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1. TITLE OF PROTOCOL: Phase I study of adoptive immunotherapy for advanced ROR1+ malignancies with defined subsets of autologous T cells engineered to express a ROR1-specific chimeric antigen receptor

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2. INTRODUCTION

The introduction of tumor-targeting receptors into T cells by gene transfer allows the rapid generation of tumor-specific T cells from any cancer patient for adoptive T cell therapy of their malignancy. A promising strategy involves engineering T cells with synthetic chimeric antigen receptors (CARs) that are comprised of a single chain antibody that is specific for a tumor cell-surface molecule linked to one or more T cell signaling molecules.[1-4] Recent trials, including FHCRC Protocol 2639 in which CAR-modified T cells (CAR-T cells) specific for the CD19 molecule are being administered to patients with B-cell malignancies, have demonstrated marked tumor regression in a subset of patients with advanced acute lymphoblastic leukemia (ALL) and non-Hodgkin's lymphoma (NHL).[5] A subset of patients with B cell malignancies recur after CD19 CAR-T cell therapy with tumor variants that lack the CD19 molecule[6,7] and it may be necessary to target additional molecules to achieve maximal efficacy. In addition, it would be ideal to extend CAR-T cell therapy to common epithelial cancers. However this poses several challenges, including the identification of molecules expressed on tumor cells that can be targeted safely with CAR-T cells.

We and others have investigated the tyrosine kinase orphan receptor ROR1 as a novel candidate target for CAR-T cells in patients with chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL) and several epithelial cancers.[8-15] We performed preclinical studies *in vitro* and *in vivo* in immunodeficient mice bearing human tumor xenografts that demonstrate ROR1 CAR-T cells have antitumor activity[16]. We have demonstrated that large doses of autologous ROR1 CAR-T cells can be administered to non-human primates without toxicity,[17] and have developed a clinical grade lentiviral vector that encodes a ROR1 CAR and can be used to transduce human T cells to re-direct their specificity to $ROR1^+$ malignancies. This phase I clinical trial will evaluate the safety and antitumor activity of adoptively transferred autologous T cells genetically modified to express a CAR that targets ROR1. Two cohorts of patients will be eligible for treatment: Cohort A will enroll patients with advanced $ROR1^+$ chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), or acute lymphoblastic leukemia **(**ALL); and Cohort B will enroll patients with $ROR1^+$ non-small cell lung cancer (NSCLC) and triple negative breast cancer (TNBC).

3. BACKGROUND

3A. ROR1 expression in cancer and normal tissues

The orphan tyrosine kinase receptor gene, *ROR1,* encodes two putative isoforms - a short 393 amino acid (aa) intracellular protein and a long 937 aa type-1 transmembrane protein (isoform 1). ROR1 isoform 1 is expressed on many tissues during embryonic development, but absent in most adult tissues except a stage of immature B-cells in the bone marrow (BM) and low levels of expression in adipocytes, pancreas, and lung.[16,18] We developed a monoclonal antibody that stains ROR1 in tissue sections and demonstrated that ROR1 was absent in vital tissues including brain, heart, lung and liver. However, we did observe some ROR1 expression in parathyroid, pancreatic islets cells, fat cells, and epithelial cells in the esophagus, stomach, duodenum, and colon. ROR1 is uniformly expressed on the surface of CLL, MCL, a subset of ALL, and on several common epithelial cancers including non-small cell lung cancer (NSCLC) and breast cancer.[8-16,19-21] The role of ROR1 in tumor cells is not completely defined; however knockdown of ROR1 expression in leukemia, lung, and breast cancer cell lines causes growth arrest and/or apoptosis.[9,13,20,22,23] Analyses of outcomes in breast and ovarian cancer patients also suggest that high levels of ROR1

expression are associated with a poor prognosis.[22,23] Together, these data suggest that targeting ROR1 with small molecules, monoclonal antibodies, or CAR-T cells may have therapeutic activity against ROR1$^+$ malignancies. Our group has focused on the development of CAR-T cells to target ROR1 (see Section 3G).

3B. ROR1 Expressing Hematologic Malignancies

3B.1. Chronic Lymphocytic Leukemia

CLL is the most prevalent adult leukemia and patients may have an indolent or aggressive clinical course. Asymptomatic patients without a large disease burden at diagnosis are usually observed until their disease progresses and requires therapy. Patients with high risk features including non-mutated immunoglobulin genes, expression of ZAP-70, CD38, and/or adverse genetic changes (11q23 or 17p-) detected by cytogenetics or FISH often progress more rapidly to symptomatic disease and require treatment to control disease burden and reduce symptoms.[24,25] Unfortunately, chemotherapy for CLL is not curative and patients remain at a continuous risk of relapse. The prognosis for patients relapsing after first line therapy is variable but largely dependent on the duration and depth of first remission and on the interim development of adverse genetic risk features such as 17p deletion. For patients with long remissions (> 2 years), the initial treatment can often be repeated. However, the response rate, depth and duration of remission are usually shorter with subsequent therapy.

For patients requiring antitumor therapy, the addition of monoclonal antibody therapy to combination chemotherapy has become standard of care. For medically fit patients less than 65 years of age, the CLL8 trial by the German CLL group demonstrated higher complete remission (CR) rates, longer progression free survival (PFS) and overall survival (OS) for patients randomized to fludarabine, cyclophosphamide, rituximab (FCR) chemotherapy compared to FC chemotherapy alone .[26] In this trial, lower levels of minimal residual disease (MRD) as detected by flow cytometry 2 months after the completion of therapy were correlated with improved PFS and OS.[27] Other approaches include combinations of fludarabine and rituximab or bendamustine and rituximab and are often used in older patients or those with comorbid medical conditions.

Patients beyond first or second relapse are in need of novel treatments. New agents targeting the B cell Ig receptor signaling pathway including the PI3kinase-delta inhibitor (idelalisib) and the Brutons Tyrosine kinase (BTK) inhibitor (ibrutinib) are now approved for patients with CLL and improve survival, but are not curative.[28,29] Allogeneic HCT using HLA matched related or unrelated donors following non-myeloablative conditioning may provide a curative option, however there is significant risk of non-relapse mortality of approximately 25% at 1 year due to GVHD and/or infections.[30]

CLL cells nearly universally express both CD19 and ROR1.[10,31] Adoptive T cell therapy with autologous CD19 CAR-T cells has induced complete remissions in up to 30% of patients with refractory CLL. Targeting CD19 can be associated with depletion of normal mature B cells, which express CD19, but do not express ROR1.[32-35] Moreover, some patients treated with murine antibody-based CAR-T cells can make immune responses against antibody domains, as seen with CAIX-CAR-T cells in patients with metastatic renal cell carcinoma[36] and in a subset of patients treated with our anti-CD19 CAR-T cell for patients with B-cell malignancies.[5] The R12 ROR1 scFv is from a rabbit antibody and is unlikely to be cross-reactive with the murine scFv used to construct the CD19 CAR. Thus, ROR1 CAR-T cells could provide a novel treatment option for

patients with CLL that is refractory to conventional therapy or CD19 CAR-T cells, and would not be expected to eliminate normal mature $CD19^+$ B cells.

3B.2. Mantle Cell Lymphoma

MCL is a chronic relapsing NHL with an aggressive clinical course. Current standard of care includes induction with regimens such as R-CHOP or HyperCVAD/Mtx/Ara-C.[37] Patients with responsive disease are considered for high-dose therapy and autologous HCT as consolidation in first remission. A study by the German low-grade lymphoma group demonstrated improved PFS and OS with autologous HCT in first remission compared with interferon maintenance therapy.[38] Even with the use of autologous HCT, patients appear to remain at risk of relapse with no evidence of a plateau on the PFS curve. Patients who relapse following autologous HCT, and those unable to receive autologous HCT, have a poor outcome and are considered for experimental approaches and for allogeneic HCT. Similar to CLL, Bruton's tyrosine kinase and PI3 kinase inhibitors have activity in MCL and are useful agents, however not all patients respond to treatment and relapses occur.[39,40]

Allogeneic HCT appears to provide powerful graft-versus-tumor effect against MCL and several studies have demonstrated high CR rates and a low rate of relapse.[41] This suggests MCL is susceptible to T cell recognition however as discussed above, the considerable risk of morbidity and mortality from GVHD and infection makes proceeding to an allogeneic HCT a difficult decision for many patients. Patients unable to find a donor or unwilling to accept the risk of allogeneic HCT are usually treated with single agent or combination chemotherapy with palliative intent. MCL uniformly expresses ROR1, suggesting that as with CLL, treatment with ROR1 CAR-T cells offers a novel approach to patients with disease that is refractory to conventional therapy.

3B.3. Acute Lymphoblastic Leukemia (ALL)

ROR1 is expressed on some cases of B-cell ALL,[9,11,42-44] although the uniformity of expression was not extensively characterized in these studies. Cell surface ROR1 expression was found in 45% of pediatric B-ALL cases and was not limited to any particular genotype,[11] The subset of adult and pediatric ALL cases with the t(1:19) chromosome translocation (encoding the oncogenic E2A-PBX1 fusion protein) exhibit uniform surface expression of ROR1 on leukemia blasts and silencing of ROR1 expression in t(1:19) leukemia cell lines increases their sensitivity to kinase inhibitors.[9] Thus, ROR1 could serve as a target for CAR-T cells in ALL patients that are $ROR1^+$, refractory to conventional therapies, and ineligible for or unresponsive to CD19 CAR-T cells or blinatumomab therapy.

3C. ROR1 Expressing Solid Tumors

Transcriptional profiling studies have demonstrated ROR1 expression by many different epithelial cancers, and linked expression of ROR1 to a poor prognosis.[22,23] ROR1 expression at the cell surface has been confirmed in lung and breast cancer cell lines using flow cytometry, however protein expression in primary tumors in formalin fixed paraffin embedded (FFPE) sections has been difficult to demonstrate because of the inconsistent behavior of currently available anti-ROR1 mAb that target the extracellular domain in immunohistochemistry (IHC). Our studies suggest that formalin fixation destroys the ROR1 epitope(s) targeted by these mAb and that routine antigen retrieval methods are insufficient for reproducible staining in IHC. We have developed new ROR1-specific mAb that target epitopes that are present in the intracellular domain of isoform 1 and these mAb are effective for detecting ROR1 protein in FFPE specimens. We will use this

reagent to determine ROR1 positivity in solid tumors. The initial trial of ROR1 CAR-T cells will focus on subtypes of advanced NSCLC and breast cancer because of the data indicating these tumors are frequently positive for ROR1 expression, and the clear need for novel therapies for these malignancies.[13,14,20,22]

3C.1 Non-small cell lung cancer (NSCLC)

NSCLC is the leading cause of cancer deaths in the United States and worldwide and often diagnosed at an advanced stage. Histologically, NSCLC is divided into adenocarcinoma, squamous cell carcinoma (SCC), and large cell carcinoma**.** Platinum-based doublet chemotherapy regimens are standard for first-line treatment of advanced stage NSCLC, but have significant side effects and provide only a modest overall survival benefit (range 2 to 4 months) compared to best supportive care.[45] Using conventional chemotherapy as the only treatment, survival beyond 2 years occurs in less than 20% of cases.[46] Although many patients with Stage IIIA disease are candidates for curative-intent therapy, palliation is the common goal for patients who have extensive mediastinal tumor burdens.

Driver mutations in the epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) are identified in a subset of lung adenocarcinomas. In patients with EGFR abnormalities, response rates of up to 70% can be achieved with tyrosine-kinase inhibitors (TKIs), erlotinib or gefitinib.[47-49] Other EGFR mutations are associated with lower response rates and acquired resistance to these TKIs.[50] Even responding patients eventually develop resistance, resulting in short-lived responses measured in months.[51,52] Additionally, although EGFR mutations are common in NSCLC cases in Asian populations (up to 40%), their incidence in Caucasian populations is only 5% to 15%.[53,54] Furthermore, K-RAS mutations which are present in 10% and 30% of Asian and Caucasian subjects respectively, act downstream of EGFR and tend to be resistant to erlotinib and gefitinib.[55,56]

ALK mutations are observed in only about 4% of unselected NSCLC cases,[57-59] and the therapeutic efficacy of ALK inhibitors such as crizotinib and ceritinib has been disappointing.[60] Thus, despite targeted therapy advances, treatments that safely deliver durable responses are urgently needed.

Early clinical trials using immune checkpoint blocking agents, including anti-PD-1 (nivolumab, BMS-936558) and anti-PD-L1 (BMS-936559) antibodies, have produced responses in a significant fraction of patients with NSCLC.[61-64] Other anti-PD-1 and anti-PD-L1 agents (MK-3475, MPDL3280A, MEDI4736) have also produced responses[63-65]. Although most patients have not responded to monotherapy with these agents, durable responses have been observed, supporting approaches to achieve more robust (or less inhibited) T cell mediated anti-tumor immunity, including strategies that directly target tumor-associated antigens with T cells. ROR1 is expressed on a subset of lung adenocarcinomas, where its expression may be induced by the NKX2-1 oncogene.[13] ROR1 knockdown in lung adenocarcinoma cell lines inhibited cell proliferation irrespective of EGFR status,[66] and cells that were resistant to the EGFR tyrosine kinase inhibitor, gefitinib. Thus, treatment with ROR1 CAR-T cells offers a novel therapeutic approach.

3C.2. Breast Cancer

Breast cancer is composed of different subtypes typically classified based on the expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). Triple negative breast cancer (TNBC) is the clinical term used to describe breast carcinomas that do not express the estrogen receptor (ER), progesterone receptor (PR), or human

epidermal growth factor receptor type 2 (HER2 or c-ERB2). TNBC comprises approximately 10-17% of all breast cancers.[67-69] Despite efforts of many investigators in recent years, there remain no well-defined therapeutic targets for TNBC and chemotherapy remains the standard of care in the early and advanced disease settings. As a group, TNBCs are associated with a poor prognosis, characterized by early relapse[70,71] and patients with TNBC often experience a significantly shorter survival following recurrence compared with patients with other breast cancer subtypes.[67] The triple negative phenotype is an independent predictor of distant metastasis and cause specific survival and remains an area of urgent, unmet need in breast cancer.

Recent clinical studies suggest that immunotherapy has the potential to improve clinical outcomes for patients with breast cancer. Genomic profiling has identified an immuno-modularity subtype of TNBC characterized by elevated expression of genes involved in T cell function, immune transcription, and antigen processing.[72] Gene expression profiling of breast cancer stroma has also revealed that cytotoxic CD8+T cell infiltration and the resulting immune gene signature are predictive of good prognosis.[73-75] On a histologic level, tumor infiltration with CD8+ T cells are generally associated with better prognosis while CD4+ T cells, which include T-regulatory cells, have been associated with worse outcomes.[76] Accumulating evidence suggests that basal TNBC may be the most regulated by intratumoral T cells and perhaps most response to immunotherapeutics.[77]

The immune checkpoint molecule, PD-L1, is highly expressed in ~20% of TNBCs, in association with decreased T cell proliferation and increased apoptosis,[78] supporting anti-PD-L1/anti-PD1 therapy in this patient population. In the KEYNOTE-012 phase Ib trial, pembrolizumab (MK-3475) resulted in a notable overall response rate of 18.5% in a heavily pretreated TNBC population with an acceptable safety profile (Nanda R *et al*., A phase Ib study of pembrolizumab (MK-3475) in patients with advanced triple negative breast cancer. SABCS 2014, S1-09) and is being evaluated in additional studies with chemotherapy in the metastatic and neoadjuvant settings. Vaccine strategies are being investigated[79-81] as are adoptive T cell therapies, including a mesothelin-targeting T cells.[82] CAR-T cells targeting cMet or carcinoembryonic antigen (CEA) have entered Phase 1 trials as treatments for patients with advanced breast cancers, including TNBC (NCT01837602; NCT00673829), however no data has been reported on these trials.

ROR1 is expressed in a subset of breast cancers and high ROR1 expression is associated with higher grade and more aggressive breast cancer types, including TNBC.[14,22] High levels of ROR1 in patient samples and cell lines are associated with expression of genes involved in epithelial-mesenchymal transition (EMT); silencing of ROR1 in TNBC-derived cell lines reduced EMT gene expression and inhibited *in vitro* cell migration and bone and lung foci size in xenografts[22]. High ROR1 levels in primary breast tumors predicted for shorter metastasis free survival, independent of ER and HER2 status. Together, these findings suggest a role of ROR1 signaling in the progression of breast cancer and support ROR1 as a novel therapeutic target, especially for TNBC where therapeutic options are limited.

3D. Adoptive T cell therapy for human malignancies with unmodified T cells

The potential for adoptively transferred T cells to eradicate human malignancies was first suggested by the graft-versus-leukemia effect of allogeneic T cells administered as part of a hematopoietic stem cell transplant (HCT).[83,84] The adoptive transfer of *in vitro* expanded, autologous T cells derived from melanoma tumor infiltrates (TIL) and administered after lymphodepleting chemotherapy has induced complete or partial remissions in up to 72% of

patients with metastatic melanoma.[85] Adoptive transfer of T cells can be associated with toxicity, related to immune recognition of normal cells that express the antigen(s) targeted by the infused T cells. For example, patients that receive allogeneic HCT or donor lymphocyte infusions often develop graft-versus-host disease as a consequence of donor T cell recognition of epithelial cells that express minor histocompatibility antigens[86] and melanoma patients treated with TIL may develop vitiligo due to recognition of normal melanocytes.[85] Despite these complications, T cell therapy, including HCT, has cured a subset of patients with advanced malignancy that was resistant to conventional chemotherapy and/or radiotherapy.[85,86]

The clinical trials at the National Cancer Institute demonstrated that administering lympho-depleting chemotherapy comprised of fludarabine and cyclophosphamide, or fludarabine, cyclophosphamide and total body irradiation, prior to the transfer of polyclonal melanoma-specific T cells dramatically improved both the survival of the transferred T cells and therapeutic efficacy compared to previous studies where lymphodepleting chemotherapy was not administered before cell transfer.[85,87,88] Studies in murine models subsequently confirmed the human data that lympho-depletion improves the persistence and efficacy of transferred T cells.[89] Lymphodepletion may improve the persistence of transferred T cells by several mechanisms including reducing competition for cytokines such as IL-15 and IL-7 that promote lymphocyte proliferation and survival, eliminating $CD4^+$ $CD25^+$ regulatory T cells, and activating antigen presenting cells that may promote the function of transferred T cells. The use of lymphodepletion prior to T cell transfer has now become a standard in adoptive T cell therapy studies, including those using CAR-T cells.

3E. Transfer of tumor targeting receptors into T cells to confer tumor-specificity

An obstacle for applying T cell therapy more broadly is the difficulty isolating tumor-reactive T cells from patients with cancer. An approach to overcome the low frequency of tumor-reactive T cells in patients is to redirect the specificity of T cells by expressing a transgene that encodes a receptor specific for a tumor-associated antigen (TAA). Vector systems to deliver transgenes into primary human T cells have been developed and clinical trials in which autologous T cells are modified to express a tumor-reactive T cell receptor (TCR) have shown transient clinical responses.[90,91] However, redirecting T cells to recognize tumor antigens through TCR gene transfer is inherently constrained because of the requirement for major histocompatibility complex (MHC) restricted peptide presentation by tumor cells, and because many tumors express low levels of MHC molecules to avoid T cell recognition.[92]

An alternative method for re-directing T cell recognition to tumor cells is to express an artificial chimeric non-MHC restricted antigen receptor (CAR) that recognizes a tumor cell surface molecule.[1-4] A CAR is typically comprised of a fusion gene that encodes a monoclonal antibody-derived single chain variable fragment (scFv), consisting of heavy (V_H) and light (V_L) chains joined by a flexible linker, fused through a transmembrane domain to a cytoplasmic signaling moiety consisting of CD3ζ alone, or CD3ζ combined with activation domains from costimulatory molecules such as CD28, 4-1BB or OX40.[93,94] CARs with specificity for tumor cell-surface epitopes are "universal" in that they bind antigen in an HLA independent fashion, and one receptor construct can be used to treat all patients with tumors that express the molecule targeted by the CAR. T cells obtained from the blood of cancer patients can be modified with CARs to generate anti-tumor effector cells (CAR-T cells) for adoptive therapy. CAR-T cells can recognize tumor cells that have down-regulated HLA molecules, thereby avoiding the need to isolate rare HLA-restricted tumor-reactive T cells. CARs have been constructed for many tumor-associated cell

surface molecules, including CD19, CD20, EGFR, HER2neu, GD2, PSMA, mesothelin, CAIX and ROR1.[16,93-97]

3F. Clinical experience with the adoptive transfer of CAR-T cells

3F.1. First generation CAR-T cells

The initial human trials of CAR-modified T cells employed first generation constructs that linked the scFv to the CD3ζ or FcR gamma epsilon chain as the only intracellular signaling domain. T cells engineered with first generation CARs specific for CAIX were administered to patients with renal cell cancer, and caused elevations in liver enzymes, possibly due to expression of CAIX on bile duct epithelium.[98] T cells specific for CD171 (L1CAM) were administered to patients with neuroblastoma without toxicity.[99] In each of these studies, T cell persistence was poor and sustained antitumor effects were not observed.

Patients with advanced B-cell malignancies have also been treated with first generation CARs that were specific for CD20 or CD19. These patients experienced little or no toxicity, but the CAR-T cells persisted poorly and sustained antitumor efficacy was not observed.[90,100] The suboptimal results in these initial trials may reflect several factors including the absence of signaling domains to provide costimulation necessary for sustained T cell proliferation and survival in first generation CARs; the methods used to engineer and expand T cells for adoptive transfer that required long term culture and favored infusing terminally differentiated effector cells; and the variability in phenotype of the starting population, which can influence the capacity of T cells to persist and function long term *in vivo*.[100-104]

3F.2. CD19 CAR-T cells with 4-1BB/CD3ζ or CD28/CD3ζ signaling domains

To overcome the limitations identified in the initial trials and enhance potency, costimulatory endodomains were added to CD3ζ to enhance signaling through the CD19 CAR,[32,33,105-107] and improved methods for selecting and transducing T cells of defined phenotype have been developed that do not require long-term culture.[32,33] Potent antitumor activity has been observed in patients with ALL, CLL and NHL treated with autologous CD19 CAR-T cells on FHCRC Protocol #2639 and in studies at the National Institutes of Health, University of Pennsylvania, and Memorial Sloan Kettering Cancer Center.[32-34,108] In all of these studies, lymphodepleting chemotherapy was administered to enhance T cell persistence. In some cases, IL-2 was administered after the T cell infusion although the most impressive antitumor responses were observed in patients that did not receive IL-2.[32]

The major toxicities of CD19-targeting CAR-T cells include cytokine release syndrome (CRS), neurologic toxicity, and tumor lysis that can occur early (days) or later (up to 2 weeks) after infusion of CD19 CAR-T cells. Some patients have persistent depletion of normal $CD19^+$ B cells, necessitating intravenous immunoglobulin treatment. A relationship between T cell dose, toxicity, and efficacy has not been clearly evident in most trials. The inconsistency in the behavior of the therapeutic products may be because polyclonal T cells from the patient were transduced to express the CAR, and the resulting cell products administered to each patient varied greatly in phenotypic composition (CD4, CD8) and subset derivation.

In the trial at the FHCRC, we have formulated the CAR-T cell products in a defined ratio of CD8 and CD4 T cells based on preclinical data demonstrating that cell products containing an equal ratio of CD8 and CD4 T cells had superior antitumor activity compared to either subset alone. Although the trial is not yet completed, the administration of cell products of defined composition

to the first 35 patients appears to provide a clearer relationship between CAR-T cell dose and toxicity in patients with different B cell histologies. In ALL, severe CRS has been observed in patients with a high tumor burden and in this setting, a cell dose of $2x10^5$/kg is now uniformly used to achieve high complete response rates. Patients with low tumor burdens will tolerate higher cell doses, generally without severe CRS. In NHL, severe CRS was observed at 2×10^7 cells/kg and $2x10^6$/kg selected for study even in patients with high tumor burdens. These studies demonstrate that CAR-T cells can have potent efficacy in advanced B cell malignancies and support the investigation of novel targets and extending this therapy to solid tumors.

3G. Development and preclinical validation of ROR1-specific CARs

3G.1. Development of ROR1 CAR

We developed and optimized ROR1-specific CARs from the sequences of VH and VL chains of the 2A2 murine monoclonal antibody and from the R11 and R12 rabbit monoclonal antibodies.[16,109] The 2A2 and R12 mAbs recognize an epitope in the Ig/Fz portion of the extracellular domain of ROR1, and R11 recognizes an epitope in the Kringle domain. T cells engineered by lentiviral transduction to express each of these ROR1 CARs optimized for spacer length specifically recognize $ROR1^+$ tumor cells *in vitro*. A comparison of antitumor efficacy of 2A2 and R12 CAR-T cells in immunodeficient mice engrafted with human tumor xenografts demonstrated that the R12 CAR was the most active.[109] Lentiviral vectors encoding R12 CARs containing either 4-1BB/CD3ζ or CD28/CD3ζ were constructed and validated clinical lots of lentiviral supernatant have been prepared. The clinical lentiviral vector R12/S/4-1BBζ will be used in this clinical trial and encodes the truncated EGFR molecule (EGFRt) downstream of a T2A element in the vector such that T cells that express the ROR1 CAR will also express EGFRt. The clinical anti EGFR monoclonal antibody cetuximab binds to EGFRt and mediates antibody dependent cell mediated cytotoxicity (ADCC). Thus, cetuximab could be administered to patients to eliminate ROR1 CAR-T cells if toxicity occurred.

3G.2. *In vivo* safety of ROR1 CAR-T cells in non-human primates

The safety of targeting ROR1 with R12/4-1BB/CD3ζ CAR-T cells was evaluated in a non-human primate model to determine the potential for serious toxicity from recognition of rare normal tissue cells that might express ROR1. Human and *macaca mulatta* (*M. mulatta)* ROR1 are completely homologous in the Ig/Fz region that is recognized by the R12 scFv and we demonstrated that the tissue expression of ROR1 in macaques and humans is highly similar, including expression in parathyroid, pancreatic islets, esophagus, stomach, duodenum and colon [110]. We adoptively transferred high doses ($1\text{-}5x10^8$/kg) of autologous macaque ROR1 CAR-T cells to 4 macaques without evidence of clinical toxicity or abnormalities in serum chemistry, or blood counts. ROR1 CAR-T cells did not eliminate normal mature B cells in the peripheral blood. ROR1 CAR-T cells trafficked to bone marrow and lymph nodes, and were functional as shown both by the elimination of endogenous $ROR1^+$ pre-B cells and by proliferation of CAR-T cells in response to challenging the animals with autologous T cells transfected to express ROR1. The T cell doses administered in this preclinical safety study are 2500-fold greater than the starting dose of CD19 CAR-T cells and 25-fold greater than the highest dose of CD19 CAR-T cells administered to patients on Protocol 2639.

A limitation of the primate study was that long-term safety of ROR1 CAR-T cells could not be established because the animals were not immunosuppressed prior to CAR-T cell infusion and all animals developed a T cell immune response to the CAR-T cells after 3 to 4 weeks that

coincided with elimination of the CAR-T cells. We treated 2 additional macaques with ROR1 CAR-T cells administered in cell doses of 1-5x10^8/kg following lymphodepleting chemotherapy to prolong CAR-T cell persistence. We did not observe any clinical toxicity and measurements of serum chemistry including calcium and glucose, and hepatic, pancreatic, and renal functions were normal. The CAR-T cells persisted for >40 days without any evidence of late toxicity.

Transgene product-specific immune responses may occur in humans as well because the R12 scFv is derived from a rabbit antibody, and later immune rejection might serve as a safety mechanism to avoid long-term toxicity. The duration that CAR-T cells must persist to eliminate tumors is not yet established, and if targeting ROR1 shows short term safety in humans, future studies could use a human or humanized scFv specific for ROR1 in the design of the CAR combined with EGFRt to provide a safety mechanism. These preclinical findings support the clinical evaluation of ROR1 CAR-T cells for the treatment of ROR1$^+$ hematologic malignancies and solid tumors in carefully designed clinical trials

3H. Safety considerations for adoptive therapy ROR1 CAR-T cells

There are several potential toxicities of adoptive CAR-T cell therapy. The first relates to normal cell expression of the targeted molecule. In the case of targeting cells that express the CD19 molecule, re-directed T cells do recognize and eliminate non-malignant CD19+ B-cells, potentially resulting in long-term B-cell immunodeficiency.[32,34,106,111,112] In the case of ROR1 targeting in our non-human primate model, we observed no overt clinical toxicity even after transferring ROR1 CAR-T cells doses equivalent to or exceeding those used in most human cancer immunotherapy trials, and observed no depletion of normal mature peripheral B cells (these cells do not express ROR1). We had previously shown low level of ROR1 on the surface of adipocytes differentiated from preadipocytes *in vitro,*[16] and plasma adiponectin levels were transiently elevated in our macaque model of ROR1 CAR-T cell therapy. This could suggest a low-level of recognition of adipocytes, however analysis of necropsy specimens did not indicate accumulation of ROR1 CAR-T cells in fat tissues and animals did not exhibit weight loss. It is possible that patients that receive ROR1 CAR-T cells may develop serious toxicity due to recognition of normal cells that express ROR1, including parathyroid, pancreatic islets, gastrointestinal tract epithelium, or other normal tissues.

A second potential toxicity relates to the possibility of transformation of the gene-modified T cells as a consequence of insertional mutagenesis. The development of T cell leukemia was reported in a subset of patients on two gene therapy trials for X-linked severe combined immunodeficiency syndrome (SCID), in which bone marrow derived CD34 cells were transduced with a retroviral vector encoding the common cytokine receptor gamma chain [113-117]. Animal studies have shown that mature T cells are resistant to transformation after retroviral integration[118], and leukemia has never been observed in clinical trials involving gene transfer into mature T cells, despite more than 10 years of follow-up in some studies. Our study also differs from the X-linked SCID trial in several important aspects: 1) we will use a lentiviral vector rather than a retrovirus to genetically modify cells, which reduces the risk of integrating into a transcriptionally active site; 2) the target of the genetic modification will be mature T cells and not hematopoietic progenitor cells; and 3) the genetically modified ROR1-specific T cells will not constitutively express a functional growth factor receptor as was the case with transduced cells in the X-linked SCID trial.

A third potential toxicity is that ROR1 CAR-T cells might cause cytokine release after engagement of target cells that can result in fever, hypotension, and neurologic toxicity, or induce rapid tumor cell death resulting in a tumor lysis syndrome. Both cytokine release and tumor lysis syndromes have been observed in clinical trials of CD19 CAR-T cell therapy in patients, often those with high tumor burdens, and represent the most serious common complication of therapy.[32,34] In the majority of patients the CRS is transient and subsides with tumor elimination, or if severe can be mitigated by administering corticosteroids or tocilizumab.[119-122]

3I. Overview of the Study

This is a Phase I/II, open-label, nonrandomized study that will evaluate the safety and potential antitumor activity of adoptively transferring autologous ROR1 CAR-T cells for patients with advanced $ROR1^+$ B-cell malignancies (Cohort A) or advanced $ROR1^+$ NSCLC and TNBC (Cohort B). Patients with $ROR1^+$ CLL, MCL or ALL that are refractory to conventional therapy will be eligible for Cohort A. Patients with $ROR1^+$ NSCLC or TNBC who have failed conventional chemotherapy or targeted therapy, or for whom these therapies are not effective will be eligible for Cohort B.

To provide for greater reproducibility of T cell products and to facilitate safety and efficacy evaluation, we will enrich $CD8^+$ and $CD4^+$ T cells separately from the leukapheresis product or blood of each patient, transduce each cell subset with the R12/S/4-1BBζ CAR lentivirus independently, expand and pool T cells in a 1:1 ratio to achieve the specified cell dose for each cohort of patients. The primary endpoint of the trial will be to determine the safety of ROR1 CAR-T cells using a dose escalation/de-escalation design to define a cell dose that has acceptable toxicity, and might be used for a future larger phase II trial. Because toxicity may depend on the tumor type (hematologic/solid tumor), each cohort will be evaluated separately for toxicity.

4. OBJECTIVES

4A. Primary objectives

To evaluate the safety of adoptive T cell therapy using *ex vivo* expanded autologous $CD8^+$ and $CD4^+$ ROR1 CAR-T cells for patients with advanced $ROR1^+$ hematologic (Cohort A) and epithelial (Cohort B) malignancies.

4B. Secondary objectives

To determine:

1. Duration of *in vivo* persistence of adoptively transferred T cells, and the phenotype of persisting T cells,

2. Trafficking of adoptively transferred T cells traffic to the bone marrow or other tumor site and function *in vivo,* and

3. Preliminary antitumor activity of the adoptive transfer of ROR1 CAR-T cells in patients with measurable tumor burden prior to T cell transfer

5. PATIENT SELECTION

5A. Inclusion/Exclusion Criteria for patients with CLL, MCL or ALL (Cohort A)

Inclusions

1. Patients with
   a. CLL who are beyond first remission and who have failed combination chemoimmunotherapy with regimens containing a purine analogue and anti-CD20 antibody, or who have failed tyrosine kinase or PI3 kinase inhibitors, or who were not eligible for or declined such therapy. Patients with fludarabine refractory disease are eligible.
   b. Mantle cell lymphoma patients who are beyond first remission and previously treated with chemoimmunotherapy. Patients who have relapsed following autologous HCT are eligible.
   c. ALL patients who have relapsed or have residual disease following treatment with curative intent. ALL patients must have ROR1 expressed on ≥ 90% of the leukemia blasts to be eligible.

2. Confirmation of diagnosis by internal pathology review of initial or subsequent biopsy or other pathologic material at the FHCRC/SCCA.

3. Evidence of ROR1 expression by immunohistochemistry or flow cytometry on any prior or current tumor specimen.

4. Male or female subject, greater than or equal to18 years of age.

5. Karnofsky performance status ≥70%

6. Negative pregnancy test for women of childbearing potential. Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

7. Fertile male and female patients must be willing to use a contraceptive method before, during, and for at least two months after the T cell infusion.

8. Ability to understand and provide informed consent.

Exclusions

1. Treatment with other investigational agent(s) within 30 days of planned lymphodepletion.
2. Patients requiring ongoing daily corticosteroid therapy at a dose of >15 mg of prednisone per day (or equivalent). Pulsed corticosteroid use for disease control is acceptable.
3. Active autoimmune disease requiring immunosuppressive therapy.
4. Major organ dysfunction defined as:

   a. Serum creatinine >2.5 mg/dL

   b. Significant hepatic dysfunction (SGOT >5x upper limit of normal; bilirubin >3.0 mg/dL)

   c. Patients with clinically significant pulmonary dysfunction, as determined by medical history and physical exam should undergo pulmonary function testing. Those with an FEV1 of < 50% or DL_{CO} (corrected) <40% will be excluded.

   d. Significant cardiovascular abnormalities as defined by any one of the following: congestive heart failure, clinically significant hypotension, symptomatic coronary artery disease, or a documented ejection fraction of <45%. Any patient with an EF of 45-49% must receive clearance by a cardiologist to be eligible for the trial.

5. Patients who are HIV seropositive.

6. Uncontrolled active infection (bacterial, viral, fungal, mycobacterial) not responding to treatment with intravenous antibiotics, antiviral or antifungal agents, or long-term treatment with oral agents.

7. Women who are breast-feeding.

8. Patients who have contraindication to cyclophosphamide chemotherapy.

9. Known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy.

10. Untreated central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to enrollment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases.

5.B Inclusion/Exclusion Criteria for Patients with NSCLC or TNBC (Cohort B)

5B.1. NSCLC

Inclusions

1. Patients with non-small cell lung cancer that is metastatic or inoperable and who have been treated with at least one line of prior therapy or declined conventional therapy.

2. Patients with known EGFR or ALK mutations must have been treated on at least one line of molecularly targeted therapy (e.g., erlotinib, crizotinib).

3. Patients must have measurable disease by at least one of the criteria below: (See Appendix C)

   a. Extra skeletal disease that can be accurately measured in at least one dimension as ≥10 mm with conventional CT techniques as defined by RESIST 1.1.

   b. Skeletal or bone-only disease measurable by FDG PET imaging.

4. ROR1 expression in >20% of the primary tumor or metastasis by IHC.

5. Karnofsky performance status of ≥ 70%.

6. Patients must be off chemotherapy for a minimum of 3 weeks prior to start of treatment. Targeted therapies must be stopped at least 3 days prior to start of lymphodepletion.

7. Male or female subject, greater than or equal to18 years of age.

8. Negative pregnancy test for women of childbearing potential. Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

9. Fertile male and female patients must be willing to use a contraceptive method before, during and for at least two months after the T cell infusion.

10. Ability to understand and provide informed consent.

Exclusions

1. ANC < 1000/mm^3
2. Hgb < 9 mg/dl (transfusion permitted to achieve this)
3. Platelet count < 75,000/mm^3
4. Treatment with other investigational agent(s) within 30 days of planned lymphodepletion.
5. Patients requiring ongoing daily corticosteroid therapy at a dose of > 15 mg of prednisone per day (or equivalent). Pulsed corticosteroid use for disease control is acceptable.
6. Active autoimmune disease requiring immunosuppressive therapy.
7. Major organ dysfunction defined as:

   a. Serum creatinine > 2.5 mg/dL

   b. Significant hepatic dysfunction (SGOT >5x upper limit of normal;

   bilirubin > 3.0 mg/dL)

   c. Patients with clinically significant pulmonary dysfunction, as determined by medical history and physical exam should undergo pulmonary function testing. Those with an FEV1 of < 50% or DL_{CO} (corrected) < 40% will be excluded.

   d. Significant cardiovascular abnormalities as defined by any one of the following: congestive heart failure, clinically significant hypotension, symptomatic coronary artery disease, or a documented ejection fraction of < 45%. Any patient with an EF of 45-49% must receive clearance by a cardiologist to be eligible for the trial.
8. Patients who are HIV seropositive.
9. Uncontrolled active infection (bacterial, viral, fungal, mycobacterial) not responding to treatment with intravenous antibiotics, antiviral or antifungal agents, or long-term treatment with oral agents.
10. Women who are breastfeeding.
11. Patients who have contraindication to cyclophosphamide chemotherapy.
12. Known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy.
13. Untreated central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to enrollment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases.

5B.2. **TNBC**

Inclusions

1. Histologically confirmed diagnosis of metastatic TNBC; i.e. breast cancer that is ER negative (≤ 10%), PR negative (≤ 10%), and HER2 negative (0 or 1+ by immunohistochemistry or negative for gene amplification by FISH).

2. Patients must have measurable disease by at least one of the criteria below: (See Appendix C)

   a. Extra skeletal disease that can be accurately measured in at least one dimension as ≥ 10 mm with conventional CT techniques as defined by RESIST 1.1.

   b. Skeletal or bone-only disease measurable by FDG PET imaging.

3. Patients must have received standard adjuvant, neoadjuvant, and/or metastatic chemotherapy per NCCN or institutional practice. No maximum on number of prior systemic treatment regimens.

4. Patients may receive agents to protect against skeletal related complications such as zolendronic acid or denosumab.

5. ROR1 expression in > 20% of the primary tumor or metastasis by IHC.

6. Karnofsky performance status of ≥ 70%.

7. Patients must be off chemotherapy for a minimum of 3 weeks prior to planned leukapheresis.

8. Subjects must be ≥ 18 years old.

9. Negative pregnancy test for women of childbearing potential. Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

10. Fertile male and female patients must be willing to use a contraceptive method before, during and for at least two months after the T cell infusion.

11. Ability to understand and provide informed consent.

<u>Exclusions</u>

1. ANC < 1000/mm3

2. Hgb < 9 mg/dl (transfusion permitted to achieve this)

3. Platelet count < 75,000/mm3

4. Treatment with other investigational agent(s) within 30 days of planned lymphodepletion.

5. Patients requiring ongoing daily corticosteroid therapy at a dose of > 15 mg of prednisone per day (or equivalent). Pulsed corticosteroid use for disease control is acceptable.

6. Active autoimmune disease requiring immunosuppressive therapy.

7. Major organ dysfunction defined as:

   a. Serum creatinine > 2.5 mg/dL

   b. Significant hepatic dysfunction (SGOT > 5X upper limit of normal; bilirubin > 3.0 mg/dL)

   c. Patients with clinically significant pulmonary dysfunction, as determined by medical history and physical exam should undergo pulmonary function testing. Those with an FEV1 of < 50% or DLCO (corrected) < 40% will be excluded.

   d. Significant cardiovascular abnormalities as defined by any one of the following: congestive heart failure, clinically significant hypotension, symptomatic coronary artery disease or a documented ejection fraction of < 45%. Any patient with an EF of 45-49% must receive clearance by a cardiologist to be eligible for the trial.

8. Patients who are HIV seropositive.

9. Uncontrolled active infection (bacterial, viral, fungal, mycobacterial) not responding to treatment with intravenous antibiotics, antiviral or antifungal agents, or long-term treatment with oral agents.

10. Breast-feeding women.

11. Patients who have contraindication to cyclophosphamide chemotherapy.

12. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy.

13. Untreated central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to enrollment and any neurologic symptoms have returned to baseline) and have no evidence of new or enlarging brain metastases.

6. EVALUATION AND COUNSELING OF PATIENT

Patients will be seen at the Seattle Cancer Care Alliance or the Fred Hutchinson Cancer Research Center (FHCRC) for consideration of treatment options for their disease. The protocol will be discussed thoroughly with the patient and other family members if appropriate, and all known and potential risks to the patient will be described. The procedure and alternative forms of therapy will be presented as objectively as possible, and the risks and hazards of the procedure explained to the patient. Consent from the patient will be obtained using forms approved by the Institutional Review Board (IRB) of the FHCRC. A summary of the clinic visit detailing what was covered will be dictated for the medical record.

7. PROTOCOL REGISTRATION

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 the subject on to the protocol through the Data Management Office. A screening/leukapheresis consent must be signed prior to the performance of study related assessments or procedures. The T cell therapy treatment consent will be signed after successful collection of T cells and prior to chemotherapy and treatment with ROR1 CAR-T cells.

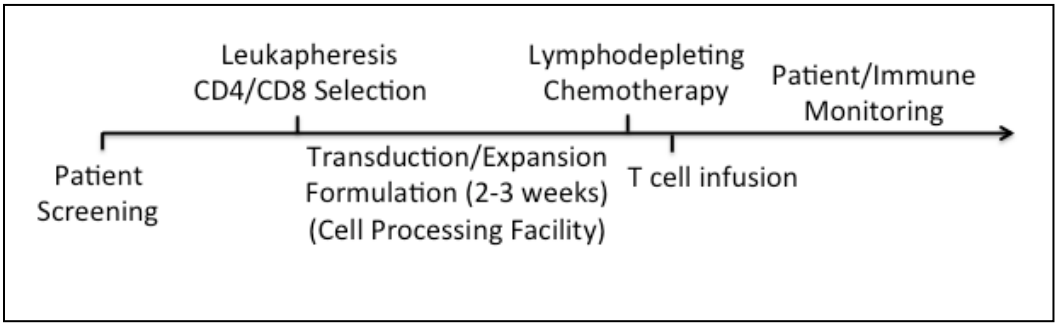
8. STUDY AGENT

8A. ROR1 CAR-T cells

1. The methods employed to derive ROR1 CAR-T cells from the patient's $CD8^+$ and $CD4^+$ T cells enriched from PBMC, and release tests of the cell products prior to infusion are outlined in the Chemistry, Manufacturing and Controls (CMC) section of the Investigational New Drug (IND) application to the FDA. Modifications to the CMC section during the course of the study must be submitted for FDA review.

2. The autologous T cell product will be prepared and administered to the patient by intravenous infusion. Patients will be pretreated with cytoreductive/lymphodepleting chemotherapy and receive the T cell infusions **36-96 hours after completing chemotherapy (Section 9.B)**.

3. A schematic of the treatment plan is shown in **Figure 1**.



9. PLAN OF TREATMENT

9A. Leukapheresis or blood-draw to obtain T cells for ROR1 CAR-T cell manufacturing

1. A leukapheresis will be performed on each patient in the Apheresis Unit at the SCCA using standard operating procedures for obtaining peripheral blood mononuclear cells. Should a technical issue arise during the procedure or in the processing of the product, or insufficient ROR1 CAR-T cells be manufactured for the prescribed ROR1 CAR-T cell dose, a second procedure may be performed.

Patients ineligible for a vein to vein apheresis may elect to have a percutaneous central venous access catheter inserted to support this collection. Patients ineligible for leukapheresis and who have a hematocrit of >38 and a total non-malignant (normal) lymphocyte count of >2000 may undergo phlebotomy of 400 ml of blood to obtain PBMCs necessary to establish the T cell cultures. This approach will only be taken in patients that would be enrolled at dose levels 1 and 2 and if the absolute CD8 and CD4 T lymphocyte counts are deemed to be sufficient by the Clinical PI to obtain enough T cells for successful manufacturing.

2. The leukapheresis or phlebotomy product will be delivered to the Cell Processing Facility (CPF) at the FHCRC or the Cell Therapy Laboratory (CTL) at the SCCA. $CD8^+$ and $CD4^+$ cell selections may be performed in the FHCRC CPF or SCCA CTL. PBMC not required for cell selections may be archived for research.

If lymphocyte subset counts are considered adequate, the product will be divided into two aliquots. One aliquot will be enriched for $CD8^+$ T cells and the second aliquot will be enriched for $CD4^+$ T cells using clinical grade reagents and SOPs developed at the FHCRC/SCCA. Subsequent processing, after selection of $CD4^+$ and $CD8^+$ T cells and cryopreservation (if required), is performed in the FHCRC CPF.

If processing of $CD8^+$ and $CD4^+$ T cells is not considered suitable due to lymphopenia, low $CD8^+$ T and $CD4^+$ cell counts or other reasons, we may use other strategies that are approved for use in this protocol by the FDA to manufacture ROR1 CAR-T cells without selection of subsets.

3. Quality control and release testing will be performed on the ROR1 CAR-T cell product prior to its release for patient infusion**.**

4. Patients may receive chemotherapy after screening and leukapheresis to control disease during production of ROR1 CAR-T cells. Decisions regarding chemotherapy in this interval should be discussed with protocol Principal Investigator (PI).

5. Patients will be scheduled for cytoreductive and/or lymphodepleting chemotherapy to be completed 36-96 hours prior to the first T cell infusion.

9B. Cytoreductive/Lymphodepleting Chemotherapy

1. Prior to the first infusion of ROR1 CAR-T cells, patients will receive chemotherapy as determined by the referring physician in consultation with the protocol Principal Investigator (PI). The objectives of administering chemotherapy are to provide lymphodepletion to facilitate T cell survival and to reduce the tumor burden prior to infusion of ROR1 CAR-T cells.

2. Refer to **Appendix A** for suggested chemotherapy regimens based on disease cohort.

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

9C. T Cell Infusions

1. ROR1 CAR-T cells should be administered between 36 and 96 hours after the completion of lymphodepleting chemotherapy.

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

3. Patients deemed not appropriate for T cell administration may receive a T cell infusion outside the 36-96 hour window if they subsequently resolve the clinical and/or laboratory concerns which deemed them inappropriate.

4. Patients who receive lymphodepleting chemotherapy, but who are unable to undergo CAR-T cell infusion within the intended 36-96 hour window due to CAR-T cell manufacturing or release issues may receive the cell infusion outside of this window if the issues can be resolved. Discussion with the protocol PI and IND holder is required.

9D. ROR1 CAR-T cells dosing

1. Cohort A (CLL, MCL and ALL) - Cell dose escalation/de-escalation

The primary objective of this study is to estimate the maximum tolerated dose (MTD) of CAR-T cells. EGFRt is co-expressed in the CAR vector and serves as a transduction marker to determine the number of CAR-T cells in the cell product to define cell dose. The MTD for these purposes will be defined as a true dose limiting toxicity rate of 25%, where DLT is defined in Section 14C.1. A modification of the continual reassessment method (CRM) will be used to estimate the MTD. The modifications include treating patients in groups of at least two (rather than one), and allowing a maximum increase of one dose level between groups. Patients will receive a single intravenous infusion of ROR1 CAR-T cells at one of four escalating dose levels beginning with dose level 1 for the first group of two patients. **Dose escalation or de-escalation is determined by the CRM algorithm, taking into account the number of patients experiencing a serious toxicity at each dose level, as described in Section 14C.1.**

Dose level 0:	up to $1x10^5$ $EGFRt^+$ cells/kg
Dose level 1:	**up to $3.3x10^5$ $EGFRt^+$ cells/kg (Starting dose level)**
Dose level 2:	up to $1x10^6$ $EGFRt^+$ cells/kg

Dose level 3:	up to $3.3x10^6$ $EGFRt^+$ cells/kg
Dose level 4:	up to $1.0x10^7$ $EGFRt^+$ cells/kg

Treatment of patients in each of the dose-escalation/de-escalation groups will be staggered with a minimum of a 21-day interval following infusion between each patient and 35 days before escalation to the next dose level. The CRM algorithm will be followed until up to 30 patients have been enrolled and treated, and the MTD will be estimated after the 30^{th} patient is evaluable for DLT. Preliminary estimates of anti-tumor efficacy will be obtained among all patients and among those who were treated at the estimated MTD.

2. Cohort B (NSCLC and TNBC) - Cell dose escalation/de-escalation

The toxicity of ROR1 CAR-T cells resulting from on-target recognition of tumor cells could differ in epithelial malignancies due to the sites of primary and metastatic disease, differences in ROR1 expression levels, and differences in the tumor microenviroment that could suppress transferred T cell immunity. Thus, we will perform the dose escalation/de-escalation of ROR1 CAR-T cells separately in patients in Cohort B. Our goal will be to have relatively balanced enrollment of TNBC and NSCLC in this cohort depending on patients meeting eligibility criteria. The primary objective for this cohort is to estimate the maximum tolerated dose (MTD) of ROR1 CAR-T cells, as above, with the MTD defined as the dose of T cells associated with a true DLT rate of 25%, where DLT is defined in Section 14C.1. **Dose escalation or de-escalation is determined by the CRM algorithm, taking into account the number of patients experiencing a serious toxicity at each dose level, as described in Section 14C.1.**

Dose level 0:	up to $1x10^5$ $EGFRt^+$ cells/kg
Dose level 1:	**up to $3.3x10^5$ $EGFRt^+$ cells/kg (Starting dose level)**
Dose level 2:	up to $1x10^6$ EGFRt+ cells/kg
Dose level 3:	up to $3.3x10^6$ $EGFRt^+$ cells/kg
Dose level 4:	up to $1.0x10^7$ $EGFRt^+$ cells/kg

Treatment of patients in the dose-escalation/de-escalation groups will be staggered with a minimum of a 21-day interval following infusion between each patient and 35 days before escalation to the next dose level.. The CRM algorithm will be followed until up to 30 patients have been enrolled and treated, and the MTD will be estimated after the 30^{th} patient is evaluable for DLT. Preliminary estimates of anti-tumor efficacy will be obtained among all patients and among those who were treated at the estimated MTD.

3. Cell administration. Individual aliquots of $CD4^+$ and $CD8^+$ ROR1 CAR-T cells will be prepared for each T cell infusion according to protocols established in the GMP Cell Processing Facility and after passing release criteria will be formulated to provide a single cell product at the specified cell dose. The specified T cell dose refers to CAR^+ T cells determined by the expression of the truncated EGFR transduction marker, which is expressed coordinately with the CAR in the vector. Each T cell infusion should be administered intravenously over approximately 20 – 30 minutes at the specified T cell dose. At least the first patient treated at each dose level will receive the T cell infusion at the University of Washington Medical Center.

4. Patient monitoring during T cell infusions. All patients will be monitored during each T cell infusion. Patients should receive the T cells under physician guidance and nursing supervision, with vital signs being monitored and recorded before, q 15 mins during and at the conclusion of

the infusion and then approximately hourly for 2 hours after the infusion. O2 saturation should be monitored continuously during the infusion and results documented before, q 15 mins during and at the conclusion of the infusion. O2 saturation should then be assessed and documented approximately hourly for 2 hours after the infusion

5. Products that cannot be formulated to meet cell dose specification. The intent for each infusion is to provide a cell product that reaches at least 85% of the targeted cell dose and contains approximately 50% (+/- 15%) of ROR1 CAR-modified $CD4^+$ T cells and 50% (+/- 15%) of ROR1 CAR-modified $CD8^+$ T cells (i.e. CAR-modified T cells in 1:1 $CD4^+/CD8^+$ ratio) If a T cell product cannot be formulated to meet this cell dose specification because of low transduction efficiency, suboptimal growth of one of the subsets, or failure of either the $CD4^+$ or $CD8^+$ T cell product to meet release criteria, the cell product should be infused at or as close as possible to the specified phenotype allocation and total T cell dose providing:

i. The total EGFRt+ CAR-T cell dose exceeds $5x10^4$ cells/kg,
ii. The cell product passes other release criteria including endotoxin, gram stain, sterility, mycoplasma, RCL and viability.
iii. Toxicity and safety data will be collected and reported for subjects receiving such products.

Such patients will not be considered evaluable for safety analysis in the assigned dose cohort and a replacement subject will be added to the cohort. Toxicity and safety data will be collected and reported on such patients, but will not be incorporated into the CRM algorithm for the cell dose cohort under study.

6. Retreatment of patients on the study. Patients enrolled in the study may be eligible to receive a second ROR1 CAR-T cell infusion with or without additional cytoreductive chemotherapy at the same (for those that received the highest cell dose) or up to the next highest dose level if adequate ROR1 CAR-T cells can be produced and the following criteria are met:

a. There is evidence of persistent disease after the first T cell infusion.
b. There were no toxicities attributed to the first infusion that were dose-limiting or required dose de-escalation (Section 14.C.1).
c. The patient is ≥ 21 days from the first T cell infusion.
d. There are no clinical and/or laboratory exclusion criteria (**Section 9C.1**).

10. MANAGEMENT OF TOXICITIES AND COMPLICATIONS

Acute infusional toxicity may occur during or shortly after T cell infusion. In addition, cytokine release syndrome, tumor lysis syndrome, and neurologic toxicity have been reported specifically after CD19 CAR-T cells and may occur after the infusion of ROR1 CAR-T cells. Recommendations for the management of these complications is addressed in **10A-10E**.Clinical interventions should be provided in accordance with the treating clinician's judgment and patient's clinical conditions. A table of proposed grading criteria for cytokine release syndrome is given in Appendix G

10A. Management of acute toxicity associated with T cell infusion

The results of our prior studies of adoptive immunotherapy for CMV, HIV, leukemia and 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 can occur very shortly after the T cell infusion in patients with a high antigen (tumor) burden.

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

- Fever, chills, and temperature elevations >101°F may be managed with acetaminophen 650 mg p.o. q 4-6 hrs. All subjects who develop fever or chills should have a blood culture drawn.
- Headache may be managed with acetaminophen.
- Nausea, vomiting may be managed with diphenhydramine 25-50 mg IV or other antiemetics (excluding corticosteroids).
- Hypotension should be managed initially by fluid administration.
- Hypoxemia should be managed initially with supplemental oxygen.

If the following signs appear during T cell infusion, the infusion should be paused and the patient assessed:

- Systolic BP < 80 mmHg or > 40 mmHg fall from baseline
- Heart rate > 140/min or increase from baseline > 40/min (confirmed by palpation or EKG)
- Respiratory rate > 35/min or increase from baseline of > 10/min
- Arterial O2 saturation < 88% on room air or fall from baseline > 5%

If after assessment by the PI/designee the patient's condition is stable then the infusion may be resumed.

If a T cell infusion is terminated due to acute toxicity, the residual T cells should be returned to the Cell Processing Lab for analysis. Investigation of possible causes of observed signs should proceed and, if necessary, additional medical treatment will be instituted.

Patients requiring discontinuation of T cell infusion may be eligible for re-treatment if the cause is deemed not related to the T cell infusion.

10B. Management of cytokine release syndrome

1. If patients become febrile or develop symptoms of cytokine release, we may measure cytokine levels, serum ferritin, C-reactive protein and markers of tumor lysis syndrome (e.g. chemistry, uric acid, LDH), and evaluate persistence and phenotype of the transgene-expressing cells, as clinically indicated.

2. Any patient who develops clinical evidence of symptoms related to cytokine release will have a work-up 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. Patients with Grade ≥ 3 CRS (severe CRS; sCRS) 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 Principal Investigator or designee and repeated doses of tocilizumab may be given if necessary. See Appendix G for cytokine release syndrome grading and Appendix H for recommended management guidelines.

10C. Management of tumor lysis syndrome

1. All patients will be considered at risk for tumor lysis and should receive allopurinol prophylaxis 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 for the first 2 weeks after the T cell infusion.

2. If tumor lysis syndrome develops, as defined by the Cairo Bishop criteria [123], the Attending Physician will direct patient management with guidance from the study staff [124]. Conservative therapy, including allopurinol, urinary alkalinization, and IV fluid hydration may be instituted immediately for suspected tumor lysis syndrome. 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.

10D. Management of neurotoxicity

Neurotoxicity, manifest as delirium, seizures and/or focal neurologic deficits, has been reported after CD19 CAR-T cell therapy. Neurotoxicity that is attributed to the T cell infusion/s should be treated with corticosteroids (e.g. dexamethasone 10 mg IV q 4-12 hours). Tocilizumab may also be given, preferably after discussion with the Principal Investigator or designee. Patients with a history of seizures or history of CNS involvement or high tumor burden ALL should receive prophylactic treatment with anti convulsants (Keppra or similar) following CAR-T cell infusion for approximately 4 weeks. Patients who develop significant neurotoxicity should be also treated with anti-convulsants if not already started. See Appendix H for recommended management guidelines.

10E. Management of other toxicities

1. If a new onset CTCAE v4.0 grade ≥ 3 toxicity is observed following any T cell infusion, the patients will receive investigation and medical treatment appropriate for the physiological abnormalities.

2. Grade ≥ 3 toxicity that is attributed to the T cell infusion/s and is unresponsive to supportive measures or persists for > 7 days may be treated with corticosteroids (e.g. dexamethasone 10 mg IV q 4-12 hours) after discussion with the Principal Investigator or designee.

3. Uncontrolled proliferation of CAR-T cells has not been observed in clinical trials to date. However, in the unlikely event uncontrolled proliferation of ROR1 CAR-T cells occurred in a study subject, initial therapy may involve treatment with corticosteroids (e.g. methylprednisolone 1

g IV). Anti-lymphocyte globulin or cytotoxic drugs would also be considered in serious cases. If we observe an increase in CAR positive cells to greater than 10% of T cells at more than 3 months after last infusion we will analyze for clonal expansion by deep sequencing of the TCR beta gene (ImmunoSeq Adaptive Biotechnology).

4. The vector encodes EGFRt and our preclinical data in animal models demonstrates that administering two doses of Erbitux (cetuximab) can eliminate CAR-T cells that express EGFRt and reverse on-target toxicity not attributed to cytokine storm, for which there is no suitable animal model. Thus, in patients without cytokine storm who have an ongoing organ toxicity that is attributed to ROR1 CAR-T cells, we could consider administering up to two doses one week apart of intravenous cetuximab in standard dosing at 400 mg/m^2 (dose 1) and 250 mg/m^2 (dose 2). Patients would be pre-medicated 30-60 minutes prior to each dose of cetuximab with 50 mg of diphenhydramine intravenously. Blood samples (30 ml in sodium heparin tubes) should be collected prior to cetuximab and on approximately days 1, 4, 7, 14, and 21 to determine if cetuximab resulted in a decline or elimination of ROR1 CAR-T cells.

11. SCHEDULE OF PATIENT EVALUATIONS (Appendix D - Table of Evaluations).
Please note that results of tests and/or procedures conducted as per standard of care purposes may be used for research purposes if conducted within the protocol-defined window prior to screening/leukapheresis (11.A.) and/or T-Cell Therapy (11.B.).

<u>11A. Evaluation for Leukapheresis (Completed within 15 days of leukapheresis procedure)</u>

1. Informed consent and HIPPA signing
2. Laboratory evaluation, including:
    a. CBC, differential and platelet count
    b. Renal function panel and hepatic function panel with LDH
    c. Blood Works NW Recipient Donor Battery Panel
    d. Serum pregnancy test for females of child-bearing potential
    e. ABO blood typing and antibody screen
    f. Patient/donor CD3 chimerism, at any time point post transplant, if prior allogeneic transplant
3. Medical history, including:
    a. Hematologic, cytogenetic, flow cytometric, and histologic findings at diagnosis and at the time of enrollment on the study
    b. Prior therapies and response to therapy if known
4. Confirmation of diagnosis by internal pathology review of initial or subsequent biopsy or other pathologic material at the FHCRC/SCCA.
5. Physical Exam and Karnofsky Performance Status
6. Research testing:

a. Confirmation of ROR1 expression on tumor cells
   i Cohort A: Flow cytometry of tumor cells from blood, lymph node, or bone marrow specimen or IHC on a prior diagnostic lymph node or other site.
   ii. Cohort B: IHC on a prior diagnostic specimen or fresh biopsy specimen.

b. 10 ml blood sample, sodium heparin (green top tube) to Berger Lab for evaluation of CD3, CD4, and CD8 T cells subsets

11.B Evaluation prior to T cell therapy (to be performed if studies in 11A. 1-7 deem patient to be eligible and T cell collected) Should be completed within 30 days of enrollment unless otherwise specified

1. Cohort A (CLL, MCL, and ALL)

   1. Laboratory evaluations

      a. CBC, differential and platelet count
      b. Renal function panel and hepatic function panel with LDH
      c. Uric acid
      d. Serum ferritin
      e. CRP
      f. DIC panel without platelets
      g. Parathyroid hormone level (PTH)
      h. G6PD screen
      i. Serum pregnancy test for females of child bearing potential
      j. Quantitative immunoglobulins (IgG, IgA and IgM)
      k. Immunophenotyping of normal and/or malignant B cells. If clinically indicated, 5 ml blood in sodium heparin should be sent to SCCA Hematopathology Laboratory for analysis of circulating normal and/or malignant B cells. This may be omitted if previously performed within 30 days of the planned T cell infusion AND the patient has not received anti-tumor therapy in the interim.

   2. A bone marrow aspirate/biopsy should be performed with pathology, flow cytometry, karyotyping, FISH and other molecular studies as indicated by the disease. This may be omitted if done within 30 days of planned T cell infusion AND the patient has not received anti-tumor therapy in the interim.
   3. A CT scan (preferably diagnostic quality) and, if possible, a PET scan should be performed to evaluate disease status in patients with lymphoma and CLL. Patients with B-ALL should undergo CT +/- PET imaging if clinically indicated. Imaging studies may be omitted in patients who have had recent imaging within 30 days before scheduled T cell infusion AND have not received anti-tumor therapy in the interim.
   4. Lumbar puncture with CSF evaluation is required for any patient with a history of CNS disease or signs and symptoms of CNS or epidural disease. This may be omitted if a lumbar

puncture performed within 30 days of scheduled T cell infusion did not show evidence of CNS disease.

5. Baseline CXR
6. Baseline EKG
7. MUGA scan or echocardiogram
8. Physical Exam and Karnofsky Performance Status
9. Research samples:
    a. A blood sample (30 ml, heparin/green top tubes) should be obtained for baselineT cell persistence and sent to the Berger Lab at the FHCRC.

    b. A blood sample (up to10 ml, serum separator tube) should be obtained for measurement of serum inflammatory cytokine levels and baseline transgene immunogenicity. Samples should be sent as soon as possible to the Berger Lab at the FHCRC.

    c. If a bone marrow is being done clinically then an additional 5 - 10 ml of marrow aspirate should be obtained and sent in heparin/green top tubes to the Berger Lab at the FHCRC.

    d. If biopsy or sampling of tissues other than bone marrow; ie CSF, pleural fluid, lymph nodes etc. can be performed and the patient has provided consent for the collection of research tissue, than these samples may be obtained and sent to the Berger lab for research studies. Please discuss the planned procedure with the PI.

2. <u>Cohort B (NSCLC and TNBC)</u>

1. Laboratory evaluations
    a. CBC, differential and platelet count
    b. Renal function panel and hepatic function panel with LDH
    c. Uric acid
    d. Serum ferritin
    e. CRP
    f. DIC panel without platelets
    g. Parathyroid hormone levels (PTH)
    h. G6PD screen
    i. Serum pregnancy test for females of child bearing potential
    j. Quantitative immunoglobulins (IgG, IgA and IgM)
2. A CT scan (preferably diagnostic quality) and if indicated a PET scan should be performed to evaluate sites and status of disease. Imaging studies may be omitted in patients who have had recent imaging within 30 days before scheduled T cell infusion AND have not received anti-tumor therapy in the interim.

3. Baseline brain CT or MRI
4. Baseline CXR
5. Baseline EKG
6. MUGA scan or echocardiogram
7. Physical Exam and Karnofsky Performance Status
8. Research samples.
    a. A blood sample (30 ml, heparin/green top tubes) should be obtained for baseline T cell persistence and sent to the Berger Lab at the FHCRC.
    b. A blood sample (up to10 ml, serum separator tube) should be obtained for measurement of serum inflammatory cytokine levels and baseline transgene immunogenicity. Samples should be sent as soon as possible to the Berger Lab at the FHCRC.

    c. If biopsy or sampling of tissues; ie CSF, pleural fluid, bone marrow, lymph node, etc. can be performed and the patient has provided consent for the collection of research tissue then these samples may be obtained and sent to the Berger lab for research studies. Please discuss the planned procedure with the PI.

11C. Evaluation Prior to Each T cell Infusion: Day 0

1. Clinical:
    a. CBC, differential and platelets
    b. Renal panel and hepatic panel with LDH
    c. Uric acid
    d. Serum ferritin
    e. CRP
    f. DIC panel without platelets
    g. Vital signs at the approximate times: before starting, every 15 minutes during the T cell infusion, and hourly for 2 hours following the T cell infusion
    h. O_2 saturation should be monitored continuously by pulse oximetry during the T cell infusion. Values should be recorded prior to initiating the infusion, approximately 15 minutes during the T cell infusion and approximately hourly for 2 hours post infusion.
2. Research:
    a. Blood samples (up to10 ml, serum separator tube) should be obtained prior to the T cell infusion for measurement of serum inflammatory cytokine levels and human anti-chimeric anti-ROR1 antibodies (HACA). Samples should be sent as soon as possible to the Berger Lab at the FHCRC.
    b. Blood samples (30 ml, heparin/green top tubes) should be obtained prior to the T cell infusion for baseline analysis of the presence of transferred T cells by q-PCR for vector

sequences and/or for expression of the EGFR and ROR1 CAR transgenes on $CD8^+$ and $CD4^+$ T cells by flow cytometry, if sufficient PBMC can be obtained.

11D. Patient Evaluations After Each T Cell Infusion

The following evaluations should be performed after each T cell infusion. If a patient receives a second T cell infusion, the day of the second infusion will be designated 'day 0' for evaluations thereafter.

1. Clinical
   a. Record new findings on history and physical exam 1 day after the T cell infusion and at least weekly for a minimum of five weeks.
   b. Laboratory studies at least twice weekly for two weeks then weekly for three weeks after each T cell infusion.
      i. CBC, differential and platelet count
      ii. Renal function and hepatic function panel with LDH
      iii. Uric acid, CRP and serum ferritin, DIC panel without platelets
   c. If patients become febrile or develop symptoms of cytokine release or tumor lysis between the indicated time points, we may measure serum ferritin, C-reactive protein, DIC panel and tumor lysis markers at additional times, as clinically indicated.
   d. PTH levels should be measured 1 and 2 months after CAR-T cells. If patients develop hypocalcemia, additional serum PTH levels may be obtained.
2. Immunoglobulin levels
   a. Patients receiving CAR T cells who develop B cell depletion may develop an IgG deficiency. Recommendations will be made for monitoring of IgG levels monthly and administering intravenous immunoglobulin (IVIG) as clinically indicated.
   b. Serum IgG, IgA and IgM levels may be measured at approximately 3 and 12 months after the final T cell infusion in patients who develop B cell aplasia.
3. Immunophenotyping

ROR1 is not expressed on mature normal B cells or plasma cells in the peripheral blood, but is expressed at a stage of B cell development in the bone marrow. The absolute numbers of normal B cells in the peripheral blood (and abnormal ROR1+ B cells in patients with MRD or relapse) should be determined on approximately days 14, 28, 60, and day 120 after the last T cell infusion, unless the patient initiates other non-CAR T cell systemic therapy. Blood, 5 ml in sodium heparin should be sent to SCCA Hematopathology Laboratory for analysis. If a deficiency of B cells develops by day 120 or there are persisting ROR1 CAR-T cells, we may monitor B cell numbers again on D180 and then every 6 months until normal B cells recover.

4. Serum Cytokines
   a. A blood sample (up to 10 ml, serum separator tube) should be obtained approximately on days 1, , 4, 7, 10, 14, 21 ,28 and 35 after each T cell infusion for measurement of serum inflammatory cytokine levels. Any patient that stays on the Immunotherapy service beyond

D35 may have weekly serum cytokine samples obtained as clinically indicated. Samples should be sent to the Berger Lab at the FHCRC.

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

5. Evaluation of persistence and phenotype of ROR1 CAR-T Cells

a. Blood samples (30 ml, heparin/green top tubes) should be obtained on approximately days 1, 4, 7, 10, 14, 21, 28, 35, 60, 120, 180, and 365 after the T cell infusion for analysis of the persistence of transferred T cells. Additional samples may be collected at other times than those indicated if required for evaluation of persistence of CAR-T cells. Persistence monitoring may be discontinued beyond day 28 in patients who proceed to allogeneic hematopoietic stem cell transplantation and/or do not have detectable transgene-expressing T cells on two consecutive occasions. Samples should be sent to the Berger Lab at the FHCRC. A subset of blood samples obtained after the infusion should also be analyzed by multiparameter flow cytometry for the phenotype of persisting CD8+ and CD4+ ROR1 CAR-T cells. Markers that may be analyzed include CD62L, CCR7, CD28, CD27, CD127, PD1, Lag3, Tim3 and PDL1.

b. 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.

6. Evaluation of human anti-chimeric anti-ROR1 antibodies (HACA)

a. A blood sample (up to 10 ml, serum separator tube) should be obtained on approximately days 7, 14, 21, 28, 35, 60, 90 and 180 after each T cell infusion for measurement of human anti-chimeric anti-ROR1 antibodies (HACA). Samples should be sent to the Berger Lab at the FHCRC.

7. Evaluation of transgene immunogenicity

Loss of persistence of transferred T cells by flow cytometry for the EGFRt marker (<0.01% of $CD3^+$ cells) is possible. Patients will be evaluated for antibody or T cell-mediated transgene immune responses as follows:

a. Blood (10ml, serum separator tube) should be collected around days 28, 60, 120, 180, 365 after the final T cell infusion and serum extracted for evaluation of antibody-mediated immune responses. Samples should be sent to the Berger Lab at the FHCRC.

b. Blood (20 ml, sodium heparin/green top tubes) should be collected around days 28, 60, 120, 180, 365 after the final T cell infusion) and PBMC isolated for evaluation of cellular immune responses. Samples should be sent to the Berger Lab at the FHCRC.

8. Archival samples for future studies of T cell function

a. Blood (60 ml, sodium heparin/green top tubes) OPTIONAL may be obtained from patients once between days 0 and 21 after ROR1 CAR-T cell infusion and once between days 21 and 90 for archival purposes.

b. Blood (20 ml, sodium heparin/green top tubes) should be obtained from patients around days 120, 180, and 365 after the final T cell infusion for archival purposes. Samples should be sent to the Berger Lab at the FHCRC.

c. Serum (up to 10 ml, serum separator tube) should be obtained from patients at around days 120, 180, and 365 after the final T cell infusion for archival purposes. Samples should be sent to the Berger Lab at the FHCRC.

9. Evaluation of migration of adoptively transferred ROR1 CAR-T cells

a. If aspirations and/or biopsies of bone marrow are performed for evaluation of tumor response or other clinical indications then additional aspirates (5 - 10 ml in heparin/green top tubes) should be obtained and sent to the Berger Lab, FHCRC.

b. If biopsy or sampling of tissues other than bone marrow is performed for clinical indications then additional tissue may be obtained during the same procedure for research studies. Please discuss the planned procedure with the PI.

10. Evaluation of tumor response

Cohort A (CLL, MCL, and ALL)

a. Bone marrow aspiration and biopsy.

i. Because the timing of disease response to ROR1 CAR-T cells is unknown, a bone marrow aspirate and biopsy should be performed between 14 days and 3 months after each T cell infusion unless the patient did not have bone marrow involvement prior to therapy. Bone marrow evaluation should be performed before the patient receives additional conventional anti-tumor therapy. Evaluation should include pathology analysis, flow cytometry, karyotyping, FISH studies and other molecular studies, according to disease-specific guidelines.

ii. A bone marrow aspirate and biopsy should also be performed at approximately 6 and at approximately 12 months after the first T cell infusion, as clinically indicated.

b. Fresh tumor biopsy: If biopsy or sampling of tissues other than bone marrow; ie CSF, pleural fluid, lymph nodes etc. can be performed and the patient has provided consent for the collection of research tissue, then these samples may be obtained and sent to the Berger lab for research studies.. Please discuss the planned procedure with the PI.

c. Imaging studies

i. A CT scan (preferably diagnostic quality) and, if possible, a PET scan of the neck, chest, abdomen and pelvis should be obtained between 1 and 3 months after each T cell infusion, and should be performed before the patient receives additional conventional anti-tumor therapy. Patients with B-ALL do not require CT/PET imaging, unless it is clinically indicated.

ii. Patients with a response to treatment should have CT or PET/CT scans performed at 6 and 12 months after the first T cell infusion, as clinically indicated.

d. CBC with diff, serum chemistry panel and LDH should be performed concurrently with restaging by biopsy or imaging.

e. Peripheral blood flow cytometry for patients in Cohort A should be performed around the time of restaging by biopsy or imaging.

f. Standard criteria (as detailed in **Appendix C**) will be used to define tumor response.

g. Evaluation of tumor response may be discontinued in patients who proceed to other systemic non-CAR T cell therapies

Cohort B (NSCLC and TNBC)

a. Imaging studies

i. A CT scan (preferably diagnostic quality) and if indicated a PET scan of the chest, abdomen and pelvis should be obtained between 1 and 3 months after each T cell infusion, and should be performed before the patient receives additional conventional anti-tumor therapy.

ii. A MRI of the brain should be obtained between 1 and 3 months after each T cell infusion for any patient with a history of brain metastases.

iii. Patients with a response to treatment should have follow-up imaging studies at approximately 6 and 12 months after the T cell infusion, and every 6 months thereafter, as clinically indicated.

b. Fresh tumor biopsy: If biopsy or sampling of tissues other than bone marrow; ie CSF, pleural fluid, lymph nodes etc. can be performed and the patient has provided consent for the collection of research tissue, than these samples may be obtained and sent to the Berger lab for research studies. Please discuss the planned procedure with the PI.

c. CBC with diff, serum chemistry panel and LDH should be performed around the time of restaging by biopsy or imaging.

d. Standard criteria (as detailed in **Appendix C**) will be used to define tumor response.

Evaluation of tumor response may be discontinued in patients who proceed to other non-CAR-T cell therapies.

11. Long Term Follow-Up (Appendix F

Enrolled patients who receive ROR1 CAR-T cells will be asked to participate in long-term follow-up (LTFU) according to the guidelines set forth by the FDA's Biologic Response Modifiers Advisory Committee that apply to gene transfer studies. 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.

12. PROTOCOL ENROLLMENT

12A. Projected Target Accrual

The number of patients required to complete dose escalation/de-escalation studies is variable, and dependent on observed toxicity. We estimate that defining a dose level in Cohorts A and B with acceptable toxicity will take up to 30 patients in each cohort. Thus, up to a total of 60 patients could be enrolled on the trial.

Table 1: Targeted / Planned Enrollment

Ethnic Category	Gender Number of Subjects		
	Females	**Males**	**Total**
Hispanic or Latino	4	2	6
Not Hispanic or Latino	32	22	54
Ethnic Category Total of All	36	24	60
Racial Category			
American Indian / Alaska	0	1	1
Asian	3	3	6
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	3	1	4
White	30	19	49
Racial Categories: Total of All Subjects*	36	24	60

13. RECORDS

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 Research Center, the University of Washington Medical Center and the Seattle Cancer Care Alliance. The investigators will ensure that data collected conform to all established guidelines for coding collection, key entry and verification. Each patient is assigned a unique patient number to assure patient confidentiality. Patients will not be referred to by name or by any other personal identifier in any publication or external presentation. The Clinical Statistics Departments at FHCRC maintain a patient database to allow storage and retrieval of patient data collected from a wide variety of sources. The licensed medical records departments, affiliated with the institution where the patient receives medical care, maintains all original inpatient and outpatient chart documents.
The primary research records are kept in access controlled office spaces or password protected computer based applications. Information gathered from this study regarding patient outcomes and adverse events may be made available to the Federal Drug Administration, NIH and Juno Therapeutics. All precautions to maintain confidentiality of medical records will be taken.

14. EVALUATION AND STATISTICAL CONSIDERATIONS

14A. Type of study

This is a phase 1/2 study to assess the safety and antitumor activity of adoptive T cell therapy with autologous $CD4^+$ T cells and $CD8^+$ T cells transduced to express a ROR1-specific CAR for patients with advanced $ROR1^+$ B cell malignancies.

14B. Definition of endpoints

1. Safety assessment (relevant data being obtained)

a. General Toxicity Assessment

- History and physical exam before and at intervals after T cell infusions.
- Pulse oximetry before and during the infusion
- CBC and chemistry battery before and at intervals after the T cell infusion
- Toxicity grading according to NCI CTCAE Version 4.0
- Serum cytokine levels
- Immunophenotyping
- Serum immunoglobulin levels
- Replication competent lentivirus
- Adverse event reporting

2. Efficacy Assessment

a. Evaluation of the duration of persistence of adoptively transferred ROR1 CAR-T cells

b. Evaluation of the migration of adoptively transferred ROR1 CAR-T cells

c. Evaluation of antitumor activity of adoptively transferred ROR1 CAR-T cells by evaluating the objective response rate of complete remission and partial remission, and determining progression free survival, and overall survival.

14C. Endpoint Evaluation

1. Safety Assessment

Patients will be treated in cohorts of two patients at one of five dose levels of ROR1 CAR-T cells starting at dose level 1 (**Section 9D.1 and 9D.2**) to determine cell doses associated with an estimated toxicity rate of less than 25%.

a. Dose limiting toxicities

DLTs will be assessed for 28 days following the infusion of the CAR T cell product. The following events will be considered DLTs if they are attributed as at least possibly related to T cell administration.
Grading will be done in accordance with the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 unless otherwise specified.

1. Grade ≥ 3 allergic reaction related to the CAR T cell infusion
2. Grade ≥ 3 autoimmune reactions
3. Any Grade 3 or 4 non-hematologic event that has not resolved to < grade 3 by day 28 post T cell infusion
4. Grade ≥ 3 neurotoxicity of greater than 7 days duration
5. Grade ≥ 3 neurotoxicity that does not revert to Grade 1 or baseline within 28 days
6. Grade ≥ 3 seizures that do not resolve to < grade 3 within 3 days
7. Grade ≥ 4 CRS (using criteria modified from Lee 2014)
8. Grade 3 CRS that does not resolve to < grade 3 within 7 days
9. Any other toxicity not meeting the above criteria that is deemed by the PI to represent a DLT

b. Stopping and Suspension Rules

If there ever exists sufficient evidence to suggest that the true probability of treatment-related **death by day 100** exceeds 20% (regardless of dose), enrollment of patients 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 whose lower 80% confidence limit exceeds 20%. Operationally, any of the following ratios of treatment-related deaths to patients treated would trigger such a rule: 2/2-4, 3/5-7, 4/8-11, 5/12-15, 6/16-20, 7/21-24, 8/25-28, or 9/29-30. If the true probability of treatment-related death is 0.10, then the probability of stopping after 15 or 30 patients is approximately 0.07. If the true probability of treatment-related death is 0.40, then the probability of stopping after 15 or 30 patients is approximately 0.85 and 0.91, respectively (probabilities estimated from 5,000 simulations).

2. Assessment of efficacy of transferred T cells

Data should be collected for persistence, migration and efficacy of transferred T cells and descriptive statistics will be used to summarize the changes from baseline where possible. For those patients with measurable disease at the time T cell therapy commences, responses will be evaluated using standard response criteria based on CT or PET imaging and histologic analysis of bone marrow or other tissue samples.

15. GUIDELINES FOR REPORTING ADVERSE EVENTS AND DATA SAFETY AND MONITORING PLAN

15A. General issues and IRB reporting requirements

1. Definitions associated with reportable events and reporting requirements can be found on the FHCRCs Institutional Review Office (IRO) extranet website (Table 1).

2. The review and reporting of adverse events will be in accordance with the Cellular Immunotherapy AE reporting for separate FH Sponsor and Investigator SOP.

3. The NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40) will be used for grading and analysis of adverse events. All grade 3 or greater adverse events will be collected during and for 48 hrs post leukapheresis and again from the start of lymphodepletion through day 35 after each T cell infusion. The collection of adverse events will stop at the time of commencement of new anti-tumor therapy.

To ensure that investigative treatment-related conditions are distinguished from disease-related conditions, attribution of causality will be established in grading adverse events. For each event, the Principal Investigator or designee, in conjunction with the physician or research nurse who examined and evaluated the research participant, will assign the attribution. Data managers who are removed from the clinical assessment of the research participant should not perform this.

Attribution of adverse events attributed to the infused genetically modified T cells should be determined using the following criteria:

- Definite - the adverse event is clearly related to the infused T cells
- Probable - the adverse event is likely related to the infused T cells

- Possible - the adverse event may be related to the infused T cells
- Unlikely - the adverse event is doubtfully related to the infused T cells
- Unrelated - the adverse event is clearly not related to the infused T cells

Deaths occurring 35 days or later from a T cell infusion and death due to progression of known malignancy will be scored as a grade 5 toxicity, but with a low level of attribution, unlikely or not related, unless a serious toxicity that was attributed to the T cell infusion occurred prior to day 35, did not resolve and was considered a significant factor in the patient's demise.

4. The FHCRC IRB will be notified of reportable events by the FHCRC Principal Investigator (PI) or study nurse according to current reporting obligations as found on the FHCRC Institutional Review Office extranet website.

5. Reporting of unanticipated adverse effects to the FDA will be the responsibility of the IND sponsor.

6. The FHCRC PI and Research Nurse and study personnel should meet regularly (in person or via teleconference) to review all reported events.

Table 2: FHCRC IRB Policies for Reportable Events.
(Relevant FHCRC policies include, but are not limited to, the following documents. Please also refer to the FHCRC IRO website)

IRB Policy 2.6	Adverse Events and Other Unanticipated Problems Involving Risks to Subjects o Others	http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html
IRB Policy 1.9	Noncompliance with the Office of the Director's Human Research Protection Program Policy	http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html
IRB Policy 1.1	Reporting Obligations for Principal Investigators	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html
IRB Policy 2.2	Continuing Review	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html
IRB Policy 1.13	Investigational New Drugs (IND), Biologics and Investigational Device Exemptions (IDE)	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html

Table 3: FHCRC IRB Forms for Reporting

Adverse Event Reporting Form	http://extranet.fhcrc.org/EN/sections/iro/irb/forms/index.html
Unanticipated Problem Reporting Form	http://extranet.fhcrc.org/EN/sections/iro/irb/forms/index.html
Noncompliance Reporting Form	http://extranet.fhcrc.org/EN/sections/iro/irb/forms/index.html

15B. Reporting to the FDA

As a study conducted under IND (Investigational New Drug) regulations we will comply with the FDA regulations regarding safety reporting 21CFR312.32 including the following requirements:

1. A sponsor must promptly review all information relevant to the safety of the drug 21CFR312.32 (b).
2. A sponsor must notify FDA in an IND safety report of potential serious risks, as soon as possible but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting under 21 CFR312.32 (c)(1). Information that is required to be reported includes, but is not limited to, a. Serious and unexpected adverse reactions and b. An increased rate of occurrence of serious suspected adverse reactions.
3. The IND safety report must be completed and sent to the FDA in a narrative format, on FDA Form 3500A, or an electronic format.
4. A sponsor must also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information 21CFR312.32 (c)(2).

15C. Data and Safety Monitoring Plan

1. Definition of Risk Level

This phase I/II trial involves genetic modification of somatic cells and requires an IND. At the FHCRC, this type of trial has independent monitoring through the Clinical Research Support Office at the FHCRC, and has a Data and Safety Monitoring Board (**Section 15C. 3**).

2. Monitoring and Personnel Responsible for Monitoring

a. The Principal Investigator (P.I.) is responsible for every aspect of the design, conduct and final analysis of the protocol. Regulations defining the responsibilities for assessment and reporting of adverse events (AE), serious AE and unexpected AE are defined by the Code of Federal Regulations: 21 CFR 312.32 and Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 published by the Cancer Therapy Evaluation Program (CTEP), a division of the NCI/NIH. Sponsor responsibilities include providing the PI with the necessary information to conduct the investigation, ensuring proper monitoring, ensuring that the investigation is conducted in accordance with the protocol, conducting ongoing review of the safety and efficacy of the investigational product, and reporting to the FDA consistent with 21 CFR 312 Subpart D.

b. This clinical study will rely upon the monitoring of the trial by the P.I. in conjunction with a Study Physician(s), Physician Assistant(s) (PA) or Nurse Practitioner(s), Research Nurse(s), Research Coordinator(s), statistician, and an independent Study Monitor assigned by the FHCRC Clinical Research Support Office (CRSO). Institutional support of trial monitoring will be in accordance with the FHCRC/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan. Under the provisions of this plan, FHCRC Clinical Research Support coordinates data and compliance monitoring conducted by consultants, contract research organizations, or FHCRC 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), FHCRC Scientific Review Committee (SRC) and the FHCRC/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.

c. Continuous monitoring of the data and safety of this study should be performed by the Protocol Management Team (PMT), which consists of the Principal Investigators, Research Nurse, and Statistician. Monitoring by the PMT should be performed at least monthly or more often if necessitated by the development of adverse events, and should include review of the data on all enrolled patients with a summary of grade 3+ adverse events and follow up information for each patient. This list of patients should be included with the PMT report form. The PMT will be responsible for implementation of the stopping rules for safety if necessary.

d. A Case Report Form (CRF) should be completed for every patient that was registered for participation in the study. The Principal Investigator or a Co-Investigator will sign and date the indicated places of the CRF. This signature will indicate that thorough inspection of the data therein has been made, and will certify the contents of the form.

3. Data and Safety Monitoring Board

The study will be monitored by the Immunotherapy Integrated Research Center (IIRC) DSMB. The DSMB will be responsible for safeguarding the interests of trial participants and assessing the safety and efficacy of the interventions during the trial. This responsibility will be exercised by providing recommendations about stopping or continuing the trial. To contribute to enhancing the integrity of the trial, the DSMB may also formulate recommendations relating to the selection, recruitment and retention of participants and their management; adherence to protocol-specified regimens; and the procedures for data management and quality control.

The DSMB will be advisory to the study Sponsor and the PI, who will be responsible for prompt review of the DSMB recommendations to guide decisions regarding continuation or termination of the trial and whether amendments to the protocol or changes in study conduct are required.

The DSMB is an independent, multidisciplinary group consisting of clinical experts and a statistician who collectively have experience in leukemia, lymphoma, hematology, biostatistics, and the conduct and monitoring of clinical trials. The DSMB will meet approximately every 6 months to review data. The current members are listed in the IIRC DSMB charter.

16. TERMINATION OF THE STUDY

The study may be terminated at any time by the Protocol PI, the IND Sponsor, the FHCRC IRB, or the FDA.

17. REFERENCES

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18. APPENDICES

APPENDIX A - Suggested Cytoreduction/Lymphodepletion Chemotherapy Regimens

The following is a guide to chemotherapy regimens that may be used immediately prior to adoptive T cell therapy. The selection of appropriate chemotherapy regimen to provide tumor cytoreduction and lymphodepletion should be discussed with the study PI's. The preferred chemotherapy regimen for patients on the trial is Regimen C however other regimens may be used depending on PI discretion. Chemotherapy regimens that employ prolonged administration of corticosteroids that would continue after the T cell infusion cannot be used for prior to T cell infusion. Patients should be scheduled for T cell infusions starting 36-96 hours after the last dose of chemotherapy.

Regimen A
Cyclophosphamide 30-60 mg/kg on day 1; fludarabine 25 mg/m^2 on days 2-4

Regimen B
Cyclophosphamide 30-60 mg/kg on day 1; fludarabine 25 mg/m^2 on days 2-6

Regimen C (Preferred)
Cyclophosphamide 300 mg/m^2 on day 1-3; fludarabine 30 mg/m^2 on days 1-3

Regimen D
Cyclophosphamide 2-4 gm/m^2 on day 1

APPENDIX B - PERFORMANCE STATUS SCALES

These indices allow patients to be classified as to their functional impairment and can be used to compare effectiveness of different therapies and to assess the prognosis in individual patients.

KARNOFSKY PERFORMANCE STATUS SCALE

Karnofsky DA Burchenal JH. (1949). "The Clinical Evaluation of Chemotherapeutic Agents in Cancer." In: MacLeod CM (Ed), Evaluation of Chemotherapeutic Agents. Page 196.

DEFINITIONS	RATING (%)	CRITERIA
Able to carry on normal activity and to work; 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 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 active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

ECOG PERFORMANCE STATUS SCALE

Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone P.P. (1982) Am J Clin Oncol 5:649.

GRADE	CRITERIA
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

APPENDIX C – RESPONSE CRITERIA

For Patients with Chronic lymphocytic leukemia (CLL) *

Complete remission (CR): Normal imaging studies (X-ray, CT, MRI) (nodes, liver, and spleen), peripheral blood by flow cytometry has no clonal lymphocytes, bone marrow by flow cytometry has no clonal lymphocytes, bone marrow by morphology has no nodules (or if present, nodules are free from CLL cells by immunohistochemistry), and the duration is at least 2 months.

CR with minimal residual disease (CR-MRD): CR by above criteria except peripheral blood or bone marrow by flow cytometry with >0 - <1 CLL cells/1000 leukocytes (0.1%)

Partial remission (PR): Absolute lymphocyte count in peripheral blood at least 50% decreased and physical exam/Imaging studies (nodes, liver, and/or spleen) at least 50% decreased. Duration is at least 2 months.

Progressive disease (PD): at least one of: Physical exam/imaging studies (nodes, liver, and/or spleen) >50% increase or new, circulating lymphocytes by morphology and/or flow cytometry >50%increase, and lymph node biopsy with Richter's transformation

Stable disease (SD): Did not meet any of the above criteria for complete or partial remission or progression.

* Hallek et al. (2008) Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood. 111(12): 5446-56.

For Patients with Mantle Cell Lymphoma (Cheson Criteria) ^

Response	Definition	Nodal Masses	Spleen, Liver	Bone Marrow
CR	Disappearance of all evidence of disease	(a) FDG-avid or PET positive prior to therapy; mass of any size permitted if PET negative (b) Variably FDG-avid or PET negative; regression to normal size on CT	Not palpable, nodules disappeared	Infiltrate cleared on repeat biopsy; if indeterminate by morphology, immuno-histochemistry should be negative
PR	Regression of measurable disease and no new sites	≥ 50% decrease in SPD of up to 6 largest dominant masses; no increase in size of other nodes (a) FDG-avid or PET positive prior to therapy; one or more PET positive at previously involved site (b) Variably FDG-avid or PET negative; regression on CT	≥ 50% decrease in SPD of nodules (for single nodule ir greatest transverse diameter) no increase in size of liver o spleen	Irrelevant if positive prior to therapy; cell type should be specified
SD	Failure to attain CR/PR or PD	(a) FDG-avid or PET positive prior to therapy; PET positive at prior sites of disease and no new sites on CT or PET; (b) Variably FDG-avid or PET negative; no change in size of previous lesions on CT		

Relapsed disease or PD	Any new lesion or increase by ≥ 50% of previously involved sites from nadir	Appearance of a new lesion(s) > 1.5 cm in any axis, ≥ 50% increase in SPD of more than one node, or ≥ 50% increase in longest diameter of a previously identifed node > 1 cm in short axis	> 50% increase from nadir in the SPD of any previous lesions	New or recurrent involvement

Abbreviations: CR, complete remission; FDG, [18F]fluorodeoxyglucose; PET, positron emission tomography; CT, computed tomography; PR, partial remission; SPD, sum of the product of the diameters; SD, stable disease; PD, progressive disease.
^ Cheson BD et al. (2007) JCO 25(5): 579.

For Patients with Acute lymphoblast leukemia (ALL)

Remission status will be determined by restaging of bone marrow and other involved sites by morphology, flow cytometry and molecular studies, as appropriate.

For Patients with NSCLC and TNBC ~

Tumor response and progression will be evaluated in this study using the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee (Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *European journal of cancer (Oxford, England : 1990).* Jan 2009;45(2):228-247). Tumor flare has not been observed in our experience with CD19 CAR-T cells; however, patients with an increase in tumor burden at initial restaging will have confirmation by a repeat assessment at ≥ 4 weeks from restaging (Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clinical cancer research : an official journal of the American Association for Cancer Research.* Dec 1 2009;15(23):7412-7420) Measurable lesions are defined as those that can be measured in at least one dimension as ≥ 20 mm with conventional CT techniques (cuts of 10 mm or less in slice thickness) or as ≥ 10 mm with spiral CT (5 mm contiguous algorithm). All tumors must be recorded in millimeters or decimal fractions of centimeters. All other lesions are considered non-measurable. Measurable lesions will be identified as target lesions. A sum of the longest diameter for all the target lesions will be calculated and reported as the baseline sum. The baseline sum will be used as a reference by which to characterize the objective tumor response. Given potential for differential trafficking of CAR T cells to nodal sites, site of target lesions will be carefully captured to assess for possible differential radiographic response in visceral versus nodal disease sites.

Target lesion responses will be described as (1) Complete response (CR): disappearance of all target lesions; (2) Partial Response (PR): at least 30% decrease in the sum of the longest diameter (LD) of target lesions; (3) Progressive disease (PD): at least a 20% increase in the sum of the LD of the target lesions, taking as a reference the smallest LD recorded prior to receiving the first T cell infusion; and, (4) Stable Disease: neither sufficient shrinkage to qualify for PR nor sufficient growth to qualify for PD. Non-target lesions will be described as CR: disappearance of all non-target lesions and normalization of tumor-marker; Incomplete response/SD: persistence of one or more non-target lesion(s) and or persistent abnormal tumor marker; PD: Appearance of ≥1 new lesions and/or unequivocal progression of existing non-target lesion.

We anticipate some patients may have bone-only disease. The RECIST system incorporates CT imaging and considers bone disease to be "non-measurable". FDG PET imaging may be useful to monitor response to therapy in bone-only lesions. PET scans are interpreted quantitatively by using the maximum SUV of the most conspicuous bone lesion at baseline PET scan and monitoring change in that value. Based on recommendations proposed by the European Organization for Research and Treatment of Cancer (EORTC) regarding the use of FDG-PET for disease assessment, we will use FDG PET imaging for monitoring response to T cell infusions in patients with bone-only metastasis as outlined in the following Table.

EORTC Criteria for Assessment of Response by FDG-PET ~
Progressive Metabolic Disease • Increase of SUV >25% • Visible increase of FDG uptake (>20% of longest dimension) • Appearance of new focus
Stable Metabolic Disease • Increase of SUV <25% or decrease <15% • No visible increase of the extent of FDG uptake
Partial Metabolic Response • Reduction of a minimum of 15-25% of SUV after 1 treatment cycle; >25% after more than 1 treatment cycle
Complete Metabolic Response • Complete resolution of FDG uptake

APPENDIX D - Patient evaluations over the course of the study

Approximate days post T cell infusion

Study Assessments/ Testing	Screen	Enrolled	0	During and following each T cell infusion	1	2	4	7	10	14	21	28	35	60	90	120	180	365
History and Physical[1]	X	X		Day 1 and weekly x 5	X			X		X	X	X	X					
Karnofsky performance status	X																	
Vitals including O2 sats			X	q 15 min during then hourly x 2														
Automated CBC with differential, platelet count	X	X	X	2 x in first two weeks then weekly x3	X			X	X	X	X	X	X					
Renal panel and Hepatic panel with LDH	X	X	X	2x in first two weeks then weekly x3	X			X	X	X	X	X	X					
Uric acid, ferritin, CRP		X	X	2 x in first two weeks then weekly x3	X			X	X	X	X	X	X					
DIC panel without platelets		X	X	2 x in first two weeks then weekly x 3	X													
Pregnancy Test[2]	X	X																
Bloodworks NW Recipient Donor Battery Panel	X																	
G6PD screening		X																
Patient/Donor CD3 chimerism[3]	X																	
ABO blood typing and antibody screen	X																	
Muga scan or echocardiogram		X																
ROR1 expression confirmed	X																	
Quantitative IgG, IgM, IgA[4]		X													X			X
EKG		X																
CXR		X																
Lumbar Puncture with CSF evaluation[5]		X																

Study Assessments/ Testing	Screen	Enrolled	0	During and following each T cell infusion	1	2	4	7	10	14	21	28	35	60	90	120	180	365
Tumor/tissue biopsy[6]		X										X						
Bone marrow aspirate[7]		X								X							X	X
CT/PET scan[8]		X										X					X	
Brain CT or MRI		X										X						
PB review of lymphocyte subsets	X																	
PBMC collection	X																	
PTH levels												X		X				
Cytokine levels		X	X		X		X	X	X	X	X	X	X					
HACA			X					X		X	X	X	X	X	X		X	
Immunophenotyping[9]		X								X		X		X		X		
T cell persistence[9]		X	X		X		X	X	X	X	X	X	X	X		X	X	X
Transgene immunogenicity_serum		X										X		X		X	X	X
Transgene immunogenicity_PBMC												X		X		X	X	X
Serum archive[10]																X	X	X
PBMC archive[10]				0-21 and 21-90					X				X			X	X	X

1. History to include hematologic, cytogenetic, flow cytometric and histologic findings at diagnosis and time of enrollment as well as prior therapies and response to therapy.
2. If clinically indicated.
3. In patients who have had an allo transplant only.
4. Timepoints after enrollment may occur in patients who develop B cell aplasia.
5. Required for any patient with a history of CNS disease or signs and symptoms of CNS or epidural disease, unless a negative lumbar puncture was performed within 30 days prior to scheduled T cell infusion.

6. As clinically indicated. or as consented by patient for research only
7. 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 sodium heparin should be sent to the Berger Lab for research if done in conjunction with clinical procedure or if patient consented to reseach only
8. Diagnostic CT/PET scan to include neck, chest, abdomen and pelvis, as clinically indicated by disease and status and according to the protocol. Staging evaluations may be ceased if the patient proceeds to other anti-tumor therapy.
9. Immunophenotyping (Cohort A) may be omitted after day 28 in patients who proceed to other systemic therapies, with a recommendation to be made to the treating physician to follow Ig levels and replace if necessary.
10. Blood for archival purposes may be collected as follows: day 0-21 and 21-90, days120, 180 and 365.

T cell infusion time points represent guidelines for performance of required evaluations. Due to numerous factors influencing scheduling (pt and provider availability, testing services limitations etc.), variation in evaluation performance dates is anticipated and acceptable to the protocol (e.g., within +/- 7 days of time points $\leq$ day 35; +/- 30 days for time points > day 35).

APPENDIX E - Research sample checklist

RECIPIENT RESEARCH EVALUATIONS BEFORE T CELL INFUSION

SAMPLE	TIME	TEST	TUBE	VOL.	LAB
Blood, lymph node, tumor specimen or bone marrow	Time of initial Screening	Confirmation of ROR1 expression by flow cytometry or IHC	Sodium heparin (green)	5 ml	UW Hematopath lab
Blood	Screening	Review of lymphocyte subsets	Sodium heparin (green)	10 ml	Berger, D3-276
Blood	At Enrollment	Baseline T cell persistence	Sodium heparin (green)	30 ml	Berger, D3-276
Blood	At Enrollment	Serum cytokine levels	Serum separator	5ml	Berger, D3-276
Blood	At Enrollment	Baseline transgene immunogenicity	Serum separator	5 ml	Berger, D3-276
Bone Marrow (if collected)	At Enrollment	Research studies	Sodium heparin (green)	5-10 ml	Berger, D3-276
Any tissue (if collected) (requires specific consent if for research only)	At Enrollment	Research studies	Formalin	varies	Berger, D3-276
Blood	Day 0	Serum cytokine levels	Serum separator	5 ml	Berger, D3-276
Blood	Day 0	Baseline HACA	Serum separator	5 ml	Berger, D3-276
Blood	Day 0	T cell baseline persistence	Sodium heparin (green)	30 ml	Berger, D3-276

RECIPIENT RESEARCH EVALUATIONS AFTER T CELL INFUSION.

SAMPLE	TIME AFTER FINAL INFUSION	TEST	TUBE	VOL.	LAB

Blood	Day 1	Serum cytokine levels	Serum separator	10 ml	Berger, D3-276
Blood	Day 1	T cell persistence	Sodium heparin (green)	30 ml	Berger, D3-276
Blood	Day 4	Serum cytokine levels	Serum separator	10 ml	Berger, D3-276
Blood	Day 4	T cell persistence	Sodium heparin (green)	30 ml	Berger, D3-276
Blood	Day 7	Serum cytokine levels	Serum separator	10 ml	Berger, D3-276
Blood	Day 7	T cell persistence	Sodium heparin (green)	30 ml	Berger, D3-276
Blood	Day 7	HACA	Serum separator	10 ml	Berger, D3-276
Blood	Day 10	Serum cytokine levels	Serum separator	10 ml	Berger, D3-276
Blood	Day 10	T cell persistence	Sodium heparin (green)	30 ml	Berger, D3-276
Blood	Day 14	Serum cytokine levels	Serum separator	10 ml	Berger, D3-276
Blood	Day 14	T cell persistence	Sodium heparin (green)	30 ml	Berger, D3-276
Blood	Day 14	HACA	Serum separator	10 ml	Berger, D3-276
Blood	Day 21	Serum cytokine levels	Serum separator	10 ml	Berger, D3-276
Blood	Day 21	T cell persistence	Sodium heparin (green)	30 ml	Berger, D3-276
Blood	Day 21	HACA	Serum separator	10 ml	Berger, D3-276
Blood	Day 0-21 (optional)	PBMC archive	Sodium heparin (green)	60 ml	Berger, D3-276
Blood	Day 28	Serum cytokine levels	Serum separator	10 ml	Berger, D3-276
Blood	Day 28	T cell persistence	Sodium heparin (green)	30 ml	Berger, D3-276

Blood	Day 28	HACA	Serum separator	10 ml	Berger, D3-276
Blood	Day 28	PBMC transgene immunogenicity	Sodium heparin (green)	20 ml	Berger, D3-276
Blood	Day 28	Serum transgene immunogenicity	Serum separator	10 ml	Berger, D3-276
Any tissue (requires specific consent if for research only)	At restaging	Research studies	Formalin	varies	Berger, D3-276
Blood	Day 35	Serum cytokine levels	Serum separator	10 ml	Berger, D3-276
Blood	Day 35	HACA	Serum separator	10 ml	Berger, D3-276
Blood	Day 35	T cell persistence	Sodium heparin (green)	30 ml	Berger, D3-276
Blood	Weekly after day 35	Serum cytokine levels	Serum separator	10 ml	Berger, D3-276
Blood	Day 60	T cell persistence	Sodium heparin (green)	30 ml	Berger, D3-276
Blood	Day 60	HACA	Serum separator	10 ml	Berger, D3-276
Blood	Day 60	PBMC transgene immunogenicity	Sodium heparin (green)	20 ml	Berger, D3-276
Blood	Day 60	Serum transgene immunogenicity	Serum separator	10 ml	Berger, D3-276
Blood	Day 21-90 (optional)	PBMC archive	Sodium heparin (green)	60 ml	Berger, D3-276
Blood	Day 90	HACA	Serum separator	10 ml	Berger, D3-276
Blood	Day 120	PBMC archive	Sodium heparin (green)	20 ml	Berger, D3-276
Blood	Day 120	Serum archive	Serum separator	10 ml	Berger, D3-276
Blood	Day 120	T cell persistence	Sodium heparin (green)	30 ml	Berger, D3-276
Blood	Day 120	PBMC transgene immunogenicity	Sodium heparin (green)	20 ml	Berger, D3-276
Blood	Day 120	Serum transgene immunogenicity	Serum	10ml	Berger,

			separator		D3-276
Blood	Day 180	T cell persistence	Sodium heparin (green)	30 ml	Berger, D3-276
Blood	Day 180	HACA	Serum separator	10 ml	Berger, D3-276
Blood	Day 180	PBMC transgene immunogenicity	Sodium heparin (green)	20 ml	Berger, D3-276
Blood	Day 180	Serum transgene immunogenicity	Serum separator	10 ml	Berger, D3-276
Blood	Day 180	PBMC archive	Sodium heparin (green)	20 ml	Berger, D3-276
Blood	Day 180	Serum archive	Serum separator	10 ml	Berger, D3-276
Blood	Day 365	T cell persistence	Sodium heparin (green)	30 ml	Berger, D3-276
Blood	Day 365	PBMC transgene immunogenicity	Sodium heparin (green)	20 ml	Berger, D3-276
Blood	Day 365	Serum transgene immunogenicity	Serum separator	10 ml	Berger, D3-276
Blood	Day 365	PBMC archive	Sodium heparin (green)	20 ml	Berger, D3-276
Blood	Day 365	Serum archive	Serum separator	10 ml	Berger, D3-276
Blood	Clinical events	T cell persistence	Sodium heparin (green)	30 ml	Berger, D3-276
Blood	Clinical events	Serum cytokine levels	Serum separator	10 ml	Berger, D3-276
Bone marrow	Any clinical time point	Migration	Sodium heparin (green)	5-10 ml	Berger, D3-276
Tissue samples	Any clinical time point	Migration	RPMI	varies	Berger, D3-276

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 final 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:
    - Adverse event screening guidance for the primary physician in the form of a gene therapy LTFU-directed screening survey may be available.
    - 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 Berger Lab for archival purposes, and for analysis for transgene and vector persistence, and RCL, as dictated by studies of transferred T cell persistence.
2. Annual phone call survey or questionnaire to the participant to screen for adverse events.
3. Offer the opportunity to return to FHCRC for an annual LTFU clinic visit.
4. Compliance with 21 CFR 312.32 in adverse event reporting.
5. Research studies
    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 may 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. 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 QPCR. If there is no evidence of transgene persistence, RCL assays may be suspended after one year and samples may be archived.

APPENDIX G– Grading Criteria for CRS

Grade	Description of Symptoms
1: Mild	Not life-threatening, require only symptomatic treatment such as antipyretics and anti-emetics (e.g., fever, nausea, fatigue, headache, myalgia, malaise)
2: Moderate	Require and respond to moderate intervention: • Oxygen requirement < 40%, or • Hypotension responsive to fluids or low dose of a single vasopressor, or • Grade 2 organ toxicity (by CTCAE v4.03)
3: Severe	Require and respond to aggressive intervention: • Oxygen requirement ≥ 40%, or • Hypotension requiring high dose of a single vasopressor (e.g., norepinephrine ≥ 20 μg/min, dopamine ≥ 10 μg/kg/min, phenylephrine ≥ 200 μg/min, or epinephrine ≥ 10 μg/min), or • Hypotension requiring multiple vasopressors (e.g., vasopressin + one of the above agents, or combination vasopressors equivalent to ≥ 20 μg/min norepinephrine), or • Grade 3 organ toxicity or Grade 4 transaminitis (by CTCAE v4.03)
4: Life-threatening	Life-threatening: • Requirement for ventilator support, or • Grade 4 organ toxicity (excluding transaminitis)
5: Fatal	Death

Adapted from Lee et al., 2014 (Lee 2014)

APPENDIX H – Recommended management guidelines for CRS and neurotoxicity