new findings on history and physical exam 1 day after the T cell infusion and at least weekly for 4 weeks.

8.4.2 Karnofsky performance status should be recorded for the day 28 evaluation.

8.4.3 The following laboratory studies at least twice weekly for 2 weeks, then weekly until 4 weeks after the infusion:

a. CBC, differential, and platelet count
b. Basic metabolic panel
c. Hepatic function panel with LDH
d. Uric acid
e. Phosphate
f. C-reactive protein (CRP)
g. Serum ferritin
h. Serum IL 6 level
i. DIC panel without platelets (PT, PTT, fibrinogen, D-dimer)

8.4.4 Additional time points for CBC with differential and platelet count: at 3, 6, and 12 months after the T cell infusion. These may be discontinued if patients proceed to other systemic anti-lymphoma therapy.

8.4.5 If patients become febrile or develop symptoms of cytokine release or tumor lysis between the indicated time points, we may measure serum ferritin, CRP, DIC panel, and tumor lysis markers at additional times, as clinically indicated.

8.4.6 Immunoglobulin levels

a. Recommendations will be made to monitor IgG levels at day 28 (± 5 days), and 3, 6, and 12 months after T cell infusion, and to administer intravenous immune globulin (IVIG) as clinically indicated for hypogammaglobulinemia.

b. Serum IgG, IgA, and IgM levels may be measured at approximately 3 and 12 months after the T cell infusion in patients who develop B cell aplasia.

8.4.7 Serum protein electrophoresis with immunofixation (for WM patients only) at day 28 (± 5 days), and 3, 6, and 12 months after T cell infusion.

8.4.8 Flow cytometry for MRD: Blood (5 ml in EDTA [lavender top] tube) should be sent to the Fred Hutch Hematopathology laboratory around day 28, and at approximately 3, and 6, and 12 months after the T cell infusion for measurement of minimal residual disease (MRD) by flow cytometry. Except for day 28 flow cytometry, which is required in all patients, the MRD tests will only be done at the above-mentioned time points if the prior test showed evidence of disease. This assessment may cease if the patient initiates other non-CAR T cell therapy.

8.4.9 Research tumor biopsy: between approximately 7 and 16 days following the T cell infusion, all patients with pathologic, palpable or radiologically accessible lymphadenopathy or extranodal solid tumor will be asked to undergo either an excisional or a core biopsy of the tumor site. The requirement for a tumor biopsy will be waived for patients who do not have a tumor site amenable to either excisional or core needle biopsy. The timing of the biopsy may be delayed to beyond day 16 if it is deemed unsafe due to clinical concerns such as cytopenias. The tumor biopsy is optional for patients with primary or secondary CNS lymphoma, and for CLL/SLL and WM patients who did not undergo a baseline tumor biopsy.

8.4.10 Patients may also be asked to undergo a tumor biopsy at the time of relapse.

a. Image guided biopsies: should be core needle biopsies, if possible the equivalent of 6-8 cores, 1 cm in length, using a 16-18 gauge needle, as well as 2-4 FNAs, should be obtained. All biopsy cores and FNAs should be placed in RPMI medium and sent directly to the Fred Hutch pathology lab for processing via a dedicated STAT courier. A portion of the tissue will be submitted for clinical evaluation and assessment of CD20 expression by flow cytometry and/or immunohistochemistry (IHC), and the remainder will be used for correlative research assays. One or more of the following tests will be performed, depending on the number of available tumor cells: gene expression profiling by RNA Seq and/or 10x Genomics single cell RNA-seq analysis, flow cytometry for CAR T cells, immune cell subsets, and CD20 expression, multiplex immunohistochemistry, and deep sequencing of the TCR-β locus (Adaptive Biotechnologies). If any tissue remains after the correlative tests, it may be banked for future studies.

b. Excisional biopsies: the tissue should be placed in RPMI medium and sent directly to the Fred Hutch pathology lab for processing via a dedicated STAT courier. A portion of the tissue will be submitted for clinical evaluation and assessment of CD20 expression by flow cytometry and/or immunohistochemistry (IHC), and the remainder will be used for correlative research assays. One or more of the following tests will be performed, depending on the number of available tumor cells: gene expression profiling by RNA

Seq and/or 10x Genomics single cell RNA-seq analysis, flow cytometry for CAR T cells, immune cell subsets, and CD20 expression, multiplex immunohistochemistry, and deep sequencing of the TCR-β locus (Adaptive Biotechnologies). If any tissue remains after the correlative tests, it may be banked for future studies.

c. Patients with CLL/SLL with suspected marrow relapse may have a bone marrow biopsy instead of a tumor biopsy. A core biopsy should be obtained and sent to the Fred Hutch pathology lab for clinical evaluation and assessment of CD20 expression by IHC. A 3 ml aspirate sample should be sent to University of Washington Hematopathology for clinical flow cytometry. An additional 3 ml aspirate sample should be sent in an EDTA/lavender tub to the Till Lab (D3-367), and a 5 ml aspirate sample should be sent in an EDTA/lavender tube to the Fred Hutch Specimen Processing/Research Cell Bank for storage for future studies as noted above.

8.4.11 Patients achieving a partial or complete response who subsequently develop disease progression or suspected disease progression may be asked to undergo a research biopsy (optional) to understand the biological changes in the tumor after treatment that allowed tumor re-growth. Patients who were not able to undergo a day 7-16 biopsy or who had a biopsy but inadequate tissue was obtained, may also undergo an additional biopsy at the time of disease progression or suspected disease progression. The biopsy may be either an excisional biopsy or core needle biopsy. If the biopsy is being performed for clinical purposes, attempts should be made to acquire additional tissue for research purposes. Samples should be processed as described in **8.4.9**.

8.4.12 Bone marrow aspirate: For patients who had detectable lymphoma (by morphology, flow cytometry, or molecular testing) in the baseline bone marrow biopsy, a repeat bone marrow biopsy and aspirate will be performed at day 28 (± 5 days, with pathology and flow cytometry. PCR for IgH clonality and/or FISH and other molecular studies should be performed if positive at baseline. If a marrow biopsy is performed, an additional 5 ml in a lavender top tube may be sent to the Specimen Processing/Research Cell Bank Lab at Fred Hutch for research purposes (optional, see below).

8.4.13 Lumbar puncture (for primary and secondary CNS lymphoma patients) at day 14 (± 3 days) and day 28 (± 5 days). Clinical samples for cell count and flow cytometry should be collected, as well as a 2 ml research sample that should be sent to the Till Lab (D3-367). Additional research samples may be obtained on any lumbar punctures that are done at later timepoints for clinical reasons.

8.4.14 <u>Research labs</u>:

a. <u>Serum storage for measurement of serum cytokine levels</u>

i. Serum cytokine levels: A blood sample (up to 10 ml, serum separator tube) should be obtained on approximately days 1, 4, 7, 10, 14, 21, and 28 after the T cell infusion for measurement of select cytokine levels. Samples should be sent to the Specimen Processing/Research Cell Bank Lab at Fred Hutch.

ii. If patients become febrile, develop signs of cytokine release syndrome, or cytokine assessment is clinically appropriate at times other than those indicated, we may measure cytokine levels at additional times.

b. Evaluation for persistence and phenotype of CD20 CAR T cells
    i. PBMC for CAR T cell persistence: Blood samples (20 ml, EDTA [lavender top] tube) should be obtained on approximately days 1, 4, 7, 10, 14, 21, and 28 during the first month after the T cell infusion, and also at 3, 6, and 12 months after the T cell infusion for analysis of the persistence and phenotype of transferred T cells. Samples should be sent to the Specimen Processing/Research Cell Bank Lab at Fred Hutch. Additional samples may be collected at other times than those indicated, including beyond 12 months, if required for evaluation of persistence of CAR T cells. Conversely, persistence monitoring may be discontinued beyond day 28 in patients who proceed to allogeneic stem cell transplantation and/or who do not have detectable transgene-expressing T cells on two consecutive occasions. The cells from these blood samples may also be analyzed by multiparameter flow cytometry for the phenotype of persisting CAR T cells (optional). Markers that may be analyzed include CD19, CD4, CD8, CD62L, CCR7, CD28, CD27, CD127, PD1, Lag-3, Tim-3, and PD-L1.
    ii. If patients become febrile, develop possible signs of toxicity, or assessment of CAR T cell persistence is clinically appropriate at times other than those indicated, we may measure the persistence of transferred T cells at additional times.

c. B-cell quantitation. Blood (5 ml in EDTA [lavender top] tube) should be sent to the Fred Hutch Hematopathology laboratory around day 28, and at approximately 3, and 6, and 12 months after the T cell infusion for B cell quantification by flow cytometry. B cell quantitation may be discontinued beyond day 28 in patients who proceed to other systemic therapy.

d. B cell deep sequencing – A blood sample (10 ml EDTA [lavender top] tube) should be collected for the evaluation of MRD and B cell reconstitution by deep sequencing of the IGH and IGK/L loci (Adaptive Biotechnologies) around day 14, day 28, and at approximately 3, 6, and 12 months. These samples should be sent to the Fred Hutch Specimen Processing/Research Cell Bank lab for processing. If a deficiency of B cells persists beyond 12 months or there are persisting CD20 CAR T cells, additional samples may be obtained to monitor B cell numbers every 6 months either with the Adaptive assay or by flow cytometry. B cell deep sequencing monitoring may be discontinued beyond day 28 in patients who proceed to other systemic therapy, or if CAR T cell engraftment is lost, or at the discretion of the PI.

e. Evaluation of transgene immunogenicity – patients may develop immune responses against the transgene leading to rejection of the transferred T cells.
    i. Serum transgene immunogenicity: Serum (10 ml, serum separator tube) may be collected around day 28, and for patients in whom persistence of the CAR T cells is lost before day 30, at approximately 3 and 6 months after the T cell infusion. Collected serum will be banked for potential assessment of antibody or cellular immune responses against the transgene (optional). These samples should be sent to Fred Hutch Specimen Processing/Research Cell Bank.
    ii. PBMC transgene immunogenicity: PBMC (20 ml, EDTA [lavender top] tube) may be collected around day 28, and for patients in whom persistence of the CAR T cells is lost before day 30, at approximately 3 and 6 months after the T cell infusion. Collected PBMC will be banked for potential assessment of antibody or cellular immune

responses against the transgene (optional). These samples should be sent to Fred Hutch Specimen Processing/Research Cell Bank.

f. Archival samples for future studies of T cell function

i. PBMC archive specimens: Blood (30 ml, EDTA [lavender top] tubes) may be obtained from patients once between days 0 and 21 and once between days 22 and 90 after CD20 CAR T cell infusion, and at approximately 3, 6, and 12 months after the T cell infusion for archival purposes. Samples should be sent to the Specimen Processing/Research Cell Bank lab at Fred Hutch for processing and cryopreservation as PBMC archive specimens.

ii. Serum archive specimens: Serum (up to 10 ml, serum separator tube) may be obtained from patients at approximately 3, 6, and 12 months after the T cell infusion for archival purposes. Samples should be sent to the Specimen Processing/Research Cell Bank lab at Fred Hutch for processing and cryopreservation as serum archive specimens.

g. Evaluation of migration of adoptively transferred CD20 CAR T cells

i. If bone marrow aspirations are performed for evaluation of tumor response or other clinical indications, then additional aspirates (5-10 ml in EDTA [lavender top] tubes) may be obtained and sent to the Specimen Processing/Research Cell Bank lab at Fred Hutch (optional).

ii. If biopsy of other tissues is performed for clinical indications, including lumbar puncture for patients who develop neurological symptoms, then additional tissue may be obtained during the same procedure for research studies. Please discuss the planned procedure with the PI.

h. Evaluation for development of endogenous anti-tumor immune responses and epitope spreading

For patients who experience at least a partial remission, we may evaluate whether any cellular or humoral anti-tumor immune responses resulting from activation of endogenous immune cells have occurred. For this purpose, we will collect the following:

i. Serum anti-tumor immune responses: Serum (10 ml, SST tube) for humoral immune responses around day 28, and at approximately 3, 6, and 12 months after T cell infusion to the Specimen Processing/Research Cell Bank lab at Fred Hutch.

ii. PBMC anti-tumor immune responses: (20 ml, EDTA [lavender top] tubes) for cellular immune responses around day 28, and at approximately 3, 6, and 12 months after T cell infusion to the Specimen Processing/Research Cell Bank lab at Fred Hutch.

iii. T-cell repertoire by deep sequencing: Blood (10 ml EDTA [lavender top] tubes) around day 28, and at approximately 3 and 6 months after the T cell infusion to the Specimen Processing/Research Cell Bank lab at Fred Hutch to evaluate the T-cell repertoire by deep sequencing of TCR-β sequence (Adaptive Biotechnologies).

Whole exome sequencing and RNA Seq: For patients in whom endogenous immune responses are detected, more detailed evaluation may be performed to characterize T cell responses by predicting which tumor mutations are likely to be immunogenic and then evaluating peripheral

blood T cells for reactivity to these epitopes. To identify mutations and determine the patient's HLA alleles, whole exome sequencing and RNA Seq of baseline tumor as well as whole exome sequencing of normal tissue may be performed (optional).

i. RCL testing

Blood samples (10 ml, EDTA [lavender top] tube) should be collected at approximately 3, 6, and 12 months after the CAR T cell infusion and sent to the Specimen Processing Lab for RCL testing, as per FDA guidelines.

8.5 Response Assessment

Objective responses to the therapeutic regimen will be assessed using physical examination, imaging, and if necessary, bone marrow biopsies according to standard response criteria as previously published (**Appendices D, E, J, and K**).[127,128] A CT scan of the neck, chest, abdomen, and pelvis, preferably of diagnostic quality, and, if possible, a PET scan, should be obtained at day 28 (± 5 days) after the T cell infusion, and should be performed before the patient receives additional anti-tumor therapy. The PET scan is optional for patients with CLL/SLL. The day 28 PET-CT is also optional for primary CNS lymphoma patients without evidence of systemic disease on baseline PET-CT. The imaging can be done earlier in patients with concerns for clinical progression. If a PET/ CT is not performed then the CT scan must be of diagnostic quality. Patients with a response to treatment may have CT or PET-CT scans performed at 6 and 12 months after the T cell infusion, as clinically indicated. Patients with CNS lymphoma must also undergo a brain/spine MRI with and without contrast at day 28 (± 5 days) and at 6 and 12 months. Additional brain and spine MRIs may be performed at the discretion of the treating physician per standard clinical practice.

Response assessment in CLL/SLL patients will be done per criteria published by international workshop for CLL (iwCLL).[129] (**Appendix E**)

Response assessment in WM patients will be done per criteria published by the 6th International Workshop on WM.[130] (**Appendix J)**

Response assessment in CNS lymphoma patients will be done per published CNS lymphoma response criteria[80] (**Appendix K**). A bone marrow aspirate and biopsy will be performed at day 28 (± 5 days) as per **Section 8.4.9** after the T cell infusion if the baseline bone marrow aspirate/biopsy had any detectable lymphoma by morphology or flow cytometry. Additional bone marrow procedures may be performed at 6 and 12 months only if the prior bone marrow samples showed evidence of disease.

A peripheral blood flow cytometry for MRD should be performed around the time of restaging by imaging as per **Section 8.4.8**.

B cell deep sequencing: A blood sample (10 ml EDTA [lavender top tube] should also be collected at the time of restaging as per **Section 8.4.10.c**, which will be used to assess minimal residual disease by deep sequencing of the IGH and/or IGK/L locus (Adaptive Biotechnologies).

All evaluations of response may be discontinued in patients who proceed to other lymphoma-directed therapies.

8.6 Long-term follow-up

Enrolled patients who receive CD20 CAR T cells will be asked to participate in long-term follow-up (LTFU) according to guidelines set forth by the FDA's Biologic Response Modifiers Advisory Committee that apply to gene transfer studies (**Appendix F**). Current recommendations from the FDA suggest a minimum of 15 years of follow-up. Recommendations will be made for an autopsy to be conducted if the research participant dies.

9 TOXICITIES AND COMPLICATIONS

9.1 T cell-related toxicities

9.1.1 Management of toxicities during or immediately following T cell infusion

The results of prior studies at our center of adoptive immunotherapy for CMV, HIV, leukemia, lymphoma, and melanoma, suggest that serious acute toxicities resulting simply from infusing the numbers of T cells proposed in this study are unlikely to occur. However, fevers and other transient constitutional symptoms are often observed with T cell infusions and may occur in patients being treated with CD20-specific T cells, particularly in patients with a high antigen (tumor) burden.

Examples of potential symptoms/signs due to T cell infusions and their initial management are:

a. Fever, chills, and temperature elevations >38.3° C may be initially managed with acetaminophen as clinically indicated (e.g. 650 mg PO q4-6 hrs), ibuprofen p.o. (400 mg) for breakthrough fevers, and meperidine 25 mg i.v. for rigors. Additional methods such as cooling blankets may be employed for fevers resistant to these measures. Since fevers may also be caused by an infection, all subjects that develop fever or chills should have a blood culture drawn, and empiric broad-spectrum antibiotics may be given at the discretion of the treating physician if clinically indicated.
b. Headache may be managed with acetaminophen.
c. Nausea and vomiting should be treated with antiemetics (excluding corticosteroids) per institutional Standard Practice Guidelines.
d. Hypotension should initially be managed by intravenous fluid administration; however, patients with persistent hypotension will be admitted to the hospital for definitive medical treatment.
e. Hypoxemia should be managed initially with supplemental oxygen; however, patients with persistent hypoxemia will be admitted for definitive medical treatment.
f. For severe symptoms concerning for cytokine release syndrome, see **Section 9.1.3** below.

If a subject experiences an acute T cell infusion reaction, the infusion should be stopped for patient assessment and treatment.

If assessment by the treating investigator determines that the subject's condition is stable, then infusion may be resumed as long as subject stability is confirmed within 2 hours after cell thaw. If the subject cannot resume treatment within 2 hours due to ongoing clinical instability, the PI will decide whether the

subject may continue cell infusion and whether the subject is DLT-evaluable. If an infusion is terminated due to infusion reaction or acute toxicity, the residual T cells will be returned to Fred Hutch Therapeutic Products Program Quality Control Department for analysis. Investigation of possible causes should proceed and, if necessary, additional medical treatment must be instituted.

Subjects requiring discontinuation of the infusion may be eligible for retreatment if the cause is deemed not related to the infusion, after discussion with the PI.

9.1.2 Tumor lysis syndrome

All patients will be considered at risk of tumor lysis syndrome and should be treated prophylactically with allopurinol before chemotherapy begins, unless contraindicated. Allopurinol should be continued for as long as the medical team determines appropriate after the T cell infusion. They may receive additional hydration and urine alkalinization if clinically indicated.

If tumor lysis syndrome develops, as defined by the Cairo Bishop criteria,[131,132] the attending physician will direct patient management with guidance from the study staff and according to published guidelines.[132,133] Consideration should be given to hospitalization, and conservative therapy, including urinary alkalinization and i.v. fluid hydration, may be instituted immediately. Hyperkalemia may be treated with potassium binding resins, diuresis, or insulin/dextrose therapy. Hyperphosphatemia may be treated with phosphate binding resins. In severe cases, rasburicase (in non-G6PD-deficient individuals) or renal dialysis may be necessary.

9.1.3 Cytokine release syndrome (CRS)

Prior studies of CD19-targeted T cells have demonstrated that administration of CAR T cells is associated with CRS. CRS is characterized by one or more of the following symptoms: high fever, fatigue, nausea, headache, dyspnea, tachycardia, rigors, hypotension, hypoxemia, myalgias, arthralgias, rash, and anorexia. Although it is often self-limited or reversible, CRS can be severe and in rare cases, fatal.

If a patient becomes febrile or develops clinical evidence of cytokine release syndrome, we may obtain additional blood samples to measure cytokine levels (such as IL-2, IL-6, IL-7, IL-10, IL-12, IL-15, IFN-γ, TNF-α, and MIP1-α.), serum ferritin, C-reactive protein, and markers of tumor lysis syndrome (e.g. chemistry, uric acid, LDH) and coagulopathy (INR, PTT, platelet count, fibrinogen), and evaluate persistence and phenotype of the transgene-expressing cells, as clinically indicated.

Symptoms and grading of cytokine release syndrome are provided in **Appendix C**. Patients developing clinical evidence of symptoms related to cytokine release will also have a workup to exclude infection or other causes. Initial treatment should consist of supportive measures as dictated by the clinical and laboratory findings, and may include fluid replacement, medications to support blood pressure, antipyretics, oxygen supplementation, and broad-spectrum antibiotics if infection cannot be excluded as a potential etiology for the signs and symptoms.

Management of CRS should be based on institutional Standard Practice Guidelines. In general, patients with grade 3-4 CRS (severe CRS) and/or grade 2 CRS with progressive symptoms and signs should be treated with tocilizumab (4-8 mg/kg IV) and corticosteroids (dexamethasone 10 mg IV every 12 hours). Higher doses of steroids may be given after discussion with the PI or designee, and repeated doses of tocilizumab may be given if necessary. Additional anti-inflammatory agents such as siltuximab, ruxolitinib, or anakinra may also be considered.

Additional measures would also be considered in serious cases after discussion with the PI, such as anti-thymocyte globulin, cytotoxic agents, or infusion of high-dose rituximab (e.g. 1000 mg/m^2) or other CD20-targeting antibodies such as ofatumumab or obinutuzumab to block CAR binding, since our preclinical data suggest that at high concentrations of rituximab or low concentrations of ofatumumab, cytokine secretion of CAR T cells is reduced.[100] Another possible intervention for refractory cytokine release syndrome would be to administer ruxolitinib based on data suggesting that blockade of JAK signaling mitigates hemophagocytic lymphohistiocytosis.[134]

9.1.4 Neurologic Toxicities

Neurotoxicity, manifesting as delirium, seizures, and or focal neurologic deficits, has been reported after CD19 CAR T cell therapy. Management of neurotoxicity should be based on institutional Standard Practice Guidelines. In general, neurotoxicity that is attributed to the T cell infusion may be treated with corticosteroids (e.g. dexamethasone 10 mg IV q 4-12 hours). Tocilizumab or other anti-inflammatory agents such as siltuximab, ruxolitinib, or anakinra may also be given, preferably after discussion with the study PI or designee. Patients with a history of seizures or history of CNS involvement by lymphoma should receive prophylactic treatment with anticonvulsants (levetiracetam or similar) following CAR T cell infusion for approximately 4 weeks. Patients who develop significant neurotoxicity should also be treated with anti-convulsants if not already started. Rare cases of fatal cerebral edema have been reported in ALL patients receiving CD19 CAR T cells, but the mechanism of this complication and interventions to prevent or treat it are unknown at this time.

9.1.5 Prolonged B cell aplasia

Prolonged absence of mature B cells is an expected potential consequence of CD20 directed T cell therapy. Patients experiencing prolonged B cell aplasia may have depressed immunoglobulin levels. If clinically indicated, they may be managed with i.v. immunoglobulin (IVIG) infusions at the discretion of the treating physician.

9.1.6 Persistent uncontrolled T cell proliferation

Uncontrolled proliferation of CAR T cells has not been observed in clinical trials to date. However, in the unlikely event of uncontrolled proliferation of CD20 CAR T cells occurring in a study subject, initial therapy may involve treatment with corticosteroids (e.g. methylprednisolone 1 g IV). Subsequent measures may include cytotoxic agents. Additionally, the truncated CD19 transduction marker provides a potential therapeutic target to eliminate CAR T cells. Thus, CD19-directed agents such as blinatumomab (or other anti-CD19 targeting agents, if they become approved) may be considered. If we observe an increase in CAR$^+$ cells to greater than 10% of T cells at more than 3 months after the last infusion we will analyze for

clonal expansion by deep sequencing of the TCR beta gene (Immunoseq by Adaptive Biotechnology) as an investigational test.

9.1.7 Replication-competent lentivirus (RCL)

Patients should be monitored for RCL for up to 15 years after the last infusion of CAR T cells per FDA guidelines (Food and Drug Administration 2006, and also the 11/19/2010 FDA Gene Therapy Advisory Committee Meeting – see **Appendix F**). All patients should be monitored for evidence of unexpected CAR T cell expansion and the emergence of a new malignancy, particularly one of T cell origin.

9.1.8 Other toxicities

Patients experiencing other toxicities, excluding expected hematologic toxicities associated with cyclophosphamide and other specific toxicities discussed above, should be evaluated and treated according to standard medical practice.

9.2 Chemotherapy

All patients will receive anti-emetic prophylaxis prior to cyclophosphamide, and/or fludarabine or bendamustine infusions. If hemorrhagic cystitis occurs, it will be managed with i.v. hydration, correction of any underlying coagulopathy, and other measures as clinically indicated. Other side effects with cyclophosphamide or fludarabine will be managed per standard clinical practice.

10 SUBJECT DISCONTINUATION

10.1 Screen Failure

Subjects who sign the screening consent will be documented as a screen failure for the following reasons:

- Determined to be ineligible per the eligibility criteria outlined in **Sections 3.1 and 3.2**.

For screen failures, the signed screening consent form and documentation of subject ineligibility must be documented in the subject's research chart.

10.2 Withdrawal and Off-study Criteria

Subjects may withdraw from this study at any time at their discretion. Subjects may also be removed from this protocol if they develop any untoward side effects from the study medications.

If a subject withdraws consent to participate in the study or aspects of the study, attempts should be made to obtain permission to record survival data up to the protocol-described end of the subject follow-up period. Survival data are important to the integrity of the final study analysis. Documentation in the medical record should state that the subject is withdrawing from the study and what, if any, selected data and research samples the subject will permit the investigator to obtain and continue to collect.

Subjects in this study will be considered off study for the following reasons:

- If the Sponsor decides to stop the study
- If leukapheresis is incomplete or insufficient
- Insufficient generation of T cell product
- At Investigator's discretion for any other reason that is not explicitly listed here

- Subject is unable to complete regimen or follow the protocol as outlined in the consent form
- Lost to follow-up
- Patient death

Patient Withdrawal:
Patients may decide to withdraw from the study at any time and for any reason. If a patient expresses a desire to withdraw then the PI will discuss with the patient the desired level of withdrawal (i.e. complete, study related procedure, etc.) and determine which research-related procedures will cease. Depending on the level of withdrawal requested, we may continue to follow patients for disease status and toxicity. If patients are willing, we may continue to draw research blood.

Long-term follow-up should commence at patient withdrawal from the study or one year after the T cell infusion.

11 ADVERSE EVENT REPORTING

11.1 Adverse Event Definitions

- **Adverse Event**
  An Adverse Event (AE) is any untoward medical occurrence in a clinical investigation subject administered a medicinal product; the event does not necessarily have a causal relationship with study drug administration or usage. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

- **Serious Adverse Event**
  A serious adverse event (SAE) is defined as an untoward medical occurrence that results in any of the following outcomes:
  1. Death.
  2. Life-threatening situation (i.e., with an immediate risk of death from the event as it occurred but not including an event that, had it occurred in a more serious form, might have caused death).
  3. In-patient hospitalization or prolongation of existing hospitalization. Inpatient hospitalization comprises formal admission to a hospital for medical reasons, for any length of time, whether or not hospitalization extends overnight. However, hospital admissions for administration of the study drug, procedures required by the study protocol, or tumor-related diagnostic procedures are not considered serious.
  4. Persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
  5. Congenital anomaly/birth defect.
  6. An important medical event that requires intervention to prevent one of the above outcomes.

- **Unexpected Adverse Event**
  An unexpected adverse event is defined as an event that has a nature or severity, or frequency that is not consistent with the investigator brochure, protocol, or consent form. In addition, the events described in **Section 9** of this protocol have been shown to be associated with other CAR T products in previous clinical trials and therefore will not be considered unexpected for the purposes of this protocol.

11.2 Monitoring and Recording AEs

Adverse events will be assessed by the investigator or qualified designee and recorded in the CRFs. The investigator should attempt to establish a diagnosis of the event on the basis of signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the adverse event and/or serious adverse event and not described as the individual signs or symptoms. The following information should be recorded:

- Description of the adverse event using concise medical terminology
- Description as to whether or not the adverse event is serious
- The start date (date of adverse event onset)
- The stop date (date of adverse event resolution or return to baseline)
- The severity (grade) of the adverse event
- A description of the potential relatedness of the adverse event to study drug or a study procedure
- Expectedness of the adverse event based on prior observed and documented adverse events
- The outcome of the adverse event

11.3 Grading of the Severity of an Adverse Event

AEs will be graded in severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. If a CTCAE criterion does not exist, the investigator should use the grade or adjectives: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal) to describe the maximum intensity of the adverse event. For cytokine release syndrome, the CRS Grading Scale provided in **Appendix C** will be used to grade severity. While the ASTCT 2019 consensus grading system[26] will be used as the primary grading system for this protocol (including for DLT purposes), we will also capture the CRS grading using the Lee 2014 criteria.[27] For neurotoxicity, we will use the ASTCT Immune effector cell-associated neurotoxicity syndrome (ICANS) consensus grading system for adults **(Appendix I**).

11.4 Attribution of Adverse Event

Association or relatedness to the study agent will be assessed by the investigator as follows:

Definite (must have all 4)	• Has a reasonable temporal relationship to the intervention • Could not have readily been produced by the subject's clinical state or have been due to environmental or other interventions

	• Follows a known pattern of response to intervention • Disappears or decreases with reduction in dose or cessation of intervention and recurs with re-exposure
Probable (must have 3)	• Has a reasonable temporal relationship to the intervention • Could not have readily been produced by the subject's clinical state or have been due to environmental or other interventions • Follows a known pattern of response to intervention • Disappears or decreases with reduction in dose or cessation of intervention
Possible (must have 2)	• Has a reasonable temporal relationship to the intervention • Could not have readily been produced by the subject's clinical state • Could not readily have been due to environmental or other interventions • Follows a known pattern of response to intervention
Unlikely (must have 2)	• Does not have a temporal relationship to the intervention • Could readily have been produced by the subject's clinical state • Could have been due to environmental or other interventions • Does not follow a known pattern of response to intervention • Does not reappear or worsen with reintroduction of intervention

For general AE assessment, an AE is considered related if it is assessed as definitely, probably, or possibly related; unrelated if it is assessed as unlikely related or unrelated.

For determination of IND safety reporting, AE attribution will be assessed according to the suspected adverse reaction definition described in 21 CFR 312.32 as an AE for which there is a reasonable possibility that the drug caused the adverse event where “reasonable possibility” means there is evidence to suggest a causal relationship between the drug and the AE. The IND Sponsor will report suspected adverse reactions that are both serious and unexpected to the FDA as an IND safety report, in accordance with regulations under 21 CFR 312.32.

11.5 Adverse Event Reporting Period

Adverse events of ≥ grade 2 and all grades of nervous system adverse events will be monitored and recorded in study-specific case report forms (CRFs) throughout the investigative phases of the study: during and for 48 hours after invasive study related procedures, then during and for 48 hours after leukapheresis and then from the start of lymphodepletion chemotherapy through day 28 after the T cell infusion. Adverse events will continue to be monitored and recorded in conjunction with standard follow-

up time points during the first year following T cell infusion. The detailed collection of AEs will stop at the time of commencement of new anti-tumor therapy. However, limited AEs will continue to be recorded annually during long-term follow-up as described in **Appendix F**.

AEs with an onset date prior to apheresis will not be recorded, except in the case of clinically significant worsening of the AE during the specified monitoring time frame. A subject withdrawn from the study because of an adverse event must be followed until the clinical outcome from the adverse event is determined.

The following events are *not* identified as AEs in this study:

- Disease progression or relapse. However, clinical events associated with progression/relapse may be reportable as AEs during the first 28 days after the T cell infusion.
- Hospitalization for the purpose of facilitating lymphodepleting chemotherapy and/or T cell infusion is not considered an AE. Any AE requiring prolongation of this hospitalization will be recorded and subject to applicable SAE reporting.
- Medical or surgical procedures in and of themselves, including those that require hospitalization (e.g., surgery, endoscopy, biopsy procedures) are not considered AEs. However, an event or condition requiring such procedures may be an AE.

11.6 Adverse Event Reporting Requirements

11.6.1 Research Site Reporting Requirements

Classification of an event as serious or non-serious (see **Section 11.1**) determines the reporting procedures to be followed by the site for reporting the event to the IND Sponsor. The investigator must report events to the Fred Hutch IRB in accordance with the policies of the IRB.

TABLE 11.1: PI to IND Sponsor Reporting Requirements for Adverse Events

Classification		Reporting Time	Reporting Action	Contact Information
Serious Adverse Event (SAE)	Fatal or life-threatening	Within 24 hours of research team awareness	Email notification to IND Sponsor's Medical Monitor and ISIOC Administrator	Medical Monitor email: mperciva@uw.edu ISIOC email: ISIOC@fredhutch.org
	All SAEs	Within 2 business days of research team awareness	Submit completed Institution-Sponsored IND SAE Reporting Form signed by PI or designated sub-Investigator	ISIOC email: ISIOC@fredhutch.org
Non-serious Adverse Event		Per CRF completion guidelines	Record information on appropriate CRFs	N/A

*Research team is defined as the individuals listed on the delegation of authority log. Physicians listed on the study's delegation of authority log as attending physicians with delegated authority to administer informed consent will not be considered part of the research team unless additional responsibilities related to the conduct of the study have been delegated to them by the Principal Investigator.

The information in the Institution-Sponsored IND SAE Reporting Form must match or be reconciled with the information recorded in the adverse events section of the CRF and study database. For example, the same adverse event term should be used on both forms.

The IND sponsor or its designee will provide all investigators with a safety letter notifying them of an event that meets FDA IND Safety Reporting criteria. A courtesy copy will also be provided to the study-specific DSMB.

In the event of study closure due to toxicities, or activation of any other stopping/suspension rule, the PI would notify the IRB, DSMB, IBC, and OSP within 7 days of the determination.

11.6.2 Fred Hutch IND Sponsor Reporting Requirements

The sponsor assumes responsibility for IND safety reporting to the FDA and participating investigators, in accordance with regulations under 21 CFR 312.32.

Each serious adverse event report received from the investigator will be evaluated by the Medical Monitor who will assess the seriousness of the event (see **Section 11.1**), the expectedness of the event (see **Section 11.1),** and the relationship to participation in the study (see **Section 11.4**). For regulatory reporting purposes, the IND Sponsor will determine expectedness relating to the investigational product using safety information specified in the Investigator Brochure. An event will be classified as related if either the investigator or the IND Sponsor determines that the event may be related to the study drug.

The IND Sponsor or its designee will provide all investigators with a safety letter notifying them of an event that meets FDA IND Safety Reporting criteria. Investigators will be requested to provide written notification of safety report to the Fred Hutch IRB as soon as is practical, consistent with IRB requirements.

12 STATISTICAL CONSIDERATIONS

12.1 Study Design and Objectives

This is a phase I/II study. The primary objective of this study is to estimate the maximum tolerated dose (MTD) of adoptively transferred autologous $CD4^+$ and $CD8^+$ T cells transduced to express a CD20-specific CAR and infused following lymphodepletion chemotherapy for patients with relapsed or refractory $CD20^+$ B cell lymphomas or CLL/SLL. Secondary objectives include an assessment of antitumor activity of adoptive T cell therapy with autologous $CD4^+$ and $CD8^+$ T cells transduced to express a CD20-specific CAR as measured by complete and partial remissions, progression-free survival, and overall survival.

These dose levels will be initially evaluated in combination with CY alone, evaluating the CR rate to determine if CY alone has sufficient activity or if fludarabine will be added (CY/flu). See **Section 12.3** for details. The following table describes the dose levels for CD20-specific CAR infused following lymphodepletion chemotherapy.

Table 12.1.1 Dose levels of CD20 CAR T cell infusions

Dose Level	CAR T cell Dose
Level 0	1×10^5 $tCD19^+$ T cells/kg
Level 1	**3.3×10^5 $tCD19^+$ T cells/kg**
Level 2	1×10^6 $tCD19^+$ T cells/kg
Level 3	3.3×10^6 $tCD19^+$ T cells/kg
Level 4	1×10^7 $tCD19^+$ T cells/kg

Dose-limiting toxicity (DLT) is a binary outcome (yes/no) and will be determined by adverse events occurring after CAR T cell infusion through day 28 post infusion (see **Section 12.2.1** for DLT definition). The MTD is defined as the dose combination with a DLT rate closest to the target DLT rate of 25%. **Section 12.4** describes the method for dose allocation and MTD estimation. Initially, the study will accrue participants in cohorts of 2 patients each. Once 10 patients have been treated at a given dose level, the study can accrue patients in cohorts of up to 4 patients (minimum 2). The starting dose level for the study will be dose level 1. If the recommended dose level for the next cohort would not be changed by the occurrence of a DLT in the current cohort, the first patient in the next cohort may be treated without waiting for completion of the 28-day DLT period from the infusion of the last patient in the current cohort.

Due to the manufacturing process, a patient may not be able to receive their assigned dose level of cells. Such patients will be evaluated within the study design (using DLTs to estimate the MTD) at the dose level received. The following table defines the range of cells that will be associated with a given dose level. Doses between -15% of a dose level to +85% of the next dose level up will be attributed to that dose level.

Table 12.1.2 Dose levels Range for Dose Attribution

Dose Level	CAR T cell Dose	CAR T cell Dose Inclusion Range
Level 0	1 x 10^5 $tCD19^+$ T cells/kg	8.51 x 10^4 – 2.80 x 10^5 $tCD19^+$ T cells/kg
Level 1	**3.3 x 10^5 $tCD19^+$ T cells/kg**	2.81 x 10^5 – 8.50 x 10^5 $tCD19^+$ T cells/kg
Level 2	1 x 10^6 $tCD19^+$ T cells/kg	8.51 x 10^5 – 2.80 x 10^6 $tCD19^+$ T cells/kg
Level 3	3.3 x 10^6 $tCD19^+$ T cells/kg	2.81 x 10^6 – 8.50 x 10^6 $tCD19^+$ T cells/kg
Level 4	1 x 10^7 $tCD19^+$ T cells/kg	> 8.5 x 10^6 $tCD19^+$ T cells/kg

The lowest cell count allowed is 5 x 10^4 $tCD19^+$ T cells/kg. If any patient receives between 5 x 10^4 and 8.50 x 10^4 $tCD19^+$ T cells/kg those patients' DLT and adverse event data will be evaluated as a separate "Level -1" dose level cohort.

12.2 Definition of endpoints

12.2.1 Primary Endpoint

The primary endpoint for this study is dose-limiting toxicity (DLT). DLT is defined as the occurrence of any of the following that is attributed as at least possibly related to the T cell infusion. Grading will be done in accordance with the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. CRS and neurotoxicity will be graded per ASTCT consensus guidelines 2019 [26] unless otherwise specified.

a. Grades 3-5 allergic reaction related to the CAR T cell infusion.
b. Grades 3-5 autoimmune reactions, excluding B cell depletion, which is expected to occur as a consequence of elimination of $CD20^+$ B cells.
c. Any Grade 3 or 4 non-hematologic event that has not resolved to < grade 3 by day 28 post T cell infusion
d. Grade ≥ 3 neurotoxicity of greater than 7 days duration
e. Grade ≥ 3 neurotoxicity that does not revert to Grade 1 or baseline by day 28 post T cell infusion
f. Grade ≥ 3 seizures that do not resolve to < grade 3 within 3 days
g. Grade ≥ 4 CRS
h. Grade 3 CRS that does not resolve to < grade 3 within 7 days

i. Any other toxicity not meeting the above criteria that is deemed by the PI to represent a DLT

The DLT evaluation period is 28 days from infusion. All patients who receive an infusion will be considered evaluable for DLTs.

12.2.2 Secondary Endpoints

Secondary endpoints include: complete remission (CR), progression-free survival (PFS), overall survival (OS), and toxicity. These endpoints are defined as:

a. Complete remission, based on the Lugano criteria (ref[128] and **Appendix D**).
b. Progression-free survival (PFS): the duration from study enrollment to progression or death due to any cause (whichever comes first). PFS for patients last known to be alive and progression free will be censored at the date of their last disease assessment.
c. Overall survival (OS): the duration from study enrollment to death due to any cause. OS for patients last known to be alive will be censored at the date of last contact.
d. Toxicity: Toxicity grading according to NCI CTCAE version 4.0.

12.3 Interim Analyses to Evaluate Lymphodepletion Regimen

At the time of study initiation, interim analyses were proposed to allow escalation in the lymphodepletion regimen, as outlined in **Table 12.3** below. The goal of the analyses was to determine if the study would proceed with CY alone or if fludarabine would be added to the lymphodepletion regimen. CR was chosen as the criterion to escalate lymphodepletion since it encompasses multiple potential reasons for suboptimal efficacy including low homeostatic cytokines, inadequate depletion of inhibitory cells, and anti-CAR immune responses. Patients who did not receive the target dose specification for T cells as defined in **Section 7.3.1** would not be included in this assessment of CR rate (i.e. are not evaluable for CR).

The interim analyses proposed at study initiation are summarized below for reference in the following table. Based on interim analysis #1 in July 2018 after the first 4 patients were treated, the switch from CY to CY + fludarabine was made.

Table 12.3 Interim analyses to assess whether to intensify lymphodepletion chemotherapy.

Interim	Interim Sample Size*	Decision Rules	Action
#1	First 4 patients	If 0 patients achieve a CR	Switch to CY + fludarabine†
		If 1 or more patients achieve a CR	Continue with CY alone
#2	First 8 patients	If 1 patient achieves a CR	Switch to CY + fludarabine†
		If 2 or more patients achieve a CR	Continue with CY alone
#3	First 16 patients	If 3 or fewer patients achieve a CR	Switch to CY + fludarabine†
		If 4 or more patients achieve a CR	Continue with CY alone

#4	First 20 patients	If 7 or fewer patients achieve a CR AND < 40% of patients treated at the interim recommended dose** achieve a CR	Switch to CY + fludarabine†
		If 8 or more patients achieve a CR OR at least 40% of patients treated at interim recommended dose**	Continue with CY alone

* Assessment is based on patients evaluable for CR as defined above
** See the following paragraph for the definition of the interim recommended dose
† See **section 7.5** for dosing of CY and fludarabine

After the first 4 patients, criteria were met to switch to CY/fludarabine. The cell dose allocation (**Section 12.4**) was reinitiated starting at one dose level below the interim recommended dose (with CY alone) in combination with CY/fludarabine. The interim recommended cell dose was defined as the lower of the maximum dose evaluated to date at time of interim analysis 1 (treatment of 4 patients) or the next cell dose that would have been selected. Once the switch was made to CY + fludarabine, analysis of the lymphodepletion regimen was discontinued.

A simulation study was conducted to estimate the switching/continuation probabilities for the interim analyses, and simulations were repeated 1,000 times. It is challenging to provide an exhaustive summary of the properties as the number of patients at dose levels will depend on the observed data, and the CR rates may increase with increasing dose levels. Therefore, we provide a summary of a few scenarios. Assuming the true CR rate is 10% for all dose levels, the cumulative probability of recommending adding fludarabine by patients 4, 8, 16, or 20 is estimated to be 66%, 84%, 96%, and 100%, respectively. Similarly, if the true CR rate is 50%, then the cumulative probability of recommending adding fludarabine by patients 4, 8, 16, or 20 is estimated to be 7%, 8%, 9%, and 19%, respectively.

12.4 Dose Allocation

Dose allocation will be based upon a Bayesian continual reassessment method (CRM),[135] which uses a model for the probability of DLT and the accumulated DLT data at each dose level to sequentially allocate each new patient cohort. Toxicity assessment is based on the occurrence of DLTs, and the minimum follow-up for determination of escalation is 28 days. The DLT probabilities are modeled using a one-parameter working model $R(d_i) = Pr(\text{DLT at dose level } i) \approx \alpha_i^{\exp(a)}$, where α_i is the skeleton of the working model. The skeleton values are pre-specified constants and were chosen according to the algorithm of Lee and Cheung,[99] which yields a working model that results in well-performing operating characteristics in a broad range of scenarios. For this study, the skeleton values are $\alpha_i = (0.01, 0.05, 0.10, 0.15, 0.25)$. The prior distribution $f(a)$ on the parameter a is specified by a Normal distribution with a mean of 0 and a variance of X; $a \sim N(0, 0.4)$, which was chosen according to the algorithm of Lee and Cheung[99] to the yield the least informative normal prior so that each dose level has approximately equal prior probability of being the MTD. Based on the available DLT data $D = \{(y_i, n_i); i = 0, \ldots, 4\}$, where where y_i is the number of DLT's and n_i is the number of subjects treated on study dose level i, the likelihood is given by

$$L(D|a) \propto \prod_{i=0}^{4} \left(\alpha_i^{\exp(a)}\right)^{y_i} \left(1 - \alpha_i^{\exp(a)}\right)^{n_i - y_i}.$$

After accrual of each patient cohort into the trial, the DLT probability estimates, $\tilde{R}(d_i)$, are updated via

$$\tilde{R}(d_i) = \alpha_i^{\exp(\tilde{a})}; \quad \tilde{a} = \int \frac{a\, L(D|a) f(a)}{\int L(D|a) f(a)} da$$

These estimates are used to sequentially allocate patients to the dose indicated by the modeling to have the estimated DLT rate closest to 25%. The study will sequentially repeat the estimation procedure after the enrollment and follow-up of each cohort (see **Section 12.1** for definition of cohort size). The approach will be used to identify recommended dose levels for the next cohort, but a recommendation to dose escalate can be overridden by the PI for clinical considerations. The allocation procedure will be repeated until sufficient information about the MTD has been obtained according to the stopping rules described in the following section.

12.5 Stopping and Suspension Rules

In addition to the interim evaluations described in **Section 12.3**, if at any point during study conduct there exists sufficient evidence to suggest that the true probability of treatment-related death by day 100 exceeds 20% (regardless of dose), enrollment of patients will be suspended pending a detailed review by the PI, study monitor and statistician, and DSMB. Sufficient evidence for this purpose will be defined as any observed outcome with lower 80% confidence limit exceeding 20%. Operationally, the observation of the following ratios of treatment-related deaths to patients treated would trigger such a rule:

Table 12.5. Stopping guidelines for Treatment-related Deaths < 100 days

Number of participants	Boundary
2-4	≥ 2
5-7	≥ 3
8-11	≥ 4
12-14	≥ 5
15-18	≥ 6
19-22	≥ 7
23-26	≥ 8
27-30	≥ 9
31-34	≥ 10
35	≥ 11

12.6 Patient Accrual and Expected Duration of Trial

The study is expected to be open for enrollment for 4-5 years to enroll up to 50 DLT-evaluable subjects, and follow up will continue for an additional 15 years following patient treatment to provide data on secondary malignancies or neurologic, autoimmune, or hematologic disorders. It is possible that some enrolled patients may either not be treated due to treatment exclusion criteria, or that treated patients may not be DLT-evaluable due to an inadequate dose of CAR T cells (i.e. < 5 x 10^4 $tCD19^+$ cells/kg). Such patients will be replaced, and thus the total accrual may be higher than 50 to achieve 50 DLT-evaluable subjects.

12.7 Operating Characteristics of Study Design

Simulations were run to display the performance of the design characteristics using the CRM web application located at http://uvatrapps.uvadcos.io/crmb/. For each scenario, 1000 simulated trials were run using a random seed of 1973. The confidence level used to define the safety stopping guidelines was 80%. Each table reports the true DLT probability at each dose level, the percentage of trials in which each dose level was recommended as the MTD, the average number of participants treated at each dose level, the average sample size, and the percentage of trials stopped early for safety. The results displayed in **Table 12.7** were based upon a maximum target accrual of 50 subjects.

Table 12.7. Operating characteristics of statistical design

		Operating characteristics for Bayesian continual reassessment method (CRM) with cohorts of size 2. **Bold** font indicates the level that is the MTD corresponding to a target DLT rate of 40%					
Scenario		Dose level 0	Dose level 1	Dose Level 2	Dose Level 3	Dose Level 4	% stopped for safety
1: Little toxicity	True DLT prob:	0.01	0.1	0.15	**0.20**	0.25	0%
	MTD selection %	0%	2.1%	20.7%	**42.1%**	35.1%	
	Avg # of patients	< 1	5.3	9.4	**11.2**	7.4	
2: Moderate toxicity	True DLT prob:	0.10	0.15	**0.2**	**0.25**	0.30	0.6%
	MTD selection %	1.5%	16.0%	**33.0%**	**32.3%**	16.6%	
	Avg # of patients	2.1	8.9	**10.5**	**8.8**	3.6	
3: High toxicity	True DLT prob:	0.20	**0.25**	0.35	0.45	0.55	11.7%
	MTD selection %	19.9%	**48.1%**	18.5%	1.7%	0.1%	
	Avg # of patients	8.1	**14.1**	7.6	1.6	<1	
4: Too Toxic	True DLT prob:	**0.30**	0.35	0.45	0.55	0.65	41.4%
	MTD selection %	**38.9%**	17.3%	2.3%	0.1%	0%	

	Avg # of patients	**13.9**	8.9	2.6	< 1	< 1	
5: All safe	True DLT prob:	0.01	0.05	0.10	0.15	**0.2**	0%
	MTD selection %	0	0.1%	4.5%	28.8%	**66.6%**	
	Avg # of patients	< 1	3.2	6.2	11.3	**13.1**	

12.8 Data Analysis

All subjects who are put on-study will be assessed for inclusion in the final report.

12.8.1 Safety

All subjects who receive any protocol treatment will be monitored for adverse events. Adverse events will be described beyond the summary of DLTs. At study conclusion frequency, proportion and severity of adverse events, and DLTs by study dose level will be tabulated.

12.8.2 Efficacy

Secondary objectives of the study include an examination of efficacy (in terms of rate of remissions, progression-free survival, and in vivo persistence of T cells). These analyses will be performed using patients treated with all doses combined with the final lymphodepletion regimen (either CY alone or CY/fludarabine), modeling outcomes as a function of dose. A logistic regression model will be used to evaluate binary outcomes (CR and CR/PR). A Cox proportional hazards model will be used to evaluate time-to-event outcomes (PFS, OS). No formal statistical hypotheses will be tested with respect to these endpoints, rather estimates and associated confidence interval will be provided descriptively. Moreover, the data will be depicted graphically. Depending on the outcome of lymphodepletion regimen evaluation (see **Table 12.3**), 10, 14, 22, or 50 patients will be evaluable for these outcomes; the associated precision (based on 95% confidence interval width) for binary estimates with these sample sizes are: 32%, 27%, 21%, and 17%.

Tertiary objectives are to evaluate of the duration of persistence of adoptively transferred CD20 CAR T cells and the migration of adoptively transferred CD20 CAR T cells. To evaluate the persistence of the CAR T cells, the patient-level area under the curve (AUC) will be estimated and the summary statistics of the AUCs will be evaluated. Migration (if CAR T cells are present post treatment), is defined as the presence of CAR T cells in the tumor at day 10-16 and, if applicable, the BM at approximately day 28. The association between AUC and migration with clinical outcomes will be mostly descriptive in nature, including graphical presentation.

To evaluate the tertiary objectives associated with evaluating biological causes of treatment resistance, the following analyses will be performed. A paired t-test will be used to compare the biomarker profiles

between baseline tumors and post-treatment tumors with appropriate transformation if needed. A logistic regression model will be used to evaluate the association between baseline biomarker values and response. A landmark analysis among patients achieving a CR or PR at 1 month, measuring survival times (PFS and OS) from the landmark time, using a Cox proportional hazard regression model to evaluate the association of correlates measured at the time of CR/PR for patients in whom a biopsy is acquired at that time. Models will include values for all patients/dose levels and include a variable for dose level.

With respect to the endpoint of assessing development of endogenous anti-tumor responses and epitope spreading, the analysis will be largely exploratory. Data at each time point will be summarized, and with sufficient data, a mixed effect model will be used to model time-varying outcomes. Differential gene expression analysis will be conducted between patients with and without demonstrated epitope spreading to identify the biomarker associated with immune response.

12.9 Ethnic and Gender Accrual Estimates

All eligible patients will be included in this study without regard to gender or ethnicity. It is expected that the distribution of these 50 patients will reflect the general demographic distribution of lymphoma patients seen at our institution.

Table 12.9: TARGETED / PLANNED ENROLLMENT: Number of Subjects

Ethnic Category	Sex / Gender		
	Females	Males	Total
Hispanic or Latino	2	1	3
Not Hispanic or Latino	18	29	47
Ethnic Category Total of All Subjects	20	30	50
Racial Categories			
American Indian / Alaska Native	1	1	2
Asian	1	1	2
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	0	1	1
White	18	27	45
Racial Categories: Total of All Subjects*	20	30	50

13 DATA AND SAFETY MONITORING PLAN

13.1 Overall Scope of Monitoring Activities

Institutional support of trial monitoring will be in accordance with the Fred Hutch/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan (DSMP). Under the provisions of this plan, Fred Hutch Clinical Research Support coordinates data and compliance monitoring

conducted by consultants, contract research organizations, or Fred Hutch employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), Fred Hutch Scientific Review Committee (SRC) and the Fred Hutch/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating patients. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state and federal guidelines. The conduct of this trial will be further monitored by an independent Data and Safety Monitoring Board (DSMB) in accordance with an approved DSMB Charter.

Any temporary or permanent suspension of this clinical research trial, as determined by the Principal Investigator, IRB, DSMC, IND Sponsor or FDA, will be reported to the NCI grant program director by the Principal Investigator.

13.2 Monitoring the Progress of Trial and Safety of Participants

The first level of trial oversight for this protocol will be provided by the principal investigator and the research nurse, who will provide continuous oversight of the trial. These individuals will meet at least monthly to review recently acquired data, stopping rules, and adverse events. Serious adverse events will be reviewed upon occurrence to ensure prompt and accurate reporting to the IND Sponsor, appropriate committees, and regulatory agencies as described above. The data recorded in the research charts and protocol database will be compared with the actual data available from the medical record and/or clinical histories. Data detailed in the research case report forms (CRFs) will include the nature and severity of all toxicities. Dr. Shadman and all other investigators on the protocol have received formal training in the ethical conduct of human research.

The IND Sponsor will ensure routine trial monitoring as described above, and will review Serious Adverse Events and other reports of safety issues promptly upon receipt from the PI.

14 DATA MANAGEMENT/CONFIDENTIALITY

The medical record containing information regarding treatment of the patient will be maintained as a confidential document, within the guidelines of the Fred Hutchinson Cancer Center, and the University of Washington Medical Center. The investigators will ensure that data collected conform to all established guidelines for coding collection, key entry and verification. Each subject is assigned a unique subject number to assure subject confidentiality. Information forwarded to the FDA, NCI, OSP, or other agencies about patients on this protocol refers to patients by a coded identifier and not by name. Subjects will not

be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The licensed medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents. Additional clinical data may be made available from the Fred Hutch core database (Gateway), which is managed and verified independent of the research group.

The research team will maintain Case Report Forms (CRFs) and associated research documentation for each patient treated under the protocol. This documentation includes both clinical data and study-specific documents for each patient. The Principal Investigator or a designee will verify completed CRFs against source documentation on an ongoing basis as they are completed for individual patients. CRFs should be complete and data entered into the study database within 30 days after the day 28 restaging tests are completed. Data required for analysis of patients treated on this protocol will be maintained in a password-protected study-specific database. Data from the CRFs are keyed directly into the database by authorized research staff and verified on an ongoing basis.

15 TERMINATION OF STUDY

The study will terminate after the last treated patient has completed 15 years of follow-up as described in this protocol.

The PI or IND Sponsor may terminate the study at any time. The IRB and FDA also have the authority to terminate the study should it be deemed necessary.

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APPENDICES

Appendix A: Study Calendar
Appendix B: Karnofsky Performance Status Scale
Appendix C: Grading Criteria for CRS
Appendix D: Lugano Response Criteria
Appendix E: iwCLL Response Criteria
Appendix F: Long-term follow-up
Appendix G: Long term follow-up provider questionnaire
Appendix H: Long term follow-up subject questionnaire
Appendix I: ASTCT ICANS consensus grading of neurological toxicity
Appendix J: iwWM Response Criteria
Appendix K: CNS Lymphoma Response Criteria

APPENDIX A: STUDY CALENDAR

	Screening	Leuka-pheresis	Prior to lympho-depleting chemo-therapy	Lympho-depleting Chemo-therapy	Prior to T cell infusion	CD20 CAR T cell infusion	Days after T cell infusion							Months after T cell infusion[27]			Annually for 15 years following T cell infusion
							1	4	7	10	14	21	28	3	6	12	
Informed Consent	x	x															
Medical History, Physical Exam[1,3]	x		x		x		x		x		x	x	x				
Karnofsky performance status	x		x										x				
Vital Signs including pulse oximetry	x					x[24]											
CBC w/ differential	x		x		x		x	x	x	x	x	x	x	x	x	x	
Basic metabolic panel	x		x		x		x	x	x	x	x	x	x				
Hepatic panel w/LDH	x		x		x		x	x	x	x	x	x	x				
Fred Hutch (PSBC) Donor Battery Panel	x																
Serum pregnancy test[2]	x		x														
Beta-2 microglobulin	x																
Serum ferritin[25]	x		x		x		x	x	x	x	x	x	x				
Serum CRP[25]	x		x		x		x	x	x	x	x	x	x				
Serum protein electrophoresis & immunofixation[27]	x		x[28]										x	x	x	x	
Uric acid	x		x		x		x	x	x	x	x	x	x				
Phosphate	x		x		x		x	x	x	x	x	x	x				
Serum IL-6	x				x		x	x	x	x	x	x	x				
PT/PTT	x																
DIC panel without platelets[4]					x		x	x	x	x	x	x	x				
IgG level													x	x	x	x	
IgA, IgG, IgM	x													x[5]		x[5]	
12-lead EKG	x																
Chest x-ray	x																
Echo or MUGA	x				x[23]												

	Screening	Leuka-pheresis	Prior to lympho-depleting chemo-therapy	Lympho-depleting Chemo-therapy	Prior to T cell infusion	CD20 CAR T cell infusion	Days after T cell infusion							Months after T cell infusion[27]			Annually for 15 years following T cell infusion
							1	4	7	10	14	21	28	3	6	12	
Flow cytometry for MRD	x												x	x[17]	x[17]	x[17]	
CT scans of neck, chest, abdomen and pelvis	x[6]												x		x[7]	x[7]	
PET scan[6, 7]	x[6,7]												x[6,7, 31]		x[6,7]	x[6,7]	
MRI[29]	x		x										x		x	x	
BM biopsy/aspirate[8]	x[32]												x[9]		x[9]	x[9]	
Lumbar puncture[10]	x										x		x				
Tumor biopsy	x[11, 30]								x[12]								x[12]
Immunophenotyping for CD3, CD4, CD8 10 mL EDTA (lavender top) tube[13]	x																
PBMC baseline samples 60 mL EDTA (lavender top) tube	x																
Serum baseline sample 20 mL serum separator tube	x																
Serum cytokine levels 10 mL serum separator tube[14]	x				x		x	x	x	x	x	x	x				
Leukapheresis		x															
Lymphodepleting chemotherapy				x													
CD20 CAR T cell infusion						x											
PBMC for CAR T cell persistence 20 mL EDTA (lavender top) tube[15]							x	x	x	x	x	x	x	x	x	x	x[16]

	Screening	Leuka-pheresis	Prior to lympho-depleting chemo-therapy	Lympho-depleting Chemo-therapy	Prior to T cell infusion	CD20 CAR T cell infusion	Days after T cell infusion							Months after T cell infusion[27]			Annually for 15 years following T cell infusion
							1	4	7	10	14	21	28	3	6	12	
B cell deep sequencing 10 mL EDTA (lavender top) tube											x		x	x[17]	x[17]	x[17]	x[18]
Bone marrow aspirate 5-10 mL EDTA (lavender top) tube	x[19]						x[17,19]										
Other tissue e.g. CSF, pleural fluid	x[19]						x[17,19]										
PBMC transgene immunogenicity 20 mL EDTA (lavender top) tubes													x	x[20]	x[20]		
Serum transgene immunogenicity 10 mL serum separator tube													x	x[20]	x[20]		
PBMC anti-tumor immune response 20 mL EDTA (lavender top) tube													x	x[21]	x[21]	x[21]	
Serum anti-tumor immune response 10 mL serum separator tube													x	x[21]	x[21]	x[21]	
T cell repertoire by deep sequencing (PBMC) 10 mL EDTA (lavender top) tube													x	x[21]	x[21]		
B cell quantitation 5 ml EDTA (lavender top) tube	x[26]												x	x[17]	x[17]	x[17]	
PBMC archive sample 30 mL EDTA (lavender top) tube	x		x				x					x		x	x	x	

	Screening	Leuka-pheresis	Prior to lympho-depleting chemo-therapy	Lympho-depleting Chemo-therapy	Prior to T cell infusion	CD20 CAR T cell infusion	Days after T cell infusion							Months after T cell infusion[27]			Annually for 15 years following T cell infusion
							1	4	7	10	14	21	28	3	6	12	
Serum archive sample 10 mL serum separator tube	x													x	x	x	
RCL testing by VSV-G Q-PCR 10 mL EDTA (lavender top) tube	x													x	x	x	x[22]
Long-term follow-up																	x[22]

1: History to include hematologic, cytogenetic, flow cytometric, and histologic findings at diagnosis and at present (if available) as well as prior therapies and response to therapy.
2: For women of childbearing potential, within 14 days of leukapheresis and lymphodepletion.
3: Adverse Events should be monitored as outlined in section 11 of the protocol.
4: Additional PT/PTT, fibrinogen, and D-dimer labs may be drawn if patients develop symptoms of cytokine storm.
5: Recommended testing as per 8.4.5.
6: PET obtained if possible. PET/CT scan and diagnostic contrast-enhanced CT scans of the neck, chest, abdomen, and pelvis within 42 days of enrollment. PET scan is optional for patients with CLL/SLL. Post-treatment scans should be performed at day 28 (± 5 days).
7: Post-treatment PET-CT scans do not need to be repeated after a CR is achieved. Subsequent imaging after a CR should be a CT scan only. Further imaging studies may be ceased if the patient proceeds to other anti-tumor therapy.
8: Bone marrow aspirates/biopsies should be sent for pathology analysis as clinically indicated and according to the protocol. A 5-10 ml aliquot of the bone marrow aspirate in an EDTA [lavender top] tube should be sent to the Specimen Processing / Research Cell Bank Lab at Fred Hutch.
9: A day 28 BM biopsy is needed if there was lymphoma detectable in the screening BM biopsy. See section 8.4.9. Other BM biopsies may be necessary at 6 or 12 months if the prior bone marrow samples showed evidence of disease
10: Lumbar puncture (for primary and secondary CNS lymphoma patients). Clinical samples for cell count and flow cytometry should be collected, as well as a 2 ml research sample that should be sent to the Till Lab.
11: See section 8.1.12.
12: See sections 8.4.7 and 8.4.8
13: Fresh sample to Till Lab.
14: In addition to these times, blood samples should be sent to the Specimen Processing / Research Cell Bank Lab at Fred Hutch if there is a suspicion of cytokine storm or macrophage activation syndrome. Discuss with PI or designee.
15: In addition to these times, blood samples should be sent to the Specimen Processing / Research Cell Bank Lab at Fred Hutch if there is a significant clinical event that may be related to CAR T cells, in the opinion of the treating physician. Discuss with PI or designee.
16: Monitor PBMC by PCR every 6 months for years 1 to 5 and every year for years 6-15 until the transgene becomes undetectable.
17: May be discontinued after day 28 in patients who proceed to other systemic therapy, or if CAR T cell engraftment is lost, or at the discretion of the PI.
18: If a deficiency of B cells persists beyond 12 months or there are persisting CD20 CAR T cells, additional samples may be obtained to monitor B cell numbers every 6 months.
19: Any time a bone marrow biopsy/aspirate/lumbar puncture/other tissue collection is performed for a clinical indication. Additional tissue may be obtained during a procedure and sent to the Specimen Processing/Research Cell Bank Lab at the Fred Hutch for research studies. Please discuss the planned procedure with the study PI.
20: In the event CAR T cell persistence is lost by day 30.

21: Only in patients who achieved at least a partial remission before this point.
22: See Appendix F.
23: Necessary if patient has received cardiotoxic chemotherapy since signing enrollment consent form.
24: Vital signs including pulse oximetry on the day of the CAR T infusion: Before infusion, every 15 minutes during infusion, and every hour for 2 hours after end of infusion.
25: Additional tests such as serum ferritin, CRP, and PFT may be performed as clinically indicated.
26: For the screening timepoint, the B cell quantitation can be performed using the same 5 ml EDTA tube as for "Flow cytometry for MRD."27: T cell infusion time points represent guidelines for performance of required evaluations. Due to numerous factors influencing scheduling (subject and provider availability, testing services limitations, etc.), variation in evaluation performance dates is anticipated and acceptable to the protocol (i.e. within ± 5 days of time points < day 30; ± 30 days for timepoints > day 30.
27: For patients with WM only
28: Only needed for WM patients who received interim therapy since baseline assessments
29: Only for patients with CNS lymphoma.
30: The tumor biopsy is optional for patients with primary or secondary CNS lymphoma, and for WM and CLL/SLL patients who have peripheral blood or bone marrow involvement that can be used to document CD20 expression.
31: The day 28 PET-CT is optional for primary CNS lymphoma patients without evidence of systemic disease on baseline PET-CT.
32: see section 8.1.21 for details. Patients with CLL/SLL and WM who did not undergo a tumor biopsy at baseline should have an additional 3 ml bone marrow aspirate sample in an EDTA tube to the Till Lab.

APPENDIX B: KARNOFSKY PERFORMANCE STATUS SCALE

General	Index (%)	Specific Criteria
Able to carry on normal activity; no special care needed	100	Normal, no complaints, no evidence of disease
	90	Able to carry on normal activity, minor signs or symptoms of disease
	80	Normal activity with effort, some signs or symptoms of disease
Unable to work, able to live at home and care for most personal needs, varying amount of assistance needed	70	Cares for self, unable to carry on normal activity or to do work
	60	Requires occasional assistance from others but able to care for most needs
	50	Requires considerable assistance from others and frequent medical care
Unable to care for self, requires institutional or hospital care or equivalent; disease may be rapidly progressing	40	Disabled; requires special care and assistance
	30	Severely disabled, hospitalization indicated, death not imminent
	20	Very sick, hospitalization necessary, active supportive treatment necessary
	10	Moribund
	0	Dead

APPENDIX C: GRADING CRITERIA FOR CRS (ASTCT CRS CONSENSUS GRADING - 2019)

CRS PARAMETER	GRADE 1	GRADE 2	GRADE 3	GRADE 4
FEVER *	TEMPERATURE ≥ 38°C	TEMPERATURE ≥ 38°C	TEMPERATURE ≥ 38°C	TEMPERATURE ≥ 38°C
		WITH		
HYPOTENSION	NONE	NOT REQUIRING VASOPRESSORS	REQUIRING A VASOPRESSOR WITH OR WITHOUT VASOPRESSIN	REQUIRING MULTIPLE VASOPRESSORS (EXCLUDING VASOPRESSIN)
		AND/OR †		
HYPOXIA	NONE	REQUIRING LOW-FLOW NASAL CANNULA ** OR BLOW-BY	REQUIRING HIGH-FLOW NASAL CANNULA,** FACEMASK, NONREBREATHER MASK, OR VENTURI MASK	REQUIRING POSITIVE PRESSURE (EG, CPAP, BIPAP, INTUBATION AND MECHANICAL VENTILATION)

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

†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.

(Adopted from Lee et al. BBMT, 2019)

Please refer to the publication for details [136]

APPENDIX D: LUGANO RESPONSE CRITERIA

Response and Site	PET-CT–Based Response	CT-Based Response
Complete Response	Complete metabolic response	Complete radiologic response (all of the following):
Lymph nodes and extralymphatic sites	Score 1, 2, or 3[a] with or without a residual mass on 5PS[b] It is recognized that in Waldeyer's ring or extranodal sites with high physiologic uptake or with activation within spleen or marrow (e.g., 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.	• Target nodes/nodal masses must regress to ≤ 1.5 cm in LDi • No extralymphatic sites of disease
Nonmeasured lesion	Not applicable	Absent
Organ enlargement	Not applicable	Regress to normal
New lesions	None	None
Bone marrow	No evidence of FDG-avid disease in marrow	Normal by morphology; if indeterminate, IHC-negative
Partial Response	Partial metabolic response	Partial remission (all of the following):
Lymph nodes and extralymphatic sites	Score 4 or 5[b] with reduced uptake compared with baseline and residual mass(es) of any size At interim, these sites suggest responding disease; At end of treatment, these findings indicate *residual* disease	• ≥ 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 mm x 5 mm as the default value • When no longer visible, 0 mm x 0 mm • For a node >5 mm x 5 mm, but smaller than normal; use actual measurement for calculation
Nonmeasured lesion	Not applicable	Absent/normal, regressed, but no increase
Organ enlargement	Not applicable	Spleen must have regressed by > 50% in length beyond normal
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). 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	
No Response or Stable Disease	No metabolic response	Stable disease

Target nodes/nodal masses, extranodal lesions	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 lesion	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	Progressive metabolic disease	Progressive disease requires at least one of the following:
Individual target nodes/nodal masses	Score 4 or 5 with an increase in intensity of uptake from baseline and/or	• An individual node/lesion must be abnormal with:

Extranodal lesion	New FDG-avid foci consistent with lymphoma	• 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 (e.g., 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

Nonmeasured lesions	None	New or clear progression of preexisting nonmeasured lesions
New Lesions		
	New FDG-avid foci consistent with lymphoma rather than another etiology (e.g., 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

5PS= 5-point scale; CT= computed tomography; FDG= fluorodeoxyglucose; GI = gastrointestinal; 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.

[a] score of 3 in many patients indicates a good prognosis with standard treatment, especially if at the time of an interim scan. However, in studies involving PET where de-escalation is investigated, it may be preferable to consider a score of 3 as inadequate response (to avoid undertreatment). 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 (e.g., 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 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 (e.g., 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 (e.g., with marrow activation as a result of chemotherapy or myeloid growth factors).

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

APPENDIX E: IWCLL RESPONSE CRITERIA

Response Definition After Treatment for CLL Patients					
Group	**Parameter**	**CR**	**PR**	**PD**	**SD**
A	Lymph nodes	None ≥1.5 cm	Decrease ≥50% (from baseline)[a]	Increase ≥50% from baseline or from response	Change of -49% to +49%
	Liver and/or spleen size[b]	Spleen size <13 cm; liver size normal	Decrease ≥50% (from baseline)	Increase ≥50% from baseline or from response	Change of -49% to +49%
	Constitutional symptoms	None	Any	Any	Any
	Circulating lymphocyte count	Normal	Decrease ≥50% from baseline	Increase ≥50% over baseline	Change of -49% to +49%
B	Platelet count	≥100,000/μL	≥100,000/μL or increase ≥50% over baseline	Decrease of ≥50% from baseline secondary to CL	Change of -49% to +49%
	Hemoglobin	≥11.0 g/dL (untransfused and without erythropoietin)	≥11 g/dL or increase ≥50% over baseline	Decrease of ≥2 g/dL from baseline secondary to CLL	Increase <11.0 g/dL or <50% over baseline, or decrease >2 g/dL
	Marrow	Normocelluar, no CLL cells, no B-lymphoid nodules	Presence of CLL cells, or of B lymphoid nodules, or not done	Increase of CLL cells by ≥50% on successive biopsies	No change in marrow infiltrate

Abbreviations: CLL=chronic lymphocytic leukemia; CR=complete response; CT=computed tomography; PD=progressive disease; PR=partial response; SD=stable disease.

Note: CR, complete remission: all of the criteria have to be met; PR, partial response: for a PR at least 1 of the parameters of group A and 1 parameter of group B need to improve if previously abnormal. If only one parameter of both groups A and B is abnormal prior to therapy, only 1 needs to improve. PD, progressive disease: at least one of the above criteria of group A or group B has to be met; SD, stable disease: all of the above criteria have to be met. Constitutional symptoms alone do not define PD.

a. Sum of the products of 6 or less lymph nodes (as evaluated by CT scans and physical examination in clinical trials, or by physical examination in general practice).

b. Spleen size is considered normal if <13 cm. There is not firmly established, international consensus of the size of a normal liver; therefore, liver size should be evaluated by imaging and manual palpation in clinical trials and be recorded according to the definition used in a study protocol.

For a detailed description of the response parameters see Hallek et al. 2018.[129]

APPENDIX F: LONG TERM FOLLOW-UP

Study participants should be asked to participate in long term follow-up, as directed by the FDA Guidance for Industry – Gene Therapy Clinical Trials: Observing Subjects for Delayed Adverse Events.

(http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm072957.htm#5)

Long term follow-up should commence one year after the T cell infusion. The planned recommendations for follow-up are as follows:

Years 1 - 15:

1. Recommendation that patients undergo at least annual history and physical examination with their primary physician:
    - A gene therapy LTFU-directed screening survey may be provided. Particular attention is paid to malignancies, hematological, neurological, and rheumatologic or autoimmune disorders. Provider questionnaire example is provided in **Appendix G.**
    - A request for the study team to be notified of all new malignancies and unexpected illnesses.
    - The primary physician may be provided with a blood draw courier kit to enable samples to be returned to the Specimen Processing Lab/Research Cell Bank for archival purposes, and for analysis for transgene and vector persistence, and RCL, as dictated by studies of transferred T cell persistence. Blood samples should be collected during a routine clinical lab draw. A prepaid shipping label will be provided for use when shipping blood samples back to Fred Hutch.

2. Annual phone call survey or questionnaire to the participant to screen for adverse events. Subject questionnaire example is provided in **Appendix H.**
3. Offer the opportunity to return to Fred Hutch for an annual LTFU clinic visit.
4. Compliance with 21 CFR 312.32 in adverse event reporting.
5. Research studies collected during a routine clinical lab draw:
   5.i. Evaluation for transgene vector sequence by PCR of PBMC every 6 months for years 1 to 5 and every year for years 6-15 until the transgene becomes undetectable.

   5.ii. If > 1% of cells express the transgene or if clonality is suggested, vector integration sites or TCRB sequence utilization will be analyzed in PBMC, CAR T cells or other tissue. If clonality is suggested, repeat testing may be performed 3 months later. Persistent monoclonality, clonal expansion or vector integration near a known oncogenic locus should precipitate careful attention to the possibility of malignancy, and should be reported to the FDA within 30 days. However, the need for additional intervention should be guided by the clinical circumstances and not solely by the presence of these factors.

   5.iii. Annual testing of PBMC for RCL by VSVG Q-PCR. If there is no evidence of transgene persistence, RCL assays may be suspended after one year and samples may be archived.

APPENDIX G: LONG TERM FOLLOW-UP PROVIDER QUESTIONNAIRE

Gene Therapy LTFU Questionnaire (Provider)

Patient: _____ / _____ / _____
Last Middle First

Date of Birth: _____ / _____ / _____
Date Month Year

UPN: | | | | | |

While at the Fred Hutchinson Cancer Research Center your patient received genetically modified T cell therapy. We are required by the FDA to collect long term follow up data for up to 15 years on patients who participated in gene therapy trials. To be in compliance with the FDA requirements, we are asking for your cooperation in monitoring this patient for potential delayed adverse events. Please complete this short questionnaire and return via US mail (return envelope is enclosed).

Developed any new or recurrent malignancies? ☐ NO ☐ YES

Type: _____

Date of onset: _____ / _____ / _____ Resolution Date: _____ / _____ / _____
Date Month Year Date Month Year

Test & Treatment: _____

_____

_____

Status: _____

Developed any new or recurrent hematologic disorders? ☐ NO ☐ YES

Type: _____

Date of onset: _____ / _____ / _____ Resolution Date: _____ / _____ / _____
Date Month Year Date Month Year

Test & treatment: _____

_____

_____

Status: _____

Developed any new incidence or exacerbation of pre-existing neurologic disorders? ☐ NO ☐ YES

Type: _____

Date of onset: _____ / _____ / _____ Resolution Date: _____ / _____ / _____
Date Month Year Date Month Year

Test & treatment: _____

_____

_____

Status: _____

Developed any new or exacerbation of prior rheumatologic / autoimmune disorders? ☐ NO ☐ YES

Type: _____

Date of onset: _____ / _____ / _____ Resolution Date: _____ / _____ / _____
Date Month Year Date Month Year

Tests &Treatment: _____

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Gene Therapy LTFU Questionnaire (Provider)

_____

_____

Status: _____

Developed any unexpected illness and hospitalization? ☐ NO ☐ YES

Nature of illness: _____

Admission Date: ____ / _____ / _____ (Date / Month / Year) Discharge Date: ____ / _____ / _____ (Date / Month / Year)

Tests &Treatment: _____

_____

_____

Status: _____

Developed any infection? ☐ NO ☐ YES

Source of infection / identification of infecting agent:

_____

Date of onset: _____ / _____ / _____ (Date / Month / Year) Resolution Date: ____ / _____ / _____ (Date / Month / Year)

Tests &Treatment: _____

_____

_____

Status: _____

Received any infusions of intravenous immune globulin (IVIG)? ☐ NO ☐ YES

How many infusions:

_____

Dates of infusions: _____

_____

_____

Form completed by: _____ Date: _____

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APPENDIX H: LONG TERM FOLLOW-UP SUBJECT QUESTIONNAIRE

Gene Therapy LTFU Questionnaire

Recipient UPN ☐☐☐☐☐ **CPF#** ☐☐☐☐ X ☐☐☐

Name: _____ **Date of Birth:** _____ / _____ / _____
Date / Month / Year

Address: _____

_____

Home Phone Number: _____ Mobile Phone Number: _____

Since your last clinic visit with your doctor, have there been:

1) **Any new malignancies (cancers)** (i.e: leukemias or other cancers)
   ☐ NO
   ☐ YES Diagnose Date: _____ (date) / _____ (month) / _____ (year)

   Type: _____

   Therapy: _____

   Status: _____

2) **Any hematologic (blood) disorders** (i.e: low blood cells count, impaired immune system)
   ☐ NO
   ☐ YES Diagnose Date: _____ (date) / _____ (month) / _____ (year)

   Type: _____

   Therapy: _____

   Status: _____

3) **Any new or worsening of pre-existing neurologic disorders** (i.e: confusion, seizure)
   ☐ NO
   ☐ YES Diagnose Date: _____ (date) / _____ (month) / _____ (year)

   Type: _____

   Therapy: _____

   Status: _____

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4) **Any new or worsening of prior rheumatologic or other autoimmune disorders** (i.e: rheumatoid arthritis, psoriasis, thyroiditis, etc.)

☐ NO
☐ YES Diagnose Date: _____ (date) / _____ (month) / _____ (year)

Type: _____

Therapy: _____

Status: _____

5) **Any unexpected illnesses, infections or hospitalizations**

☐ NO
☐ YES Date of illness or hospitalization : _____ (date) / _____ (month) / _____ (year)

Type: _____

Therapy: _____

Status: _____

6) **Since your last T cell infusion, have you had any children?**

☐ NO
☐ YES, Deliver Date: _____ (date) / _____ (month) / _____ (year)

If you answer YES, was there any birth defect?
☐ NO
☐ YES
Type: _____

Name of your current local health care provider: _____

Address: _____

Phone number: _____ Fax Number: _____

SIGNATURE

_____ Date: _____

APPENDIX I: ASTCT ICANS CONSENSUS GRADING FOR ADULTS

ICE
• Orientation: orientation to year, month, city, hospital: 4 points • Naming: ability to name 3 objects (e.g., point to clock, pen, button): 3 points • Following commands: ability to follow simple commands (e.g., "Show me 2 fingers" or "Close your eyes and stick out your tongue"): 1 point • Writing: ability to write a standard sentence (e.g., "Our national bird is the bald eagle"): 1 point • Attention: ability to count backwards from 100 by 10: 1 point
Scoring: 10, no impairment; 7-9, grade 1 ICANS; 3-6, grade 2 ICANS; 0-2, grade 3 ICANS; 0 due to patient unarousable and unable to perform ICE assessment, grade 4 ICANS

ASTCT ICANS Consensus Grading for Adults

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE score	7-9	3-6	0-2	0 (patient is unarousable and unable to perform ICE)
Depressed level of consciousness†	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse. Stupor or coma
Seizure	N/A	N/A	Any clinical seizure focal or generalized that resolves rapidly or 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

(Adopted from Lee et al. BBMT, 2019)
Please refer to the publication for details[136]

APPENDIX J: IWWM RESPONSE CRITERIA

Response Category	Definition
Complete response (CR)	• Absence of serum monoclonal IgM protein by immunofixation • Normal serum IgM level • Complete resolution of extramedullary disease, i.e., lymphadenopathy and splenomegaly if present at baseline • Morphologically normal bone marrow aspirate and trephine biopsy
Very good partial response (VGPR)	• Monoclonal IgM protein is detectable • ≥90% reduction in serum IgM level from baseline[a] • Complete resolution of extramedullary disease, i.e., lymphadenopathy/splenomegaly if present at baseline • No new signs or symptoms of active disease
Partial response (PR)	• Monoclonal IgM protein is detectable • ≥50% but<90% reduction in serum IgM level from baseline[a] • Reduction in extramedullary disease, i.e., lymphadenopathy/splenomegaly if present at baseline • No new signs or symptoms of active disease
Minor response (MR)	• Monoclonal IgM protein is detectable • ≥25% but<50% reduction in serum IgM level from baseline [a] • No new signs or symptoms of active disease
Stable disease (SD)	• Monoclonal IgM protein is detectable • <25% reduction and <25% increase in serum IgM level from baseline [a] • No progression in extramedullary disease, i.e.,lymphadenopathy/splenomegaly • No new signs or symptoms of active disease
Progressive disease (PD)	• ≥25% increase in serum IgM level [a] from lowest nadir (requires confirmation) and/or • progression in clinical features attributable the disease

[a] Sequential changes in IgM levels may be determined either by M protein quantitation by densitometry or total serum IgM quantitation by nephelometry.

Please refer to the publication for details[130]

APPENDIX K: CNS LYMPHOMA RESPONSE CRITERIA

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

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

Please refer to the publication for details[80]